Lower Extremity Arterial Disease

EDITED BY

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Series Editor's Introduction

The morbidity of peripheral arterial disease and its associated co-morbidities are becoming increasingly recognized by physicians and scientists in the 21st century. Drs. Caralis and Bakris' volume on *Lower Extremity Arterial Disease* is an up-to-date and clinically relevant contribution to the field of arterial disorders that brings together the numerous pathophysiologic, diagnostic, and therapeutic advances in the evaluation of atherosclerosis and peripheral arterial diseases involving the lower extremities.

The editors have carefully organized their volume into sections that detail the epidemiology of arterial disorders as it relates to smoking, diabetes, and hypertension as risk factors. There is ample coverage of diagnostic testing for claudication and lower extremity arterial disease using both noninvasive and arteriographic techniques. A superb chapter on the pathogenesis of arteriosclerosis using a translational approach segues the sections on diagnosis and epidemiology with those on treatment and special patient populations.

The editors have four chapters devoted to the management of claudication and lower extremity arterial disease: nonpharmacological (exercise), medical therapy, angioplasty, and endovascular stent placement and revascularization. These comprehensive chapters are very clinically oriented and help the reader to understand when nonsurgical versus surgical management should be considered.

The chapters in *Lower Extremity Arterial Disease* have been written by several well-known authors who have provided comprehensive, scientifically sound, and clinically appropriate information. As series editor of Clinical Hypertension and Vascular Diseases, I am delighted by this timely and excellent book and know that *Lower Extremity Arterial Disease* will become a highly useful textbook for all physicians interested in arteriosclerosis, diabetes, vascular medicine, interventional radiology, and general and vascular surgery.

William B. White, MD

Series Editor

PREFACE

Arterial diseases are the leading causes of morbidity and mortality in all industrialized countries, and their incidence is increasing in non-industrial countries. Among the industrialized countries, the United States in particular is in the midst of an epidemic of obesity and diabetes. One in four Americans meets the criteria for obesity and the number keeps growing.

Lower Extremity Arterial Disease (LEAD) is a common disease entity for men older than 40 and women older than 50 years of age. The prevalence of LEAD continues to increase with age, from less than 3% in the population younger than the age of 60 to more than 20% at age 75 and older. The majority of patients older than 75 with LEAD are asymptomatic. Prevention of arterial disease is key to reducing morbidity and mortality. LEAD is associated with specific risk factors, namely hypertriglyceridemia, homocysteinema, very low HDL cholesterol, physical inactivity, and above all cigaret smoking and diabetes mellitus, alone or in tandem.

LEAD, symptomatic or not, particularly when it coexists with Coronary Artery Disease (CAD) (Chapter 9), calls for polypharmacy. Polypharmacy should no longer have a bad connotation in treating patients with LEAD and CAD. In patients with metabolic syndrome and LEAD, a short-acting statin in the evening and triglyceride-reducing fenofibrate during breakfast can improve time to claudication significantly, improve endothelial function, improve the lipid profile, and at the same time decrease the probability of a coronary event. Fenofibrate and rosuvastatin or simvastatin should be given twelve hours apart to avoid an overlap of their half-lives. In treating LEAD, aggressive risk factor modification should be implemented, which includes: smoking cessation, euglycemic control of diabetes, ideal control of both systolic and diastolic pressure, dramatic improvement of the lipid profile, low calorie Mediterranean diet rich in antioxidants, and, equally important, exercise therapy, either community based or supervised (Chapter 11).

Percutaneous interventions with balloon angioplasty, bare metal stents, and the more preferable for the femoral and intrafemoral arteries, drug-diluting stents, offer dramatic improvement of the stenosed or occluded lumen (Chapter 12). Blood flow is restored and great symptomatic relief is achieved. Arterial grafting techniques have also pro-

vided tremendous advances in reinstituting peripheral blood flow with the lowest possible periprocedural complication rate. Today's therapeutic armamentarium also includes the most promising approach for advanced and distal disease (i.e., therapeutic angiogensis). The vascular growth factors can be administered intra-arterially or intramuscularly in the ischemic muscle. Therapeutic angiogenesis combined with the other current therapeutic options and aggressive risk-factor modification can remarkably improve claudication, prevent limb loss, and prolong life (Chapters 10 and 13).

The presence of LEAD, as defined by an ankle brachial index (ABI) of less than one, adversely influences the prognosis of coronary heart disease. The lower the ABI (the lowest in either ankle), the worse the prognosis. The morbidity and mortality from coronary artery bypass grafting is higher in patients with LEAD. The same increased morbidity and mortality also occurs during or after percutaneous coronary interventions, short or long term. LEAD differs from other peripheral arterial diseases by its specific medical therapy as well; the drug currently approved by the Food and Drug Administration to treat the symptoms of claudication, the phosphodiesterase III inhibitor Cilostazol, has no effect on other arterial beds like the renal or the carotid systems (Chapter 10).

Intermittent claudication can be caused by an abdominal aortic aneurysm (AAA), which is a totally different disease entity with different pathophysiology and distinct genetic mechanisms. AAAs cannot be stopped from increasing in diameter with either blood pressure control or antilipid therapy.

Lower Extremity Arterial Disease provides a comprehensive state-of-the-art review of LEAD. A detailed review of its cardinal symptom, intermittent claudication, is presented in Chapter 1. The book provides a thorough and detailed description of noninvasive and accurate assessment of LEAD with special emphasis on the ABI and its diagnostic and prognostic significance. Modern diagnostic methods, such as vascular flow patterns and magnetic resonance angiography, are eloquently presented for the educational benefit of the clinician in Chapter 2. The known risk factors for LEAD and CAD—smoking, diabetes mellitus, dislipidemia, systemic hypertension, and physical inactivity—are presented in the chapters on epidemiology and risk factors (Chapters 3–8).

The question "What do claudiants die from?" is reviewed and analyzed in the chapter on LEAD coexisting with CAD. The presence of LEAD increases significantly the probability of coexisting CAD. In patients who cannot exercise much because of claudication or in asymptomatic patients with LEAD, the preferred approach to diagnose coex-

isting CAD is dual isotope pharmacological stress testing either with adenosine IV or dipyridomole IV. Adenosine should not be given if bronchial asthma, hypotension, or profound bradycardia is present. Alternatively, dobutamine can be utilized as a pharmacologic stressor. Dobutamine should not be used in patients with LEAD and atrial fibrillation; dobutamine remarkably accelerates atrioventricular conduction. Dobutamine should be avoided as a pharmacologic stressor if blood pressure is elevated; it may precipitate a hypertensive crisis. If the clinical index of suspicion is high, then the cardiovascular physician may recommend coronary angiography (luminography is a more accurate term). Overall, single photon emission computed tomographic images of the myocardium are preferable because they can accurately assess myocardium at risk in patients with LEAD.

In morbidly obese patients (those weighing more than 350 lbs) with LEAD, neither myocardial perfusion studies nor coronary angiography can be performed for technical reasons. These patients can be evaluated with contrast echocardiography (Chapter 9). The management of patients with LEAD and CAD is the same as in every patient who has myocardial ischemia, silent or symptomatic.

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1 Claudication

Clinical Diagnosis and Differential Diagnosis

Dennis G. Caralis, MD, PhD, MPH, FAHA, FACC

CONTENTS

INTRODUCTION

Legend has it that the term *claudication* was given after the Roman Emperor Claudius, who would walk for a short distance, then stop and stand before he would start walking again. Etymologically, claudication is derived from the Latin verb *claudicare*, which means to limp. In 1858, Jean Martin Charcot described pain in the lower extremities resulting from arterial insufficiency. Intermittent claudication can inhibit walking, and cause limping due to ischemia of the lower extremity unilaterally or bilaterally. The most common cause of lower extremity ischemia is peripheral arterial disease of the major arteries supplying the legs and feet. Lower extremity ischemia may also progress to severe limb-threatening ischemia with symptoms and physical signs at rest as well. A detailed complete history should be obtained from every patient, middle-aged and the elderly, before the diagnosis of claudication is made. Vascular claudication under the age of 45 years is rare.

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Frequent symptoms of lower extremity arterial disease (LEAD) are pressure, tightness, squeezing sensation, burning, and frank pain precipitated by leg exercise and relieved by rest. Intermittent claudication also may be present as fatigue in working skeletal muscles. Continuous pain must be differentiated from intermittent pain, because continuous pain may be a result of a sudden arterial occlusion with or without preexisting stenosis. Extremely severe ischemia or ischemic neuropathy, ulceration, or gangrene can also cause continuous pain.

Persistent pain of an extremity may have a different etiology other than arterial occlusive disease, from an atheromatous process or embolization. Connective tissue diseases with arteritis can cause persistent leg pain *(1)*. Venous insufficiency, severe anemia, muscle phosphorylase deficiency, muscular dystrophy, radiation fibrosis, and retroperitoneal fibrosis have been associated with painful lower extremities *(1,2)*. Ergot toxicity and cyclist's lesion may cause pain in the legs during exercise *(2–4)*. (Ergotamine toxicity can also cause mitral valve disease *[3]* and coronary spasm.) Extra-arterial causes of persistent pain are phlebitis with or without phlebothrombosis and lymphangiitis. Intermittent tightness or pain in a muscle group is more characteristic of intermittent claudication, and is generally associated with exertion. Rarely, claudicants may report worsening of the claudication when treated with β-blockers *(5,6)*. In general though, β-blockers are safe in LEAD. If there is a definite indication for their use, they should be administered. They can cause reduction of the cardiac output and blockade of β2-adrenoreceptor mediated skeletal muscle vasodilatation. The latter effect results in unopposed β-adrenoreceptor vasocontristion. β-Blocking agents with β1-selectivity (cardioselective) should be used instead in order to avoid the effects of peripheral vasoconstriction.

Intermittent claudication typically occurs with walking and is relieved with rest or standing. Location of the symptoms can be the hip, unilaterally or bilaterally, the thighs, the calf most frequently, and the foot. Claudication of the foot caused by ischemia is less common. It may exist independently of claudication of the calf when the occlusive lesions are diffuse and involve small arterial branches of the infrapopliteal arteries distally *(4)* such as in diabetic patients. A clinical clue to the diagnosis of frank claudication of the foot is its common association with more proximal arterial occlusions and calf pain. Claudication of the foot is usually manifested as pain or ache or a *catch* or a cramp in the forefoot precipitated by walking. More observant patients also report a more cold foot at night, ipsilaterally. Prognostically, claudication of the foot, as uncommon as it may be, indicates severe and diffuse atherosclerotic disease that frequently progresses to ischemic pain at rest. The posterior tibial pulse is virtually always absent *(8–11)*.

Intermittent claudication is a clinical diagnosis. Usually lower extremity ischemia is caused by flow-limiting atherosclerotic plaques. Intermittent claudication is the presenting symptomatology in half of the patients with chronic lower extremity ischemia. The other one-third to half of patients with documented peripheral arterial occlusive disease are asymptomatic or silent *(8)*. Other symptoms and physical findings indicating more severe ischemia are pain at rest, pale and cold extremity(s), ischemic ulcers, peripheral cyanosis, and gangrene *(12)*.

Less frequently, peripheral symptoms may appear episodically as a result of an otherwise silent dissection of the aorta *(4)*. Coarctation of the aorta, thoracic, or abdominal, is another cause of intermittent claudication *(4,13)*. Episodic claudication can also be caused by distal embolization of a mural thrombus from the left cardiac ventricle or the left atrium, and less commonly from a left atrial or left ventricular myxoma. Because of its many clinical presentations cardiac myxoma has been described as the *great simulator (14)*. The importance of atrial fibrillation as a cause of embolism is well known *(15)*. Increasingly, recognized sources of emboli utilizing trans esophageal echocardiography are left atrial thrombi in patients with large left atria, atrial septal aneurysms, or fibrillating atria. Embolization from the heart can precipitate a dramatic symptomatology. Claudication can be a result of an aortic aneurysm or from an arterial aneurysm situated proximally to the site of the symptoms. An aneurysmal dilatation *(16)* or a significant ectasia can cause hemodynamically important limitation of blood flow and be the source of distal emboli as well. The prevalence of abdominal aortic aneurysms in older men varies from 0.4 to 5.4%. In carotid artery disease the prevalence of abdominal aortic aneurysms can be as high as 25.7% *(17)*. Atherosclerosis can be a generalized disease.

The symptoms of claudication is a result of LEAD confirmed by ABIs or duplex scanning can be classified on a scale of I–IV, a grading classification of the Canadian Heart Association that parallels that of angina pectoris.

Claudication, Class I is hemodynamically significant LEAD diagnosed, either by physical examination, by arterial flow studies, or by ankle brachial indices but without symptoms.

Claudication, Class II is symptomatology precipitated by moderate exertion, e.g., walking fast or for a long distance or uphill and relieved by rest, usually standing.

Claudication, Class III is characterized by symptoms precipitated by walking on a straight level and for a short distance, the equivalent of one to two city blocks, approximately 50–100 m.

Claudication, Class IV is ischemic pain of one or both lower extremities occuring even at rest.

Many patients with LEAD may deny the specific symptomology of claudication, but tend to walk very slowly even on a straight level. Limb ischemia can be significant as evidenced by physical findings of diminished pulses, atrophic skin changes, elevation blanching, dependent rubor, and delayed venous filling time. Yet, it can be painless, a condition pathophysiologically analogous to silent myocardial ischemia. Silent limb ischemia and silent myocardial ischemia can frequently co-exist with a higher prevalence in elderly men and women.

In 1962, the widely used WHO/Rose questionnaire (Table 1) on intermittent claudication was developed for use in epidemiological surveys. However, several population studies have shown that it is only moderately sensitive (60 to 68%), although highly specific (90 to 100%). Reasons for the moderate if not poor sensitivity and high specificity were determined following its application to 586 claudicants and to 61 subjects with other causes of leg pain. The results showed two important findings: (1) over half of the false negatives were produced by one question alone, and (2) only three questions were required to maintain the specificity of the questionnaire. This knowledge, in conjunction with the pretesting of additional questions, enabled a new questionnaire to be constructed, the Edinburgh Claudication Questionnaire (Table 2). This questionnaire was tested on 300 subjects aged over 55 years attending their general practitioner, and found to be 91.3% (95% CI 88.1 to 94.5%) sensitive and 99.3% (95%CI 98.9 to 100%) specific in comparison to the diagnosis of intermittent claudication made by a physician. The repeatability of the questionnaire after six months was excellent ($\kappa = 0.76$, $p < 0.001$) *(18)*.

The physician taking the history should bear in mind, as he unravels his detective work, the various risk factors and disease entities that predispose to or aggravate LEAD. Common risk factors for peripheral arterial disease are cigaret smoking, diabetes mellitus, hypertension, hyperlipidemia (particularly hypertiylyceridemia), aging, sedentary life, and obesity. Less commonly one may detect a history of gout (with hyperuricemia), homocysteinemia high sensitivity C-reactive protein, and thrombophilic states as predisposing factors for claudication. A hypertensive patient, who has none of the mentioned risk factors and complains of claudication, may have an abdominal aortic aneurysm *(16)*. The risk factors for LEAD have been well documented in the literature *(16,19–32)*. Among some less known risk factors for intermittent claudication include an elevated hematocrit *(2)*, hostile personality *(25)*, and a decreased forced vital capacity *(28)*. The best single discriminator for LEAD is a decreased or absent posterior tibial pulse *(33)*.

Table 1
The WHO/Rose Questionnaire on Intermittent Claudication

(a) Do you get a pain in either leg on walking?	1. ☐ Yes 2. ☐ No
(b) Does this pain ever begin when you are standing still or sitting?	1. ☐ Yes 2. ☐ No
(c) Do you get this pain in your calf (or calves)?	1. ☐ Yes 2. ☐ No
(d) Do you get it when you walk uphill or hurry?	1. ☐ Yes 2. ☐ No
(e) Do you get it when you walk at an ordinary pace on the level?	1. ☐ Yes 2. ☐ No
(f) Does the pain ever disappear while you are still walking?	1. ☐ Yes 2. ☐ No
(g) What do you do if you get it when you are walking?	1. Stop 2. Slow down 3. Continue at same pace
(h) What happens to it if you stand still?	1. Usually continues more than 10 minutes 2. Usually disappears in 10 minutes or less

Definition of positive classification requires all of the following responses: "Yes" to (a) and (d); "No" to (b) and (f); "stop" or "slow down" to (g); and "usually disappears in 10 minutes or less" to (h). Grade 1 = "no" to (e) and Grade 2 = "yes" to (e) *(18)*.

How prevalent is intermittent claudication? Intermittent claudication is present in about 4.5% of individuals over 60 years of age, compared with 2% of patients under 60 years of age *(34,35)*. The odds ratio for claudication in men is three times higher than in women. Diabetic patients are at five times greater risk than nondiabetic individuals. Six percent of men and 3% of women between the ages of 55–74 years have claudication *(36)*. In the diabetic patient, peripheral arteriosclerosis is 11 to 40 times higher than in the nondiabetics *(36)*. Diabetic men with intermittent claudication have double the risk for stroke. Congestive heart failure may be three times higher when both conditions co-exist, diabetes and claudication, compared with either alone. In LEAD gangrene among diabetic and nondiabetic patients was found to be 31% and 5%, respectively *(37)*. In the Framingham (Massachusetts) Heart Study *(28)*, 5209 men and women aged 35–94 years were followed for cardiovascular events. For men 35–54 years old, the prevalence of peripheral arterial disease was 7.4%; for women it was 8.2%. In the ensuing two decades, the prevalence for men was 12.5% for the sixth decade of life and 11.6% for the seventh decade, whereas for women it was 14.4% and 9.4%, respectively. In the age group 75–94 years, the prevalence decreased in men to 7.1% and 5.0% in women *(28)*. Smoking, more than any other risk

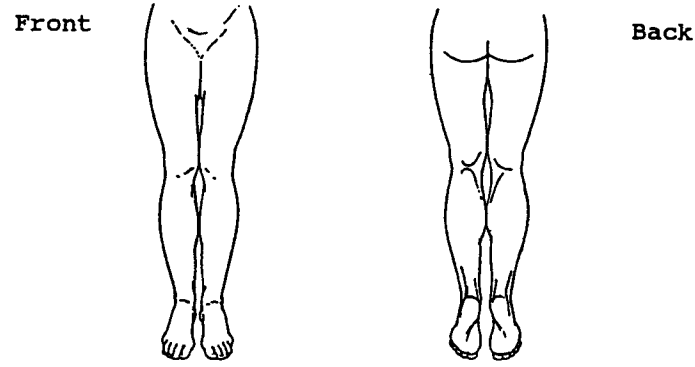
Table 2
The Edinburgh Claudication Questionnaire

(1) Do you get a pain or discomfort in your leg(s)?	Yes	☐
	No	☐
	I am able to walk	☐

If you answered "yes" to question (1) - please answer the following questions. Otherwise you need not continue.

(2) Does this pain ever begin when you are standing still or sitting?	☐ Yes	☐ No
(3) Do you get it if you walk uphill or hurry?	☐ Yes	☐ No
(4) Do you get it when you walk at an ordinary pace on the level?	☐ Yes ☐ Yes	☐ No ☐ No
(5) What happens to it if you stan still?		
Usually continues more than 10 minutes	☐	
Usually disappears in 10 minutes or less	☐	

(6) Where do you get this pain or discomfort? Mark the spaces(s) with "x" on the diagram below



Definition of positive classification requires all of the following responses: "Yes" to (1), "No" to (2), "Yes" to (4). If these criteria are fulfilled, a **definite** claudicant is one who indicates pain in the calf, regardless of whether pain is also marked in other sites; a diagnosis of atypical claudication is made if pain is indicated in the thigh or buttock, in the absence of any calf pain. Subjects should not be considered to have claudication if pain is indicated in the hamstrings, feet, shins, joints, or appears to radiate, in the absence of any pain in the calf *(18)*.

factor, can accelerate peripheral arterial disease and increase its severity *(30)*. The physician, as in all disease entities, should expand in a detailed fashion into all of the patient's past and present health aspects, family history, occupational history, detailed past medical history, operations, and medications.

A large percentage of claudicants (more than 65%) suffer from severe coronary artery disease, silent or symptomatic *(38–42)*. A truly investigative approach should be taken, in order to detect in the history, or in the symptomatology or in the patient's habits, clues for coronary artery disease. A patient may have significant coronary artery disease and LEAD as well, but may not experience effort angina because claudication may stop the patient first. In a study of 25 patients *(42)* with intermittent claudication, eight were found to also have carotid artery obstruction. It is interesting to note here that in 1914 Ramsey Hunt termed *cerebral intermittent claudication* the brain ischemia resulting from partial carotid artery occlusion *(43,44)*. If peripheral arterial disease co-exists with carotid disease, the probability of the same LEAD having coronary artery disease as well is 85%. Three hundred-twelve patients with LEAD were followed for 8- to 11- years *(45)*. Sixty-nine percent died during the follow-up and 68% of the deaths were cardiac. The 5-year mortality of claudicants is 29%. In a Japanese study *(46)*, 30% of claudicants died within 5 years and 60% of them died from cardiac or cerebrovascular disease. In claudicants, by far, the leading cause of death is cardiac.

Within 5 years from the onset of claudication, 55% of all patients will experience an improvement of their symptomatology or their symptoms will stabilize. In 14% of claudicants the ischemic process will progress. The amputation rate is 4% in 5 years. More than 80% of patients avoid ischemic complications or amputation for 5 or more years. Up to 15% of those who continue to smoke will undergo amputation within 5 years. Diabetics who smoke cigarets have an amputation rate of about 25% within 9 years *(47–49)*. In a landmark study by Criqui et al. *(50)*, patients with LEAD were followed for 10 years; 62% of men and 33.3% of women died during the follow-up, as compared with 16.9% of men and 11.6% of women without peripheral arterial disease *(50)*. Today, with our powerful therapeutic armamentarium ranging from regional thrombolysis, angiogenesis, drug eluting stents, balloon angioplasty directional and/or rotational atherectomy, and other endovascular strategies or bypass surgery, amputation should be preventable. Sadly, it is not.

CLAUDICATION IN THE ELDERLY

In the elderly, the symptoms of pain, pressure, or tightness, may be modified and more ill defined; actually they may be minimal to absent. Similarly, asymptomatic coronary heart disease with silent myocardial infarction and silent ischemia is more prevalent in the elderly. Epidemiologic studies estimate the prevalence of claudication to be as high as 10%

in patients between 65 and 70 years of age, up from 4.5% for the age group 60–64 years of age *(46)*. The true prevalence of significant LEAD may be much higher as a result of underreporting of the specific symptomatology of claudication by the elderly. In a study of more than 500 patients older than 70 years of age, there was a much higher prevalence of intermittent claudication than in younger patients *(48)*. The evaluation of the elderly for LEAD should rely more on meticulous physical examination and other objective data and less on the patient's complaints. Peripheral and coronary artery disease can co-exist more frequently and more silently in the elderly. The physician is challenged to identify patients at risk for gangrene, which is more prevalent in older patients, even though ischemic symptoms may be unimpressive or lacking. A detailed physical examination of the cardiovascular system in order to detect signs of peripheral ischemia should be an integral part of the physical examination of individuals over the age of 60 years. Today's medicine, in addition to an ever improving transcatheter therapeutic technologies, possesses risk factor modification methods to render gangrene and amputation preventable. Gangrene and amputation should be considered as a defeat for the physician(s) who have cared for the patient. The number of amputations should start declining in patients with LEAD. This has been the trend for the number of myocardial infarctions annually in the United States, downward for the past three decades, mainly as a result of aggressive risk factors modification and other therapeutic interventions.

Claudication is a clinical, easy to make diagnosis. Claudication of the upper extremities, although much less frequent than that of the lower extremities. is also a clinical diagnosis. The extremities should be examined carefully. Examination of the peripheral arterial system should include an evaluation of the volume and character of the arterial pulses of the carotids and of the arteries of the upper extremities: the subclavian, the brachial, the radial, and the ulnar. Physical examination should definitely encompass the abdominal aorta for abnormal pulsations, ectasias and/or bruits, and the arteries of the lower extremities: femoral, popliteal, dorsalis pedis, and posterior tibialis. The pulse volume can be graded on a scale of 0 to 4. In addition to palpation, physical examination of the peripheral arterial system should include auscultation over the carotids, auscultation over the subclavian arteries above, and below the midclavicular area. A bruit over the subclavian artery and disappearance of the radial pulse with compression of the subclavian artery is evidence for subclavian syndrome. On occasion, a bruit may be heard by auscultation deep in the axilla. The bruit, a composite of low frequency sounds, is better appreciated when the examiner is using the bell of the stethoscope.

Auscultation for abdominal and femoral bruits is a must in evaluating any patient.

The clinician should grade the pallor and check for elevation blanching by raising the lower extremity 30–40 degrees above the horizontal level. The examiner should press with his or her index finger to occlude capillary inflow and then, time in seconds, the return of color. Color return time of 15–20 seconds indicates moderate ischemia *(50)*. In severe ischemia, it takes more than 40 seconds for the baseline color to return. The venous filling time (in seconds) is another useful bedside index and should be measured. Elevation and dependency tests give a rough but reliable estimate of the degree of ischemia of the lower extremities. If more objective data are desired the systolic blood pressure index can be determined *(51)*. Ankle-to-arm systolic pressure ratios below 0.97 and 0.90 were found to be a highly probable sign of LEAD *(51)*. The fall in ankle systolic pressure after exercise may serve as an objective indicator of the severity of hemodynamically important LEAD. This simple evaluation can become a standard test; a fall in the leg pressure that occurs after 1 minute of walk at a speed of 4 mph at 10% elevation *(52)* indicates hemodynamically significant LEAD. In a study of 150 patients with peripheral arterial disease and claudication the ankle mean pressure was 58 mmHg; in patients with rest pain it was 33 mmHg and in patients with chronic ulceration, it was 20 mmHg *(53,54)*.

With electromagnetic flowmetry and Doppler ultrasound *(55)* it is possible to confirm and quantitate patient complaints, follow the disease progression, and document improvement following pharmacotherapy, exercise therapy, percutaneous endovascular interventions or arterial reconstructive interventions. Limb scintigraphy with thallium-201 presents advantages and great potential for clinical applications *(56)*.

Peripheral arterial occlusion can be the initial manifestation of cardiac or systemic disease. At times, patients with chronic stable claudication may experience abrupt shortening of the distance at which claudication occurs, and this may be the only symptomatic evidence of an acute arterial occlusion either by embolization of by thrombus formation on a pre-existing arterial stenosis. The situation is not chronic and stable any more, but acute and unstable. As ischemia becomes more severe, the patient with chronic peripheral arterial disease develops ischemic pain at rest. The pathophysiologic mechanisms and the clinical presentation parallel the evolution of chronic stable angina pectoris to unstable angina and acute coronary syndromes.

When limb ischemia becomes more severe, rest pain appears usually in the toes or the foot and can become *nocturnal*. The patient with rest pain at night finds some relief by sitting up on the side of the bed with

the feet in a dependent position. A reverse Trendelenburg position can be helpful. Ischemic ulceration, usually but not always, a result of trauma to the ischemic limb can be a cause of severe pain. The ischemic ulcer has a discrete edge covered with eschar and its base is pale.

In the young, two congenital lesions are noteworthy: (1) entrapment of the popliteal artery, causing calf claudication with walking but not with running, and (2) adventitial cystic disease of the popliteal or femoral arteries *(62–65)*. Both warrant surgical repair.

DIFFERENTIAL DIAGNOSIS

Epidemiologically, claudication caused by peripheral arterial disease affects primarily patients over the age of 40 years. Buerger's disease or thromboangitis obliterans is primarily a disease of younger men. It was first described by Leo Buerger in 1908 *(57–59)*. Most patients with Buerger's disease are smokers. Smokers may present with peripheral vasospasm, cold fingers and toes. Cyanosis can be observed upon exposure to cold. Smokers, both men and women, *(61)* with peripheral vasospasm (Raynaud's phenomenon) frequently have coronary spasm with chest pain or other angina equivalent symptoms at rest, particularly in the morning hours. When observed in a hospital setting, an electrocardiogram (ECG) should be taken during chest pain; the ECG may show ST elevation and much less frequently ST segment depression. The ECG is normal between the attacks of coronary spasm. Men with Buerger's disease may also have a history of phlebitis, usually superficial. They typically seek medical attention for claudication of the arch or the calf. Physical examination is remarkable for diminished pulses of the small arteries. Frequently, the upper extremity is also involved. The etiology of Buerger's disease may relate to an autoimmune process *(58,60)* rather than atherosclerosis.

The differential diagnosis of claudication should also include occupational diseases with recurrent blunt trauma. Claudication can be iatrogenic in etiology, particularly among patients who suffer from migraine attacks and consume high doses of ergot containing preparations. Ergotamine derivatives can cause coronary spasm, peripheral vasospasm, and claudication *(4)*. Arteritis associated with collagen vascular disorders, temporal arteritis *(4)*, and Takayasu's disease *(70,71)* can also be causes of claudication. Infrequently, claudication can be the presenting symptom of congenital arterial narrowing or of fibromuscular hyperplasia *(4)*. Popiteal artery aneurysm, almost always related to atherosclerosis, is another surgical disease that causes claudication, rest pain, skin ischemia, and gangrene. Ultrasonography is the most useful

diagnostic tool *(66)* for popliteal aneurysm. Thoracic outlet compression may be symptomatic or may present with intermittent ischemic symptoms of the arm and forearm, peripheral vasospasm and at times severe ischemia of the hand and of the fingers depending on the site of the arterial occlusion *(67–69)*. The cause of the distal ischemia in the thoracic outlet syndrome is distal embolization from compression of the subclavian artery. Anatomically, the thoracic outlet syndrome is caused by compression of the subclavian artery by a cervical rib or by an abnormal first rib that can be palpated in the supraclavicular fossa.

Occlusive arterial disease of the upper extremity, a rare entity, can cause arm claudication and may be associated with other ischemic symptoms. Ischemia of the upper extremity should be of particular interest to the cardiologist in view of the increasing utilization of the internal mammary arteries as coronary arterial grafts.

PSEUDOCLAUDICATION

Approximately half a century after Charcot described ischemic pain in the lower extremities, Dejerine in 1911 described a syndrome he called *intermittent claudication of the spinal cord (72–74)*. This neurogenic intermittent claudication is known as pseudoclaudication. In 1954, Verbiest reported in detail symptoms caused by the developmental narrowing of the lumbar vertebral canal *(72)*. Later, a number of cases of neurospinal compression were reported *(73–75)*. Pseudoclaudication or neurogenic claudication is bilateral or unilateral and consists of discomfort or pain in the buttocks, thighs, legs, and calves precipitated by walking uphill, on a straight level, or worse by walking downhill. Standing can also cause symptoms in neurogenic claudication. The discomfort or weakness or frank pain is relieved by sitting or by lying down *(76)*. Bending forward or adopting a flexed position can alleviate the symptoms of neurogenic claudication *(75)*. Leaning against a wall or bending forward can also improve the symptoms of pseudoclaudication. Prolonged standing has been associated with severe discomfort in neurogenic claudication, but not in vascular claudication. Walking downhill can precipitate pain in neurogenic claudication, but not in vascular claudication *(77)*. In advanced cases of neurogenic claudication there is pain both in the standing and in the supine positions. Physical examination of the peripheral pulses is usually normal. By contrast in vascular claudication, the peripheral pulses are diminished or absent, there are bruits and physical findings of peripheral ischemia. The electromyogram is abnormal in neurogenic claudication and normal in vascular claudication. Patients with neurogenic claudication often complain of leg weak-

ness and may actually fall down *(76)*. This weakness is best evoked by attempts to walk on the heels or on the toes. Any position that tends to cause the canal to become narrower will aggravate pseudoclaudication. Bending forward opens up the canal and relieves the symptoms *(76,77)*. A lordotic position hyperextends the spine and produces radicular pain. Probably the best finding on physical examination is to provoke the symptoms by having the patient stand up or walk for a few minutes and notice if they adopt a flexed position. Standing up for several minutes will cause the patient to bend forward and lean on the nearest back support. Continued walking for several minutes induces leg distress. In about 43% of patients with neurogenic claudication, the deep tendon reflexes are reduced at the ankle level and in 18% of patients there is a reduction at the knee level. In neurogenic claudication rechecking the deep tendon reflexes of the lower extremities after walking for several minutes or standing for several minutes will show a reduction as compared with sitting *(76)*.

Neurogenic claudication and vascular claudication are not mutually exclusive and can co-exist in about 9% of patients with either diagnosis. Vascular changes in the lower extremities may co-exist, because of the older age group in which vascular and neurogenic claudication occur. In one study *(72)*, up to 42% of patients with pseudoclaudication were found to have absent pedal pulses. The etiology of neurogenic claudication is lumbar stenosis *(78,79)*. In the nineteenth century, medical reports were published describing a narrow spinal canal syndrome in achondroplastic dwarfs. Other rare congenital causes of pseudoclaudication are Morquio's syndrome dysplasia, hypochondroplasia, and Down's syndrome. Apart from discogenic disease acquired causes are Paget's disease, systemic amyloidosis *(80)*, hypertrophied ligamentum flavum, and calcium pyrophosphate crystal deposition *(81)*. Syphilitic arteritis of the cord (Dejerius syndrome) in years past and the Foix Alajouanine syndrome are two very rare causes of neurogenic intermittent claudication *(90)*. Degenerative joint and ligament hypertrophy is the leading cause of lumbar stenosis. The ligamentum flavum which normally does not exceed 4 mm in thickness may measure 7–8 mm. Spinal stenosis, which is the anatomic cause of neurogenic claudication, can be either congenital with a congenitally narrow spinal canal or more frequently acquired. The diameter of the canal is narrowed by the hypertrophic skeletal changes. In the middle-aged or the elderly patient who can have both neurogenic and vascular complications, the correct diagnosis, because of the variance in the presenting symptomatology and physical signs, has to be confirmed by basic laboratory evaluation. The plain radiographs of

the lumbar spine show dense bony structures and the presence of degenerative disease. Further evaluation consists of co-axial tomography and preterably magnetic resonance imaging (MRI) *(82,83)*. Myelography is an important test *(82)*, but is less frequently indicated even when laminectomy is being considered by the consulting surgeon. Computed tomography and MRI are complementary tests for the preoperative elevation *(82,83)*. Plain X-ray films are of far less value in evaluating the lumbar spine; they are more useful for the cervical spine. Pseudoclaudication, which was not widely appreciated in the past *(84,85)*, is now part of the differential diagnosis of intermittent claudication.

Wilson *(87)* divided patients with this condition into two groups: (1) In the larger group, symptoms occurred during any activity or position involving extension of the lumbar spine that he termed *postural cauda equina claudication*, and (2) a smaller group of patients, with symptoms of the affected extremities after exercise that he described as *ischemic cauda equine claudication.*

According to Blau et al. *(88,89)*, the vascular factor is more important. In exercised animals, vessels inside the canal are dilated and if the canal is narrow increased blood supply is prevented, thus leading to ischemia of the cord and the nerve roots. However, this explanation is not shared by others *(90)*. It does not explain why neurogenic claudication occurs in the lordotic position at rest. Pseudoclaudication can be caused by other orthopedic conditions of the hips, knees, and other joints.

VASOSPASTIC CLAUDICATION

Vasospasm occurring in the digital circulation is also known as Raynaud's phenomenon. Raynaud's phenomenon is caused by abnormal vascular reactivity precipitated by exposure to cold or by emotional stress. Peripheral vasospasm was first described by the French clinician, Maurice Raynaud in 1862 *(91)*. Usually in peripheral vasospasm there are three phases: first, the main arterial branches of the digits constrict causing a marked reduction of the blood flow to the tissues; the skin of the digits becomes pale and the patient complains of numbness, pain or paresthesias. The second phase is the cyanotic phase; the digits appear blue, purple or even black in extreme cases. The cyanotic phase continues until the blood flow is re-established as the arterial branches open up again. The cyanotic color is caused by deoxygenated hemoglobin in the post arteriolar capillaries. The third and final phase, is the post-ischemic hyperemia; the increased blood flow to the skin gives a blushed coloration. Many years ago, Allen and Brown *(92)* described the minimal requisites for the diagnosis of Raynaud's disease or syndrome.

It should be noted that several patients do not exhibit the classical color changes. They may complain only of cold and numb fingers or toes; thumbs are usually spared.

As with claudication caused by atherosclerosis, Raynaud's phenomenon is a clinical diagnosis. No practical laboratory test exists to diagnose peripheral vasospasm. The physician should elicit the symptoms by taking a detailed history. Color charts can also be used. The patient is asked to identify the color of the skin during a typical episode by choosing colors from actual photographs of Raynaud's attacks. Patients with peripheral vasospasm are classified as having primary or secondary Raynaud's phenomenon. In primary Raynaud's phenomenon, the patient has symmetrical attacks in the absence of digital pitting, ulceration, or gangrene. The nailfold capillaries are normal and screening tests for connective tissue disease, like erythrocyte sedimentation rate, antinuclear antibodies, immunoglobulin electrophoresis, etc. are normal. The prognosis of primary vasospasm is usually benign, provided the appropriate protective measures from direct exposure to cold are taken. The situation is different in secondary Raynaud's phenomenon. The most common cause of secondary Raynaud's phenomenon is an underlying collagen vascular disease. Several conditions are associated with Raynaud's *(91–97)*. CREST consists of Calcinosis, Raynaud's phenomenon, Esophageal changes, Sclerodatyly, and Telangiectasias. Raynaud's phenomenon may precede a connective tissue disorder for years *(94)*. The prognosis in secondary Raynaud's phenomenon depends on the underlying connective tissue disease. The most common cause of secondary Raynaud's phenomenon is scleroderma. Approximately 90% of patients with scleroderma have Raynaud's phenomenon and it can be the first clinical expression of the disease. Peripheral vasospasm has also been associated with systemic lupus erythematosus in about 30 to 40% of patients. Approximately 20% of patients with dermatomyositis describe Raynaud's phenomenon. An association of Raynaud's phenomenon with Sjogren's syndrome has been described. The existence of Raynaud's phenomenon in collagen vascular diseases may adversely influence their prognosis. In lupus erythematosus, for example, the presence of Raynaud's symptomatology has been associated with pulmonary hypertension. Any kind of vasculitis or vascular injury can be subsequently associated with Raynaud's phenomenon. Raynaud's symptoms in patients with giant cell arteritis (polyarteritis nodosa) can evolve to cutaneous gangrene *(93–96)*.

Hyperviscocity states like cryoglobulinemia have been associated with Raynaud's phenomenon: from reversible vasospasm to frank pur-

pura. Certain pharmacologic agents can cause or aggravate Raynaud's phenomenon. The most notorious for that toxicant is nicotine. Smoking can cause both coronary and peripheral vasoconstriction and has been associated with coronary spasm in men and women *(61)*. Cocaine is another cause of both coronary and peripheral vasospasm. Ergotamine derivatives can also produce both coronary and peripheral vasospasm. Raynaud's phenomenon occurs in about 30% of cases following chemotherapy with Vinca alkaloids and bleomycin. β-Blockers have also been reported to cause Raynaud's phenomenon, but very rarely as have certain sympathomimetic agents used as over-the-counter cold preparations.

The pathophysiology of peripheral vasospasm is unclear. The author of this chapter would like to propose the hypothesis that it is a result of endothelial dysfunction. In the late 1920s, Sir Thomas Lewis hypothesized that Raynaud's disease is a result of a *local vascular fault (99)*, but after all these years, the *fault* has not been adequately defined. An attractive hypothesis is an imbalance of the endothelial function, involving endothelin *(100–106)*. Endothelin *(107)* that is produced by the endothelial cells is a potent vasoconstrictor, whereas nitric oxide, an endothelium-derived relaxing factor (EDRF), is a potent vasodilator *(104)*. Prostacycline and prostaglandins also produced by the endothelial cells normally counteract thrombaxene, which is a potent platelet derived-vasoconstrictor. A parallel mechanism leading to peripheral vasospasm can be increased sensitivity to α-adenoceptor agonists *(102)*.

Individuals with primary Raynaud's phenomenon are more likely to respond well to therapy than individuals with secondary Raynaud's phenomenon. Many patients with mild Raynaud's phenomenon are minimally disabled, but often frightened by the cutaneous color changes. The majority of patients do not need pharmacologic intervention. Protection of the upper and lower extremities from direct exposure to cold is a practical approach. Although Raynaud's phenomenon is more severe during the winter, recurrent attacks do occur in any season upon a sudden cold stimulus. Often a rapidly changing temperature is more likely to precipitate peripheral vasospasm than exposure to a lower, but constant temperature. A central body chill is as likely to provoke an attack of Raynaud's as is a direct cold exposure to the hands. In a number of patients, emotional stress is a major precipitating factor. All known aggravating factors should be avoided and by all means smoking. Ketanserin has been tried in the treatment of Raynaud's phenomenon occurring with scleroderma *(109)*.

Calcium channel blockers (CCBs) are the most widely used pharmacologic agents for the treatment of peripheral vasospasm *(110–112)*. Sympatholytic agents have also been used in the treatment of Raynaud's phenomenon. Topical nitroglycerin ointment can be applied with a nocturnal nitrate-free interval to avoid nitrate tolerance.

The treatment of claudication due to LEAD is multifacted: aggressive risk factor modification, regression therapy of the atheromas, pharmacotherapy, exercise therapy, endovascular transcatheter therapeutic interventions, angiogenesis, and vascular graft procedures.

In any patient with documented LEAD, risk assessment for cerebrovascular, and coronary heart disease of utmost importance.

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2 Noninvasive Diagnostic Evaluation of Peripheral Arterial Disease

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CONTENTS

INTRODUCTION

The pathogenesis of arterial stenosis or occlusion may be secondary to a wide spectrum of etiologic factors including atherosclerosis, thrombosis, embolic events of various etiology, fibromuscular dysplasia, vasculitidies, dissection, trauma, external compression, and vasospastic syndromes. Atherosclerosis is by far the most common cause of arterial stenosis.

Atheromatic plaques have been identified as early as the first two decades of life, but usually become hemodynamically significant after the age of 50. They can develop in most of the arteries in the body, and are most commonly located in the area of arterial bifurcations. A hemodynamically significant arterial stenosis reduces blood flow in the capillary bed distal to it. The extent of this interference is related to the severity of the stenosis and is determined by strict hemodynamic principles. Tissue perfusion distal to an arterial stenosis can be maintained by development of a network of collateral vessels around the stenosis,

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increase of the cardiac performance, and dilatation of the local arterioles and precapillary sphincters.

There are three levels of occlusive disease in the lower limb arteries: aortoiliac, femoropopliteal, and infrapopliteal disease. Disease confined to one level may be asymptomatic or it can present with intermittent claudication. The presence of two or three levels of disease are symptomatic, and patients usually present with severe claudication or rest pain. Three levels of disease are often seen in patients with skin damage and critical limb ischemia. Without an intervention most limbs with critical ischemia will be amputated within 1 year. In patients with diabetes mellitus the disease is usually confined in the infrapopliteal vessels. Such patients may develop critical limb ischemia with one level of disease because this is the most distal of the three. Usually, multiple stenoses and/or occlusions are found in at least two of the run-off arteries. Although it is known that atherosclerosis develops most often in bifurcations, in the lower extremities the most frequently involved site is the superficial femoral artery. Other common sites are the aortoiliac, iliac, femoral popliteal, and tibioperoneal trunk bifurcations.

DIAGNOSTIC EVALUATION

History and Physical Examination

The evaluation of a patient with lower extremity arterial occlusive disease starts with a detailed history and a complete physical examination *(1)*. A thorough pulse exam of both upper and lower extremities is of outmost importance. Absence of palpable pulses at any level indicates hemodynamically significant lesion(s) to the main artery proximal to that level. Thus, absence of palpable femoral pulses is suggestive of severe stenosis or occlusion of the ipsilateral iliac artery *(2)*.

Physiological Testing

Hemodynamic testing of the lower extremity includes noninvasive methods that evaluate the dynamic function of the circulation. Various techniques have been developed for noninvasive assessment including segmental pressures, ankle-brachial indices, continuous-wave Doppler waveform analysis, pulse volume recordings, transcutaneous oximetry, treadmill testing, and duplex scanning. Inexpensive equipment and a short learning curve have made hemodynamic testing widely used for the diagnosis of lower extremity arterial disease.

Systolic pressures can be taken at different locations in the lower extremities to help identify the location of arterial disease. Most commonly, pressures are taken at the high thigh, lower thigh, calf, and ankle.

A pressure gradient > 20 mmHg between cuffs is considered indicative of significant arterial disease. For example, a pressure difference of >20 mmHg between the high and low thigh cuffs indicates the presence of a hemodynamically significant superficial femoral artery stenosis. Segmental pressures can give a general idea of the location of the disease, but cannot ascertain the exact site, extent, or severity of a lesion *(3)*.

The ankle-brachial index (ABI) is the best screening test to evaluate the presence or absence of arterial disease in the lower extremities. It is a ratio between the systolic pressure at the ankle in the arms (Fig. 1). In a person with normal arterial circulation, the ankle pressures should be equal or greater than the brachial pressures. Therefore, the normal ABI value is one or higher. Any patient with an ABI <0.9 has lower extremity arterial occlusive disease. The ABI is an important tool in diagnosing and following up arterial disease *(4)*. It is helpful in the follow-up of patients who have undergone revascularization procedures such as angioplasty or bypass grafting. Success of these procedures and progression of disease can be monitored using the ABI. For example, during follow-up of patients who underwent infrainguinal bypass grafting, a drop of >0.15 in the ABI would indicate the development of significant stenosis either in the graft or in the native arteries. In patients with calcified noncompressible arteries, the ABI is of no value because erroneously high pressures are measured in the lower limb. In these occasions a toe pressure is taken instead *(1)*.

Continuous-wave (CW) Doppler waveforms are commonly used in conjunction with segmental pressures or ABIs. Waveforms can help in identifying the location of arterial disease in the lower extremity. Waveforms are usually taken from the common femoral, superficial femoral, popliteal, dorsalis pedis, and posterior tibial arteries and recorded on a strip chart recorder. Qualitative analysis of the waveforms is performed to identify abnormalities in the arterial circulation. The presence of triphasic waveforms at any level indicates absence of a hemodynamically significant arterial lesion proximal to that level. Attenuated waveforms that have lost their triphasic appearance indicate an arterial stenosis proximally.

Pulse volume recordings (PVRs) are similar to the waveforms obtained by CW Doppler and can be used to identify location of disease in the lower extremity. Blood pressure cuffs are placed on the high thigh, low thigh, calf, and ankle of the lower extremity. Cuff pressure changes reflect a change in cuff volume, which reflects changes in limb volume. Pulse volume recordings from each site can be recorded on a strip chart recorder. Differences in the PVR waveforms can be used to determine the location and severity of arterial disease.

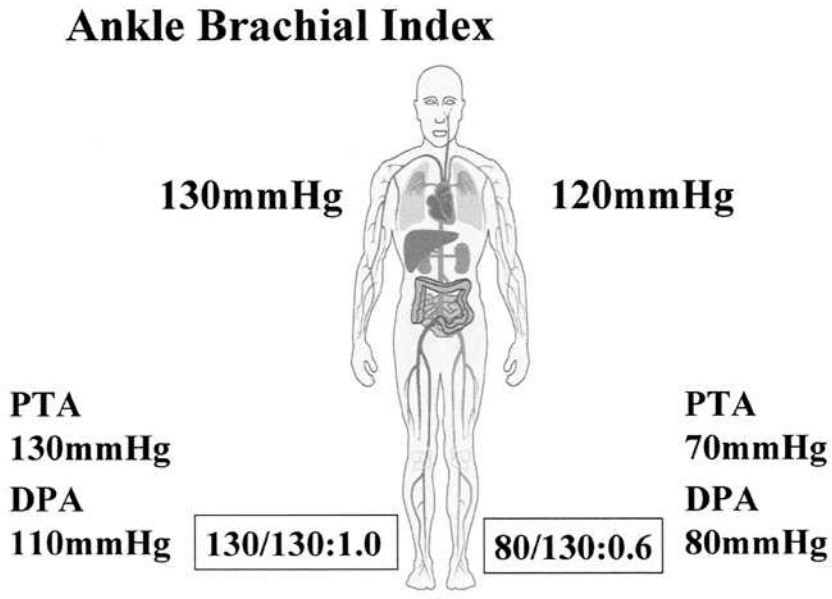

Fig. 1. The measurement of ABI involves bilateral arm pressure measurements. The highest arm pressure is used in the estimation of the ABI. Of the arteries at the ankle level the highest pressured is used.

Transcutaneous oximetry (TCPO2) can be used in the lower extremity to evaluate the oxygen supply to the skin. This procedure is used most often in patients with severe arterial disease requiring surgical reconstruction or amputation. TCPO2 values are obtained by placing an electrode on several areas in the lower extremity. Most commonly, values are taken at the chest (control) above the knee, below the knee, and at several areas on the foot. A measurement can also be taken on the dorsum of the foot with a 30 degree elevation. TCPO2 values can be helpful in patients who have nondiagnostic ABIs (falsely elevated pressures as a result of calcification of vessels). Values obtained from TCPO2 measurements can aid the surgeon in decisions regarding amputation site and healing potential.

Segmental pressures (ABIs) and CW waveforms at rest can be normal in patients with significant arterial lesions. This can be caused by collateral circulation providing adequate flow without significant reduction in pressure. CW waveforms can appear normal if the sample is taken distal to a lesion, where the flow has had a chance to normalize. By exercising a patient on a treadmill, the working muscles require increased blood flow. Symptoms and their severity can be reproduced and walking time

can be documented. In the event of a significant arterial lesion, pressures will drop after exercise. *Treadmill testing* can provide a baseline for patients who have undergone interventional or reconstructive procedures. Improvement or progression of disease can be followed with exercise testing.

Toe pressures can be taken using a photoplethysmography (PPG) probe. A small cuff (2.5 cm) is placed around the digit and the PPG probe is attached with double-stick tape to the pad. Toe pressures can be used in patients with nondiagnostic segmental pressures resulting from calcific vessels. A toe pressure >80 mmHg is normal. Pressures from 80–30 mmHg indicate mild to moderate disease and those <30 mmHg indicate critical disease.

Skin perfusion pressure measurements are taken with laser Doppler. Skin perfusion pressure is used in patients with critical limb ischemia requiring surgical reconstruction or amputation. Like the toe pressures, it is useful in patients with falsely elevated pressures caused by arterial wall calcification.

Color Flow Duplex Scanning

Duplex scanning (with or without color flow) combines a B-mode image with Doppler spectral waveform analysis. Unlike segmental pressures or continuous-wave Doppler waveforms, duplex scanning allows direct visualization of the arterial segment. Location and severity of the disease can be documented with high accuracy. Duplex scanning can also differentiate between different types of disease such as stenosis, occlusion, or thrombosis. Using velocity criteria, the degree of stenosis can be documented *(5)*. Color flow can be helpful in identifying changes in velocity resulting from an arterial stenosis. These changes are used to calculate the severity of stenosis *(5)*. Thus, duplex scanning provides a combination of anatomic and physiologic information that other imaging or physiologic testing modalities cannot offer.

Basic Principles

Inertial losses depend on the kinetic energy of the blood ($DP = K\ 1/2pv^2$). Because p is a constant changes occur only in the velocity. These changes are significant across a severe stenosis *(6)*. According to the equation of continuity, flow (cross-sectional area x velocity) is the same at any point along a tube segment with no branches

$$A1V1 = A2V2 \qquad \text{or} \qquad A1/A2 = V2/V1$$

The V2/V1 from the equation, where V2 is the peak systolic velocity (PSV) at the stenotic segment and V1 the PSV at the normal diameter

segment proximal to the stenosis, is being used to detect significant stenosis in peripheral arteries *(7)*. This ratio is not applicable in the internal carotid artery because the carotid bulb diameter is usually 1.2 times larger than the common carotid diameter, and therefore other complex flow phenomena occur *(8)*. This is one of the main reasons for which different laboratories have to establish their own criteria to detect carotid artery stenosis.

Blood flow in large vessels at rest is laminar with relatively uniform velocities. Narrowing or obstruction causes disruption of the laminar flow, creating vortices and whirls of different velocities and directions. This flow is called turbulent and generally characterizes flow across an area of stenosis *(6)*.

Critical Stenosis

A stenosis is hemodynamically significant when a reduction in the blood pressure or flow is being observed. In an experimental setting it has been shown that pressure or flow decrease when a lesion produces at least 75% *area reduction*. This decrease in the luminal area corresponds to a 50% *axisymmetric diameter stenosis (6,9–12)*.

The energy loss across a stenosis is inversely proportional to the fourth power of the minimal radius at the stenosis, and to the ratio of the fourth power of the radius at the prestenotic and stenotic site. It also depends on the blood flow velocity. In a low resistance high-flow artery, flow reduction occurs at a lesser degree of narrowing than in a low-flow (high resistance) artery. Therefore, the resistance of the vascular bed distal to the lesion also affects the energy loss across the stenosis. This phenomenon is well illustrated in the lower extremity arteries where at rest *(high-resistance low-flow system)* may be nonsignificant, but during exercise *(low-resistance high-flow system)* may become significant *(6)*.

The length of the stenosis accounts for viscous energy loss as shown by the Poiseullie's equation. However, such energy loss is less significant compared to that occurring from further reduction in the vessel's diameter. The amount of resistance offered by a stenosis depends largely upon entry and exit blood flow phenomena. This explains why a 2 cm long stenosis in an artery would produce much less resistance than two separate 1 cm long stenoses with the same degree of narrowing *(6)*.

Diagnostic Criteria for Arterial Stenosis by Duplex Scanning

Criteria for detecting significant stenosis have been developed for peripheral arteries. These criteria have been derived by correlating the ultrasound parameters to angiography, pressure measurements across the stenosis, and measurements on operative specimens. For peripheral

arteries and grafts a V2/V1 ratio >2.0 *(7,13,14)* indicates the presence of significant stenosis; this is the most widely recommended diagnostic criterion (Fig. 2). However, few centers are using as a cut-off value a ratio >2.5 for better specificity and a very small reduction in sensitivity. A pooled analysis using the above criteria showed that the sensitivity and specificity for detecting aortoiliac stenosis were 86%, 95% CI 80–91 and 97%, 95% CI 95–99, respectively *(14)*. A V2/V1 ratio >5.5 and/or an end-diastolic velocity >60 cm/s indicates that the diameter reduction is >75%. A DPSV (PSV difference across a stenosis) >160 cm/s at rest has shown a sensitivity of 85% and a specificity of 88% in the aortoiliac segment. After exercise a DPSV >160 cm/s has a sensitivity of 100% and a specificity of 82% *(15)*.

The waveform pattern obtain by duplex scanning can also be used for diagnosing proximal or distal disease *(16,17)*. A triphasic waveform in the common femoral artery indicates that the ipsilateral proximal vessels are normal. A biphasic waveform would indicate stenosis that is not significant, and a monophasic waveform would signify the presence of significant stenosis or occlusion (Fig. 3). A low-end diastolic velocity indicates a tight stenosis or occlusion distal to the measurement (Fig. 4).

Presently, there are no standards by which hemodynamic testing is used. Initially, a clinical examination is performed in an outpatient setting. Depending on the signs and symptoms of arterial insufficiency, any of the earlier discussed tests may be performed. It should be noted that in many centers, the decision to perform a noninvasive test may be linked to the type of treatment the patient will receive. For example, a physician who plans to treat a claudication patient with an angioplasty or a bypass procedure might request a duplex scan. On the other hand, a duplex scan would not be considered in a patient where no intervention is planned. The following are basic algorithms that can be helpful in deciding which method of testing is most beneficial in patients presenting with claudication and rest pain.

In a patient presenting with symptoms of an acute arterial occlusion, time is critical. If the limb is threatened on physical exam, the patient should proceed to angiography/surgery without noninvasive testing. In a nonthreatened limb with suspected occlusion, ABIs can be performed along with a duplex scan. A duplex scan can help to identify the source of occlusion or confirm a thrombotic event, such as in the territory of a peripheral aneurysm or a stenosis (Fig. 5).

Traditionally, arteriography has been the *gold standard* for diagnosis of arterial disease. The recent advancement of duplex scanning has enabled some centers to perform lower extremity revascularization procedures without angiography. Several studies in the last decade have shown

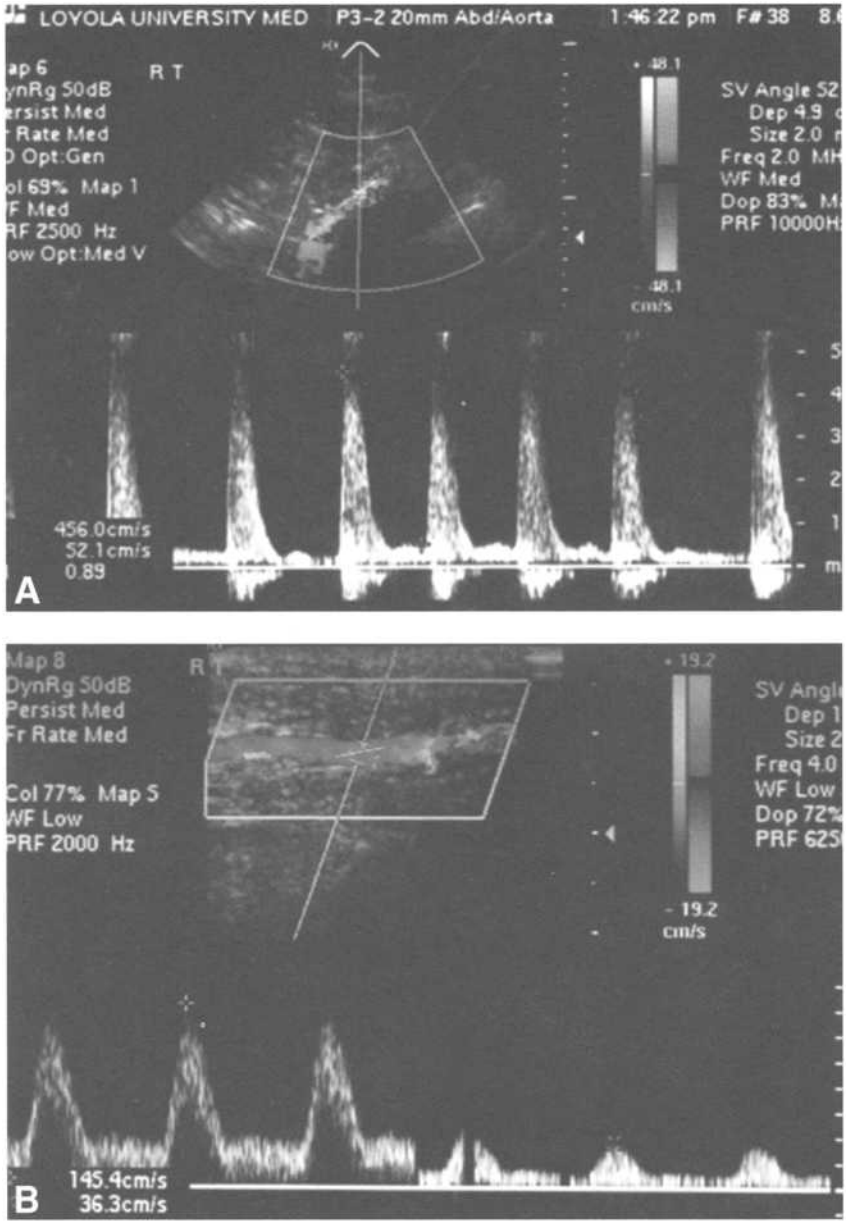

Fig. 2. Significant stenosis in peripheral arteries. **(A)** Stenosis in the common iliac artery. Both the peak systolic and end diastolic velocities are significantly increased. The V2/V1 was 3.9 (456/117) indicating a >50% diameter stenosis. **(B)** Stenosis in the anterior tibial artery. The prestenotic (36 cm/s) and the poststenotic (145 cm/s) velocities are obtained to estimate the V2/V1 ratio (4.0).

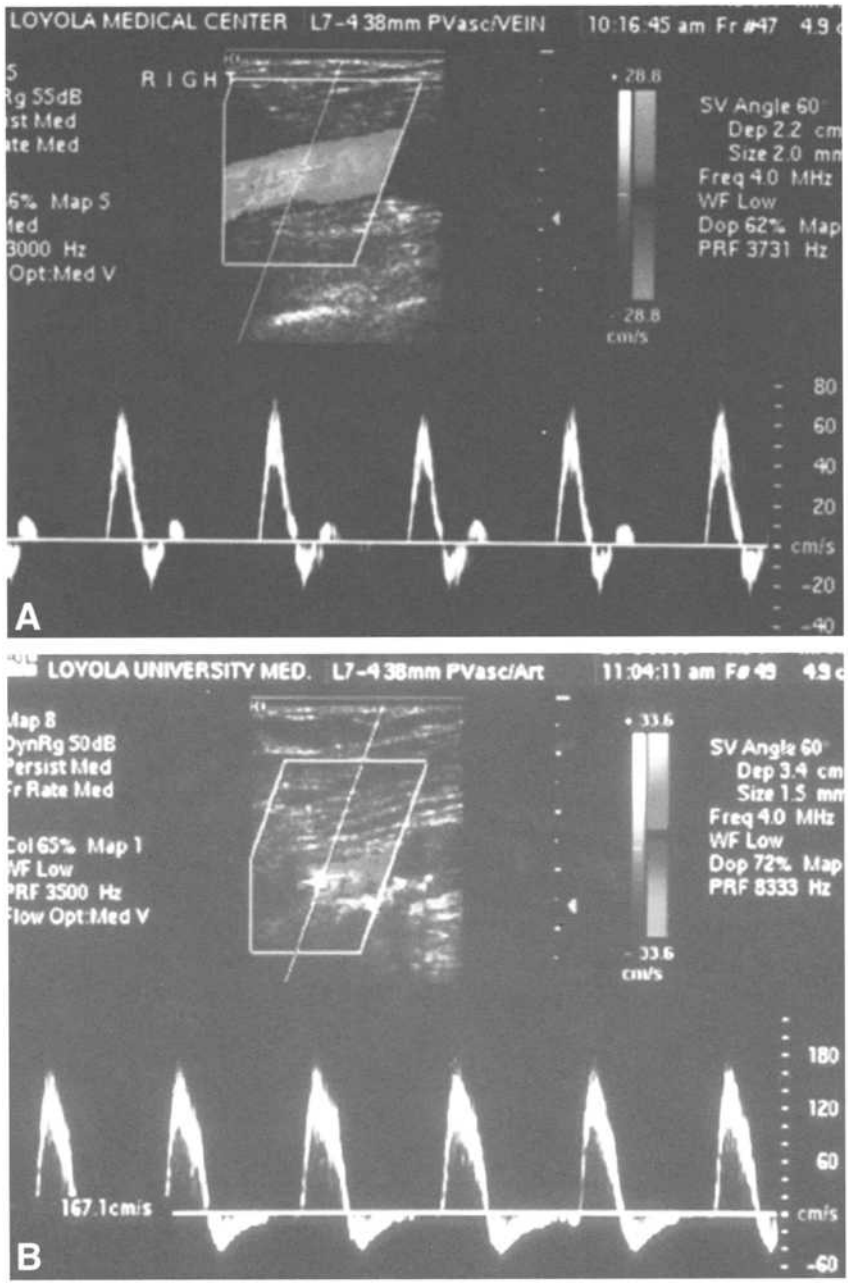

Fig. 3. Prediction of iliac stenosis from the common femoral artery (CFA) waveform. **(A)** triphasic waveform in the CFA indicates normal ipsilateral iliac arteries. **(B)** Biphasic waveform indicates mild to moderate (*continued on page 32*)

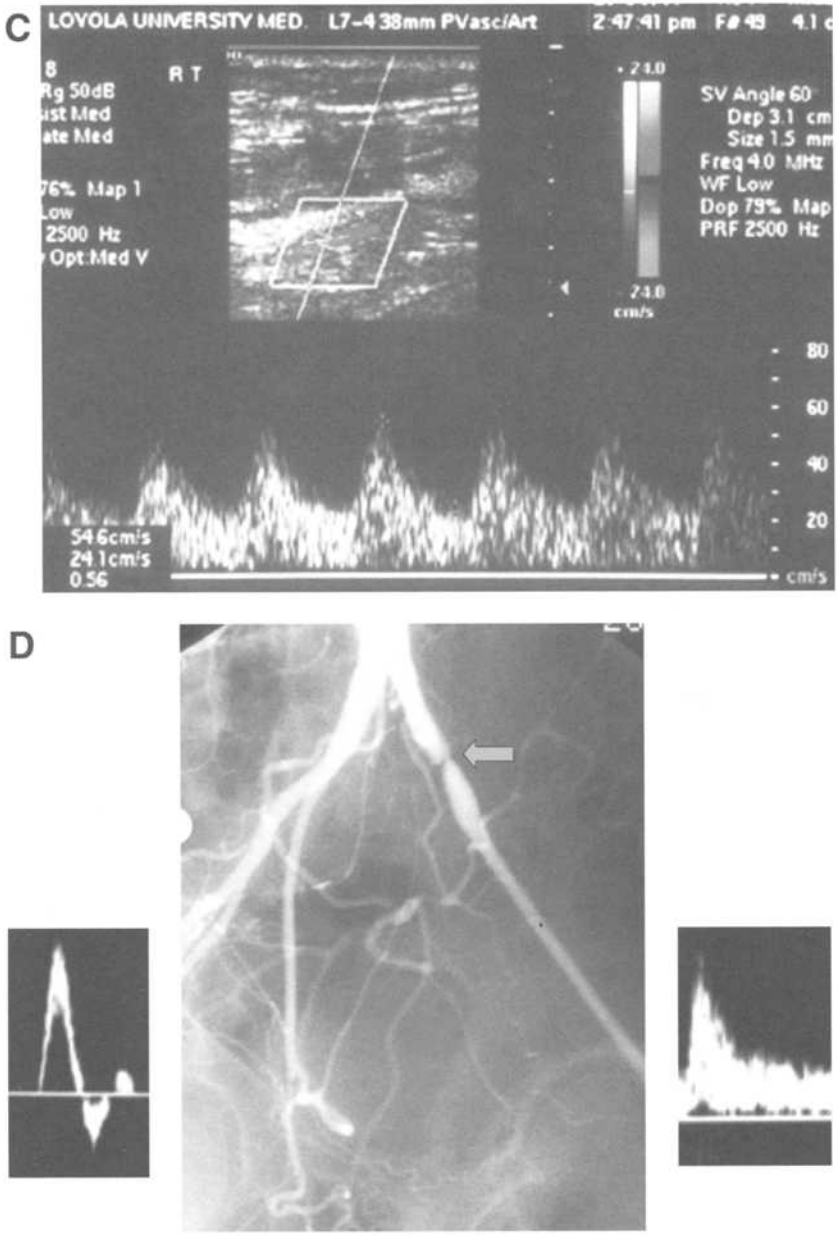

Fig. 3. (*continued from page 31*) stenosis. **(C)** Monophasic waveform denotes the presence of significant stenosis or occlusion. **(D)** Angiography and duplex scanning in a patient with intermittent claudication in the left lower extremity. In the right lower extremity the waveform is triphasic and the ipsilateral iliac artery in the angiogram is normal. In the contralateral limb the waveform is monophasic and a significant stenosis is present in the ipsilateral iliac artery (solid arrow).

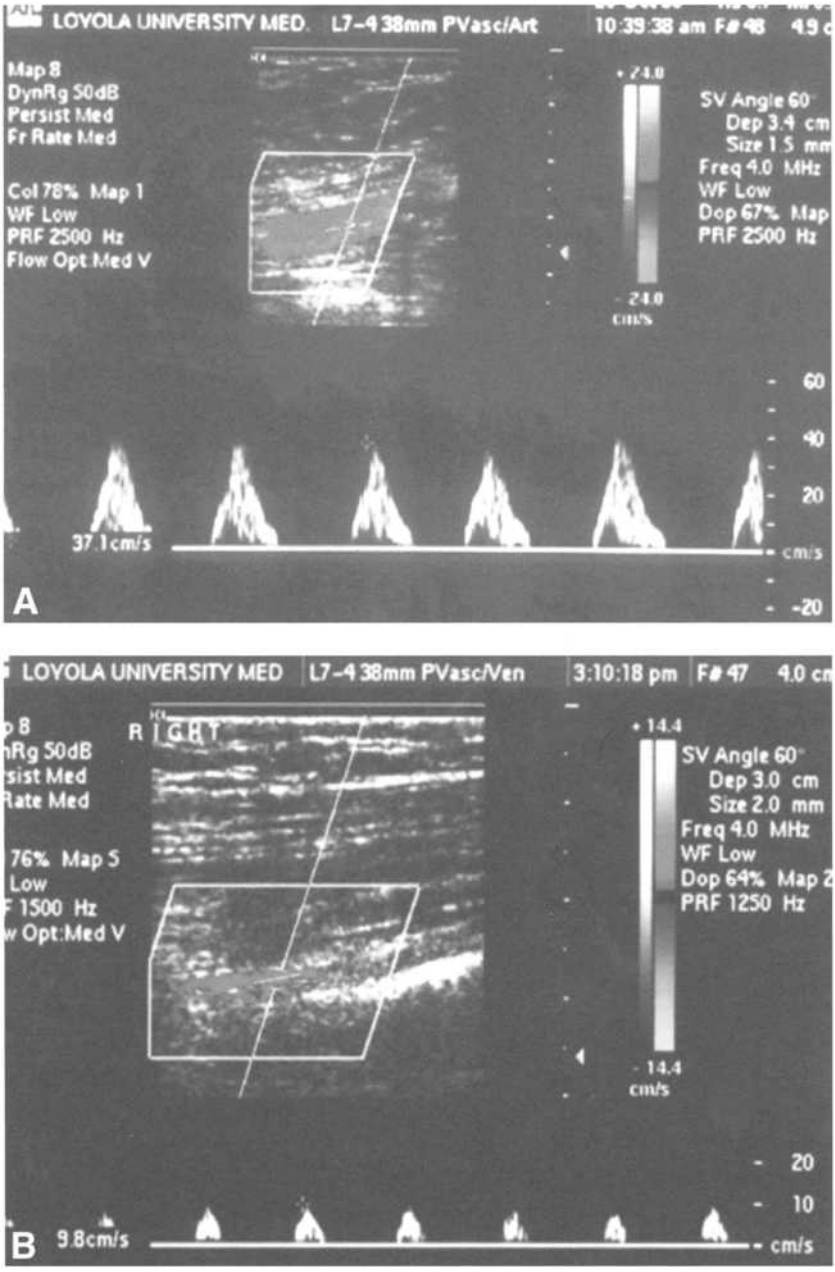

Fig. 4. Low amplitude waveform with absence of end diastolic velocity is common in patients with occluded arterial segments above and below the segment under investigation. **(A)** Low amplitude and absence of end diastolic velocity in the superficial femoral artery in the mid-thigh in a patient with common iliac significant stenosis and distal superficial femoral artery occlusion. **(B)** Very low velocities and a monophasic waveform in the posterior tibial artery of a patient with multiple stenoses and occlusions proximal and distal to the site of measurement.

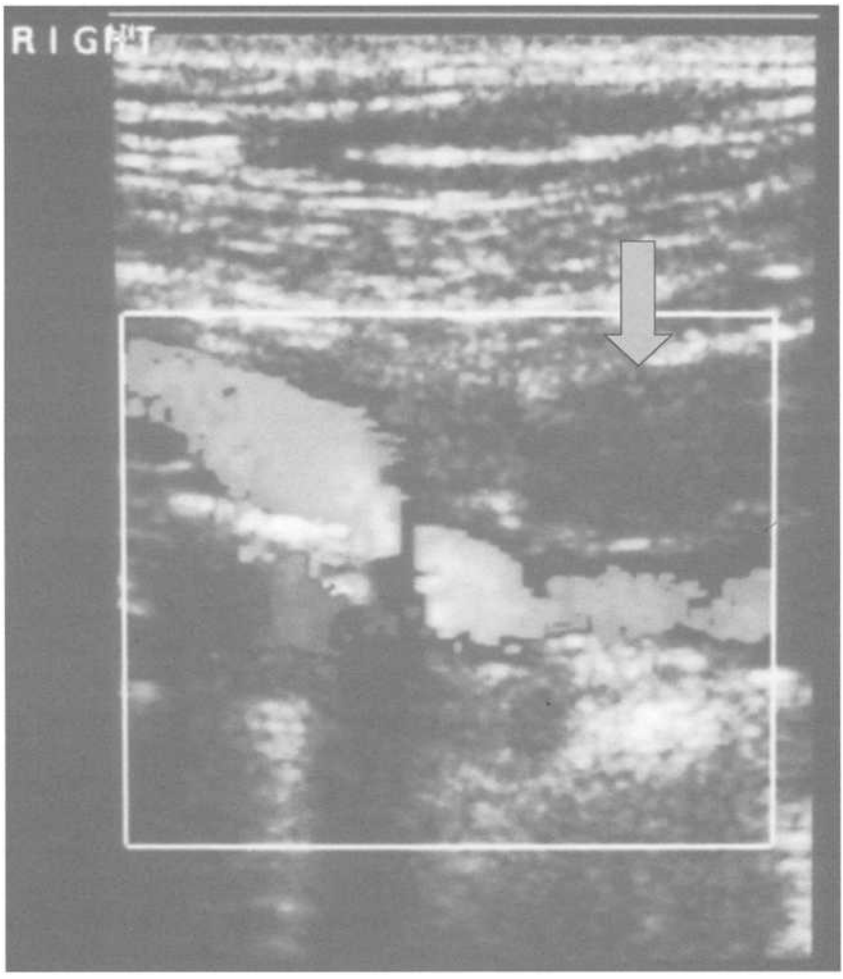

Fig. 5. Acute thrombosis of the right external iliac artery (solid arrow) in a patient with atrial fibrillation. The common iliac and internal iliac arteries are patent. The patient presented with acute lower ischemia. A thrombectomy was performed and the symptoms were resolved.

very promising results on that application. Duplex scanning can also be used with great accuracy in determining whether an iliac lesion is a good target for percutaneous intervention. The length of the lesion, degree of stenosis, and amount of calcification can all be accurately evaluated, giving the physician valuable information for decision making. In addition, duplex can be used to determine the presence of distal targets for revascularization procedures when none are visible by angiography (Fig. 6).

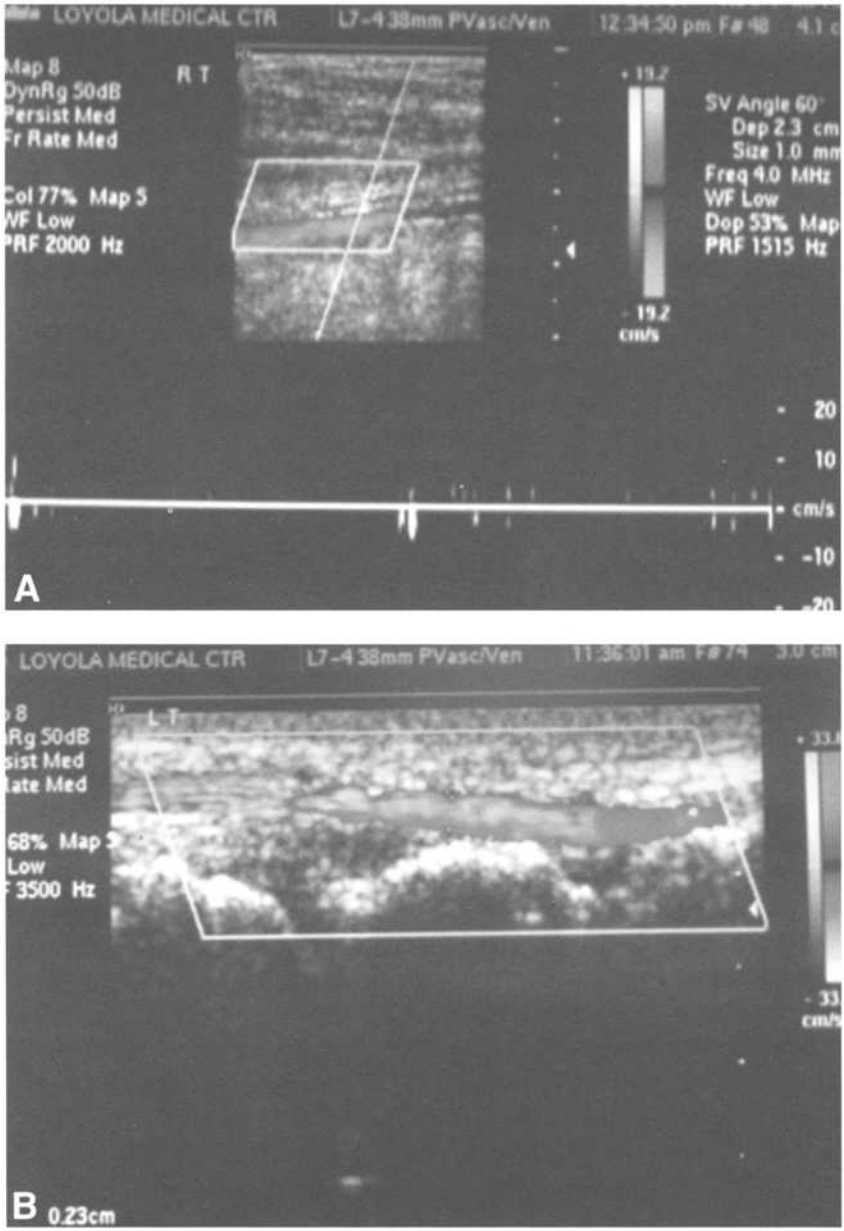

Fig. 6. Target arteries for surgery can be selected with the duplex scanning. Arterial segments with stenosis, diffused disease and heavy calcification are not selected for the distal anastomosis. **(A)** Occluded, calcified posterior tibial artery. **(B)** The dorsalis pedis artery in this patient had no significant abnormality and it was the best artery for the bypass placemet. This was a diabetic patient with typical infrapopliteal disease that underwent a popliteo-tibial by-pass with a saphenous vein.

Magnetic Resonance Angiography

In recent years, magnetic resonance imaging (MRI) and magnetic resonance arteriography (MRA) have been employed with increased frequency as diagnostic tools in the management of peripheral arterial disease *(18)*.

Basic Principles

MRI is based on the reactions of various tissues to a magnetic field followed by a radiofrequency radiation pulse. MRA imaging is generated by taking advantage of blood flow-related effects relative to the stationary surrounding soft tissues. Two techniques are currently used for MRA:

Time of flight (TOF) angiography: TOF MRA relies on signal difference between stationary protons in the vessel wall and the surrounding soft tissues, compared to moving protons (flowing blood).

Phase contrast (PC) angiography: Protons undergo a change in the phase of their rotation as they move through a magnetic field. PC MRA uses gadolinium that shortens the T1 relaxation of blood protons, thereby increasing the intravascular signal.

Background can be eliminated by subtraction protocols and its suppression is greater for PC than for TOF MRA. Because of inherent limitations, TOF MRA has never become a real alternative to contrast angiography except in very few highly experienced centers. Currently, PC MRA is considered the best alternative to contrast angiography.

Clinical Applications

MRA has evolved as a noninvasive, sensitive accurate, and cost-effective method of imaging of the peripheral arterial circulation. Although contrast angiography is still considered the gold standard, it carries an overall (major and minor) complication rate of approximately 8%. Local complications related to the arterial puncture (bleeding, hematoma, infection, thrombosis, stenosis, pseudoaneurysm, etc.) and systemic complications (contrast-induced allergic reactions or renal insufficiency) are not uncommon. MRA is an alternative noninvasive imaging method for the peripheral vessels that avoids the risk of these complications. Its sensitivity in and specificity in detecting patent segments, hemodynamically significant stenoses and/or occlusions approaches 100%. In a recent review of the existing literature, TOF MRA was found to have sensitivity and specificity of 82% and 84%, respectively, whereas PC MRA had a 96% sensitivity and specificity compared to conventional angiography *(18)*. The latter was also reported to be more sensitive in detecting patent distal run-off vessels in patients

with severely compromised distal circulation. This advantage is inherent to the mechanism of image formation in MRA, which requires only the presence of local flow with velocity as low as 2 cm/s. Presently, MRA is considered the imaging modality of choice for the diagnosis of vascular arterio-venous malformations and popliteal entrapment syndrome. In several centers, MRA is used as the sole pre-operative imaging technique for distal revascularization procedures with great success. It is currently considered to be more cost-effective compared to contrast angiography.

Nevertheless, not all patients are suitable for MRI/MRA exams. Individuals with implanted metal devices (pacemakers, cerebral vascular clips, etc.) should not be considered for such imaging. Claustrophobia remains a relative contraindication. The exam may also be limited by respiratory movements, but with contrast-enhanced MRA the acquisition time has significantly decreased (approximately 20 seconds for a complete limb exam), making *breath-hold images*possible.

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3 Smoking and Smoking Cessation

Albert J. Bellg, PhD
and Aristdis N. Mavridis, MD, FCCP

CONTENTS

INTRODUCTION

When Christopher Columbus discovered America he found the natives chewing tobacco in much the same manner as is done today *(1)*. The American Indians believed tobacco to have medicinal properties, and it was also used in native ceremonials in the New World. Once in Europe, the genus Nicotiana was named in honor of Jean Nicot, the French ambassador to Lisbon, who sent the seeds of *Nicotiana tabacum* to Catherine de Medicis, the queen of France. The word *tobacco* was derived from an American Indian word referring both to a tube for inhaling the smoke and to a cylinder of leaf prepared for smoking.

The association of smoking with health problems is almost as old as the use of tobacco. In 1604, King James I of England issued the first

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official condemnation of tobacco, "A Counterblaste to Tobacco." In 1859, it was shown that of 68 patients in a Montpelier hospital in France who had cancer of the lips, tongue, tonsils, or other parts of the mouth, all used tobacco; 66 of them smoked short-stemmed clay pipes. The earliest concern about the danger of cigaret-smoking based on modern scientific methods developed in the late 1940s, when it was realized from case-control studies that smoking is causally related to lung cancer. In 1954, the American Cancer Society and the British Medical Research Council reported independently that death rates were higher for cigaret smokers than for nonsmokers. In 1964, the Surgeon General of the United States published the landmark "Report on Smoking and Health," which was updated in 1979. This report reinforced more strongly than ever that cigaret smoking is one of the most serious health hazards faced by Americans *(2)*. The consequences of smoking have been regularly reviewed by the US Public Health Service in publications of the National Clearinghouse on Smoking and Health, as well as by other authorities *(3–7)*.

There is currently near-consensus in the medical community on the hazards of smoking. Approximately 430,700 American smokers died of premature illness each year on average from 1990–1994 *(8)*. The Centers for Disease Control and Prevention has estimated that the health care costs associated with tobacco are about $50 billion annually, with an additional estimated $80 billion added if one includes the cost of smoking during pregnancy, lost workdays, lost output from early death and retirement, and fires caused by smoking *(9)*. In the general population, most people are aware of the link between smoking and cancer; far fewer are aware of the relationship between smoking and heart disease and vascular diseases in general. Nonetheless, the risk of morbidity and mortality from coronary artery disease, stroke, sudden cardiac death, and peripheral vascular disease has been linked to cigaret smoking in many cross-cultural and national studies *(10,11)*.

SMOKING AND CARDIOVASCULAR DISEASE RISK

The risk of heart disease is directly related to the number of cigarets smoked *(12)*. Smoking one pack of cigarets per day doubles the risk for heart disease, whereas smoking more than one pack per day triples the risk. Cigaret, cigar, and pipe smoking, chewing tobacco and snuff, as well as occupational exposure to environmental tobacco smoke and passive smoking at home, are all deleterious to health *(13–15)*.

In addition, risk and type of cigaret are only loosely associated; there are no *safe* cigarets. In a study of more than a million men and women from 1960 to 1972 *(16)*, the health consequences of smoking high-tar

and high-nicotine cigarets were compared to the effects of smoking brands lower in tar and nicotine. Although total death rates were somewhat lower for low-tar and low-nicotine brand smokers than for smokers of high-tar and high-nicotine brands, those death rates were still 30 to 75% higher than those of nonsmokers. Lower-tar and low-nicotine cigarets are not *safe*.

Nonsmokers who live with or work with smokers are also at risk. It has been estimated *(17–19)* that there are 35,000 to 40,000 annual excess heart disease deaths among never-smokers and long-term former smokers as a result of their exposure to environmental tobacco smoke. By comparison, lung cancer has been estimated to cause approximately 3000 excess deaths per year among never-smokers. A census of Washington County, Maryland, in 1963 obtained information on smoking habits of all adults in the census; death certificates of all residents who died in the subsequent 12 years were coded for underlying cause of death and matched to the census *(20)*. Death rates from coronary heart disease of nonsmokers were higher among men and women who lived with smokers, as compared with those living with nonsmokers.

Smoking also contributes to the prevalence of stroke in the United States. Several large prospective studies, including the Framingham Study, have linked smoking to stroke generally and to specific stroke types, including brain infarction, intracerebral hemorrhage, and subarachnoid hemorrhage *(21)*. In the Framingham Study, even after other risk factors were taken into account, stroke incidence was 40% higher in male smokers and 60% higher in female smokers compared with nonsmokers. The impact of smoking on stroke did not decrease with advancing age. However, within 2 years of quitting smoking, the risk of stroke fell significantly. Within 5 years of quitting cigaret smoking the risk of stroke fell to that of a nonsmoker.

For large vessel peripheral arterial disease, cigaret smoking is the most important risk factor *(22)*. Surgical revascularization is significantly less effective in reducing long-term symptoms of claudication if patients continue to smoke.

PHYSIOLOGICAL MECHANISMS INCREASING RISK OF CARDIOVASCULAR DISEASE FROM SMOKING

There are strong plausible hypotheses for the relation between exposure to tobacco and vascular disease. In animal and human studies, endothelial injury has been associated with carbon monoxide and nicotine. In contrast to healthy individuals, those with ischemic heart disease are affected by carbon monoxide during submaximal exercise, in part

because carbon monoxide reduces the ability of hemoglobin to combine with oxygen and diminishes oxygen transport from the lungs to the tissues. Levels as low as 2.5 to 3% carboxyhemoglobin (COHb) have been found to decrease exercise time before the onset of angina and prolong the duration of ischemia. For maximal exercise, the critical level is approximately 4.5% COHb. Above this level, both exercise and VO2max are inversely related to CO concentration. Additionally, cigaret smokers may have baseline COHb in the 4 to 8% range *(23)*.

Platelet and clotting factors predisposing to formation of thrombus, as well as vascular smooth muscle cell proliferation have also been observed with exposure to cigaret vapor components, including carbon monoxide and nicotine. Increased platelet aggregation has been found both in humans and in animals *(24,25)*. Thrombolytic substances such as plasminogen have been found to be lower in smokers and to increase with cessation of smoking *(26,27)*.

There is a growing body of evidence suggesting that oxidation of low density lipoprotein (LDL)-cholesterol particles may be a pivotal step in atherogenesis *(28–30)*. Cigaret smoking, even for brief periods, can markedly enhance LDL-cholesterol oxidation as well as decrease high-density lipoprotein cholesterol *(31)*. It also depletes body stores of vitamin C that may be an important antioxidant protection. Intake of the anti-oxidant beta carotene has been shown to be inversely related to the risk of coronary events among current smokers (relative risk 0.30) and former smokers (relative risk 0.60), but was not beneficial in persons who had never smoked *(32,33)*.

Fibrinogen has also been implicated in atherogenesis and thrombus formation, and has been found to be elevated in smokers *(34,35)*. The Framingham Study has shown that risk of cardiovascular disease (CVD) and stroke increased with increases in fibrinogen levels. In this study, as well as in other studies, smokers had a higher risk of developing claudication compared with nonsmokers with similar risk factor profiles *(17,36–40)*.

Cigaret smoking can cause marked coronary vasoconstriction *(41,42)*, and has been strongly associated with angiographically documented coronary spasm in men and women *(43–45)*. Vascular endothelial damage from smoking can cause thromboxane A2 release, decreased prostacyclin production, α-adrenergic stimulation, and vasopressin generation in the process of provoking constriction of the coronary vessel. Cigarets elevate the circulating level of catecholamines and free fatty acids that can further alter vascular tone.

Smoking also:

- Decreases the useful blood levels of drugs like β-blockers as a result of their increased metabolism *(46)*.
- Increases both blood pressure and heart rate.
- Lowers the threshold for ventricular arrhythmias *(13)*.
- Has a direct toxic effect on the ventricular myocardium *(47,48)* and may significantly increase myocardial oxygen demand.

Passive smoking is also associated with dose-related impairment of endothelium dependent dilatation, suggesting early arterial damage *(49)*. Studies have concluded that the public health burden caused by environmental tobacco smoke (ETS) is likely to be much greater for heart disease than for lung cancer *(50)*. Although environmental tobacco smoke is less dense, it is more toxic. Twice as much nicotine is emitted in sidestream as in mainstream smoke, and the ratio for benzene is 10:1. The concentration of carbon monoxide is 2.5 times higher in sidestream smoke than it is in mainstream smoke. Polycyclic aromatic hydrocarbons present on smoke as well as in environmental air pollution are capable of inducing and accelerating atherosclerosis. Tobacco smoke may also sensitize circulating neutrophils in humans, and may cause their subsequent activation and oxidant-mediated tissue damage, leading to atherosclerosis of the coronary and peripheral vessels *(51)*.

BENEFITS OF SMOKING CESSATION

Smoking cessation significantly improves cardiovascular health. According to the World Health Organization, within 1 year of quitting the risk of CHD decreases by 50%, and within 15 years (and often less) the relative risk of dying of CHD for an ex-smoker approaches that of a long-time nonsmoker *(8,52)*. Therefore, smoking cessation is more beneficial in reducing mortality in the aggregate of smokers with coronary artery disease than medical therapy, β-blockers with bypass surgery, or coronary angioplasty *(53,54)*.

Short of revascularization, cessation of smoking is the single most important factor in the management of patients with intermittent claudication *(55)*. In fact, in up to 85% of patients, walking distance can increase as much as 200 to 300% *(56,57)*. Detection and treatment of hypertension and control of risk factors for cardiovascular disease such as smoking, diabetes, and hyperlipidemia offer substantial stroke prevention benefits as well.

SMOKING CESSATION ISSUES

As Mark Twain said, "Quitting smoking is easy—I have done it many times." Although the health benefits of smoking cessation are substantial and would seem to provide all the motivation a smoker would need to quit, long-term smoking cessation remains among the most difficult behavioral changes to sustain.

Since 1965, smoking has declined gradually by about 42%, but recent data indicate that this downward trend may have leveled off *(8)*. In the United States, approximately 25.9 million men and 22.8 million women 18 years and older are smokers, about one in four in the adult population. Smoking rates are highest in adults with less than 9–11 years of education (39.7% of men, 34.3% of women), and lowest in those with 16 or more years of education (11.5% of men, 11.2% of women). Non-Hispanic black men are marginally more likely to smoke than non-Hispanic white men (29% vs 24.7%). American Indians/Alaskan Natives are also more likely to be smokers (41.7% of men, 38.1% of women) *(8)*.

Most people who quit do so on their own. Those who repeatedly attempt to quit and fail are often discouraged and frequently require professional assistance in dealing with the powerful physiological and behavioral responses associated with smoking.

Physical dependence on nicotine is one of the two major factors driving people to their smoking behavior. In 1988, the Surgeon General's report on nicotine addiction contended that cigaret smoking meets all of the established criteria for drug addiction, that nicotine is the psychoactive drug responsible for the addiction, and that cigaret smoking is similar to cocaine abuse in terms of the behavior associated with its maintenance. Each cigaret puff delivers a dose of nicotine to the brain within 7 seconds. Thus, smoking is one of the most rapid drug delivery systems. A smoker may self-administer 50,000 to 70,000 doses of nicotine per year.

Withdrawal from nicotine can produce several effects, including craving for tobacco, anxiety, irritability, increased restlessness, difficulty in concentrating, headache, drowsiness, and gastrointestinal symptoms. These symptoms are particularly pronounced for 2 to 3 days after cessation, but usually cease after that time. It is important to note that withdrawal symptoms *per se* are not generally associated with or triggered by specific situations, although they may be more or less severe during the withdrawal period and medications and behavioral coping strategies may reduce their intensity. These general withdrawal symptoms must be

distinguished from specific conditioned cravings for cigarets that can continue for months or even years after withdrawal has been completed.

Psychological addiction is the second major factor involved in sustaining smoking behavior and is not directly related to the ex-smoker's previous physical dependency on nicotine. A classical conditioning model can explain much of the craving for cigarets that can occur long after an ex-smoker has gone through nicotine withdrawal. Repeated association of smoking (an unconditioned stimulus) with using a telephone, driving a car, drinking a cup of coffee, working at a computer, etc. can lead to these activities becoming associated with the thought or memory of cigarets and even with the physiological response to smoking (thus becoming conditioned stimuli). Smokers can also become conditioned to smoke during subjective experiences such as stress, depression, anxiety, boredom, or anger. The result of this conditioning is that the exsmoker can be reminded of smoking or experience a physiological craving for a cigaret when exposed to the normal daily activities or subjective experiences during which he or she had been accustomed to smoke.

In addition, different factors may be involved in ex-smokers who relapse after several months or years of abstinence. Relapse may result from encountering social pressure to smoke, an unaccustomed stressor such as a personal loss, or an unexpected situation that leads to a return to smoking. A key concept in preventing relapses is the distinction between a *lapse*, which is a brief use of cigarets, and a *relapse,* which is a full return to smoking in the pattern previously followed by the smoker. One of the main goals of relapse prevention is to keep a lapse from turning into a relapse.

The following recommendations for an outpatient medical clinic-based smoking cessation protocol follow current US Public Health Service recommendations *(58),* with additional suggestions to specifically distinguish and address both nicotine withdrawal symptoms, conditioned cravings for cigarets, and relapse prevention.

PHYSICIAN-DELIVERED SMOKING CESSATION PROGRAMS

In 1991, 70% of smokers in the United States had at least one outpatient visit with a physician or other health professional during the previous year *(59).* Sixty to 70% of current smokers want to stop; 70 to 80% feel that a physician intervention would help them in quitting. There is a natural window of opportunity for physicians to intervene with smok-

ers, and the effectiveness of physician advice is cost-effective and has been examined in trials of smokers in general practice *(60)*.

However, simple advice by the physician to quit smoking compared to no advice, results in only an additional 5% of smokers quitting in 1 year. Physicians are not unaware of this low rate of success, and unfortunately it has resulted in many physicians not systematically addressing smoking with their patients. Having a more effective yet practical smoking cessation protocol is likely to increase physician attention to this issue.

The following clinical practice guideline for smoking cessation is based on the guidelines recommended by the United States Public Health Service *(58)*. It suggests that *every* patient who smokes be offered an intervention for smoking. Patients who are willing to quit now should be provided with effective treatment; patients who are *unwilling* to quit now should be provided with a brief intervention designed to increase their motivation and readiness to quit.

Step 1: Ask About Tobacco Use

Clinicians should ask all patients whether or not they smoke. The goal is to screen for and identify patients in three categories:

1. Smokers who are willing to make a quit attempt within the next 30 days
2. Smokers who are unwilling to make a quit attempt at this time
3. Former smokers

Step 2: Advise All Smokers to Quit

All smokers should be advised to quit in a clear, strong, and personalized manner. At the same time, acknowledge that it is up to the patient to make the decision to quit. Ask the patient what their personal reasons for quitting might be. Look for reasons that are concrete, specific, and personal such as, "I want to be able to keep up with my grandchildren when we go to the zoo." Theoretical, vague, and impersonal reasons may sound like the *right* answer ("I want to quit for my health"), however, they are less likely to motivate behavior.

Step 3. Assist Smokers Willing to Quit Within the Next 30 Days

1. Discuss a *quit plan* with patients. The patient should:
   a. Set a quit date (ideally, within 2 weeks).
   b. Tell family, friends, and coworkers about quitting and request support.

   c. Anticipate what to do to meet challenges that occur, such as withdrawal symptoms and conditioned cravings.
   d. Remove tobacco products from their environment prior to quitting.
2. Provide practical counseling and problem solving.
   a. Total abstinence is essential. "Not a single puff past the quit date."
   b. Find other ways to accomplish the positive things that smoking does for the patient, such as provide relief from stress and boredom, keep weight down, aid in socializing with friends who smoke, etc.
   c. Review past quit attempts to identify what helped during the quit attempt and what lead to relapse.
   d. Based on past experience and the patient's self-knowledge, discuss challenges to staying smoke-free and how the patient will overcome them. Discuss specific issues such as using alcohol without smoking or quitting while other smokers are in the house.
3. Provide support for patient.
   a. Encourage patient to use clinic staff for advice and assistance during the quit period, particularly during the first week after quitting smoking. A daily phone call or brief clinic visit during the first several days (the withdrawal period) can be particularly helpful to the patient.
   b. Encourage patient to obtain support from family and friends.
4. Recommend use of approved pharmacotherapy to reduce symptoms of withdrawal and increase smoking cessation success, except when contraindicated by the patient's medical condition. There are five first-line approved medications:
   a. Bupropion SR (Zyban). 150 mg QD for 3 days, then 150 mg BID for 4 weeks up to 6 months. Contraindications: history of seizures, history of eating disorder.
   b. Nicotine replacement therapy in the form of the patch, gum, nasal spray, or inhaler. Generally used for 3–6 months. Initiate dose of nicotine replacement therapy at approximately the level of the smoker's current daily nicotine consumption. Note that nicotine replacement, in particular the nicotine patch, is safe and has not been shown to cause adverse cardiovascular effects *(58)*. Second-line pharmacotherapy (clonidine or nortriptyline) may be used if first-line therapies are contraindicated.
5. Recommend use of behavioral techniques to deal with stress, withdrawal symptoms, and conditioned cravings for cigarets.
   a. Use deep breathing and relaxation to make withdrawal symptoms easier and eliminate conditioned cravings.
   b. During withdrawal, also have patient distract himself or herself with other activities when feeling uncomfortable.
   c. Confront specific conditioned cravings by having the patient deliberately place himself or herself in the situation that evokes a craving,

and then breathe deeply and relax to make the craving go away. Repeat until the situation no longer evokes a craving. Generally, this should be done after the 3-day withdrawal from nicotine.

d. Recommend using a *nonsmoking break* for stress management. This is designed to mimic the behavioral stress management components of taking a smoke break, but without a cigaret. There are three steps: (1) get away from the stressor, (2) distract oneself with a minor activity, and (3) take several deep breaths to relax.

6. Provide supplementary information and brochures that are culturally, racially, and age appropriate for the patient.

Unless a patient has had a good experience in the past quitting with nicotine replacement therapy or is not able to tolerate Zyban, we generally do not recommend nicotine replacement in our clinic and suggest that the patient's *quit day* be the day they begin their 3-day withdrawal from nicotine. This allows the patient to deal with withdrawal *up front* during a well-defined period that includes daily clinical contact by telephone or in person and pharmacotherapy assistance from Zyban. It is our experience that patients on nicotine replacement therapy are at increased risk of resuming smoking when they finally quit nicotine replacement, and go through withdrawal several weeks or months after their quit day.

Although Zyban is generally recommended for approximately 3 to 4 months following smoking cessation, when medication costs are a consideration we have found that patients find it helpful in reducing withdrawal symptoms when they use it for as little as 2 to 4 weeks (starting about 4 days prior to the quit day). Close contact with the patient during the first several days after quitting smoking is essential, and we recommend a daily phone call to an identified clinic staff person to report on difficulties and obtain support.

Step 4. Provide a Motivational Intervention for Smokers Currently Unwilling to Quit

With smokers unwilling to quit, have a brief discussion following the *5 Rs*: relevance, risks, rewards, roadblocks, and repeat as necessary at future clinic visits.

1. Relevance. Have patient indicate their own personal reasons for quitting smoking, being as concrete and specific as possible.
2. Risks. Ask the patient to identify potential negative health consequences of tobacco use. Discuss acute, long-term, and environmental risks.

a. Acute risks: shortness of breath, exacerbation of asthma, harm to pregnancy, impotence, infertility, etc.
b. Long-term risks: myocardial infarction (MI) and stroke, peripheral vascular disease, lung and other cancers, etc.
c. Environmental risks: increased risk of heart disease and lung cancer in spouses; higher rates of smoking in children of smokers; sudden infant death syndrome, asthma, middle ear disease, respiratory infections in children of smokers.

3. Rewards. Ask the patient to identify the specific benefits that may come from quitting smoking, such as improved taste and smell, better-smelling car and clothing, feeling better physically, etc.
4. Roadblocks. Ask the patient to identify barriers to quitting (such as fear of withdrawal symptoms, weight gain, depression, enjoyment of tobacco, etc.), and use problem solving to try to address issues.

Step 5. For Former Smokers, Assist in Preventing Relapse

Relapse prevention involves anticipating potential difficulties that may lead to resumption of smoking and addressing them in advance as much as possible. Issues to be dealt with may include:

1. Feeling a lack of support from family and friends
2. Negative mood or depression
3. Weight gain, expected or unexpected
4. Prolonged conditioned cravings or difficulty withdrawing from nicotine replacement therapy
5. Flagging motivation or feeling deprived
6. Keeping a *lapse* from evolving into a *relapse*

In regards to keeping a lapse from becoming a relapse, patients should be specifically told that if circumstances lead them to smoke one or two cigarets, they have not failed and can choose to go back being smoke-free. The worst situation is for them to smoke a single cigaret, decide they have failed, and resume smoking at their previous level. An effective analogy to use is as follows: "If circumstances lead you to say something you wish you hadn't said to a person, you don't need to go on saying it. The same thing applies if circumstances lead you to smoke one or two cigarets." Simultaneously with this message, however, patients should be explicitly told that this does not imply that they are being given *permission* to smoke one or two cigarets occasionally; their health does not benefit if they become occasional smokers, and their risk of complete relapse rises substantially.

Step 6. If Necessary, Refer Patient to a Professional Smoking Cessation Program

Structuring a clinic to provide patients with an effective and comprehensive smoking cessation program may not always be possible. However, we contend that it is *always* possible to provide a referral to such services when smokers have been identified and the risks of continuing to smoke have been discussed with them. Smoking cessation programs are available throughout the United States from the American Lung Association, the American Cancer Society, and the Seventh-Day Adventist Church. The American Heart Association also offers a self-help program. Many national and local commercial programs are also available. Developing a comfortable referral relationship with programs in your area can improve the care you provide for your smoking patients.

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4 Peripheral Arterial Disease and Diabetes

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CONTENTS

INTRODUCTION

The incidence of type 2 (noninsulin-dependent) diabetes mellitus continues to increase in the United States *(1)*. Consequently, the associated morbid and mortal events from the associated microvascular and macrovascular complications of this disease have also risen *(1)*. The preponderance of research toward the understanding of vascular disease in diabetes has been directed toward the delineation of end organ injury from microvascular disease. Consequently, the focus on macrovascular peripheral vascular disease has been relatively ignored. However, several studies have elucidated both the incidence and prevalence of peripheral vascular disease associated with both type 1 (insulin-dependent) and type 2 diabetes mellitus. In general, these studies have found that the duration of diabetes is perhaps the best correlate to predict the development of coronary artery and renal disease, as well as other associated end organ injuries that have not consistently correlated with the development of peripheral vascular disease.

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This chapter will review the available studies that report the incidence and prevalence of peripheral vascular disease in both type 1 and type 2 diabetic patients. It will focus not only on risk factors associated with the development of peripheral arterial disease, but also touch on pathophysiologic changes that may help to account for some epidemiologic trends. Lastly, it will highlight differences between diabetic and nondiabetic subjects concerning localization of disease and its association with mortality and limb loss.

ANATOMIC DISTRIBUTION OF DISEASE

Prospective studies have demonstrated that peripheral arterial disease associated with diabetes predominately affects the more distal circulation when compared to nondiabetic subjects with atherosclerotic disease. Specifically, the anterior and posterior tibial and peroneal arteries are affected to a greater extent than the femoral or iliac arteries in diabetics *(2–4)*. Abbott et al. *(2)* reviewed the Framingham data base for the relationship between diabetes and development of peripheral arterial disease in the carotid and femoral circulations. They evaluated 20 years of follow-up data from the Farmingham Study involving approximately 1200 men and 1600 women. They demonstrated that the incidence of carotid bruits and nonpalpable pedal pulses increased with age. However, in both diabetic men and women there was an increased risk for peripheral arterial disease that increased across all age groups. In addition, they noted that women without diabetes had a twofold excess in femoral bruits and nonpalpable pedal pulses compared to women without diabetes. Diabetic men also had approximately a twofold greater risk of carotid bruits when compared to nondiabetic men. Diabetes was also shown to be an important risk factor for the development of intermittent claudication in this study. Those who had both diabetes and peripheral arterial disease were at the highest risk for development of cardiovascular events. Moreover, presence of diabetes resulted in a worse prognosis for subsequent cardiovascular outcomes in the Framingham study *(2–4)*. This group has a twofold excess in coronary heart disease and stroke, as well as heart failure. Lastly, presence of a femoral bruit doubled the risk of coronary disease in diabetic men in this study.

In a separate study by Witteman et al. *(5)*, the presence of aortic calcified plaques on routine chest X-ray correlated with a twofold increased risk of cardiovascular death in men and women younger than age 65. A similar relative risk was noted for the development of coronary artery disease, stroke, and intermittent claudication among middle-age women. Lastly, the risk of sudden coronary death in men with calcified

aortic plaques in the thoracic aorta was sevenfold higher at age 35 compared to age 70, where no excess risk was noted. Therefore, radiographic abnormalities in the peripheral vasculature among younger individuals clearly correlates with the presence of accelerated cardiovascular disease.

As previously noted, atherosclerotic changes associated with diabetes have a similar prevalence in the femoral arteries, but have a much higher prevalence in the tibial and peroneal arteries *(6)*. The reasons for this difference in location and extent of involvement in diabetic patients are not known. This involvement of the more distal and smaller vessels, however, correlates with a much higher incidence of amputations seen in the diabetic population when compared to the nondiabetic population with peripheral occlusive disease. In a 12-year follow-up study in 84 Pima Indians with diabetes, it was noted that the incidence of amputations increased with age *(7)*. Moreover, these investigations noted that the death rate was greater among diabetic amputees than in nonamputees. These observations confirm other investigations that report similar increases in mortality among amputees *(8,9)*.

These related studies clearly show that diabetes is associated with a more distal distribution of peripheral vascular disease when compared to nondiabetic atherosclerotic disease. While the mechanisms to explain this varied distribution are not known, it does support the clinical contention that diabetes will have more distal disease and should always have assessment of their peripheral vasculature if arterial disease is suspected.

RISK FACTORS

Many risk factors have been associated with the development of peripheral vascular disease in the diabetic patient. These are summarized in Table 1. These risk factors will each be discussed to its relative importance and contribution to the development and acceleration of pre-existing peripheral arterial disease. The prevalence of lower extremity arterial occlusive disease by noninvasive methods was examined by Beach et al. *(10)*. They found that most risk factors including smoking history, duration of diabetes, reduction in high-density lipoprotein (HDL) cholesterol, total cholesterol elevations, and systolic blood pressure elevations correlated with the progression of pre-existing lower extremity arterial lesions among diabetic patients. These findings confirm previous investigations by this group that document an association between smoking and lipid abnormalities in contributing to the development of peripheral arterial disease *(11,12)*. Moreover, it is important to

Table 1
Risk Factors for Accelerated Development of Peripheral Artery Disease in Diabetic Subjects

Smoking
Elevated systolic blood pressure
Low HDL-cholesterol
Family history of peripheral arterial disease

Note in most studies, diabetes itself is associated with a much higher prevalance of peripheral artery disease compared to nondiabetic populations.

note that lower extremity occlusive disease is 20 times more likely to occur in type 2 diabetics than in age- and sex-matched control subjects *(13)*. Although the histological changes of atherosclerosis in the diabetic is similar to the nondiabetic, the location and extent of involvement is different.

In a study of diabetic Pima Indians, Nelson et al. *(7)* identified risk factors associated with a high probability for limb amputation among diabetic individuals. These included: presence of diabetic retinopathy, nephropathy, hypertension, absent patellar tendon reflexes, and presence of medial arterial calcification. However, no single risk factorwas identified as being relatively more important than another.

The most significant risk factor that has consistently been shown to aggravate pre-existing peripheral arterial disease in diabetics is smoking. In a study by Fowkes et al. *(14)*, more than 1500 men and women between the ages of 55 and 74 years demonstrated that the only risk factor consistently associated with an increased risk of peripheral arterial disease was smoking. Moreover, smoking increased the risk for development of peripheral arterial disease in diabetics more than it did for development of heart disease. The aim of this study was to learn if certain risk factors in the general population are more strongly related to peripheral arterial disease than ischemic heart disease. Multiple regression analyses of various risk factors for the development of peripheral arterial disease was performed in both diabetic and nondiabetic and sex matched controls. These patients were obtained from ten general in Edinburgh, Scotland. The analyses showed an association between the development of peripheral arterial disease and diabetes, systolic blood pressure and serum cholesterol. This study also displayed an inverse association with the development of peripheral arterial disease and HDL-cholesterol as well as elevated triglycerides. Interestingly, diabetes alone was not a strong risk factor for development of peripheral arterial disease. In

another study by Beach *(12)*, the prevalence of atherosclerotic peripheral vascular disease among diabetics was evaluated in 141 type 1 and 289 type 2 diabetic subjects and compared to 64 age matched controls. The prevalence of peripheral arterial disease among type 1 diabetics was 18% and in the older type 2 diabetes, 41%. The prevalence increased at 7.5% per decade in smokers. Thus, concomitant risk factors superimposed on diabetes are associated with an accentuated progression of peripheral arterial disease.

MORTALITY

Clearly, diabetics who have peripheral arterial disease have higher mortality rates than those who do not have arterial disease. A recent study by Vogt et al. *(15)* evaluated the relationship between peripheral arterial disease and mortality in a population of close to 2000 individuals over a 13-year period. All patients 50 years of age and older with no history of lower extremity surgery were evaluated for the presence of peripheral arterial disease. Analysis of the data stratified by populations and comorbid conditions showed that a low ankle-brachial index is an independent predictor of all causes of mortality in both men and women with peripheral arterial disease. This increase is a relative risk and is unchanged after exclusions of all patients with a clinical history of cardiovascular disease or diabetes. Thus, a low ankle-brachial index is an important measurement to obtain to assess the risk of mortality among those who smoke and have either angina or diabetes.

It is important to note that this increased predisposition for the development of peripheral vascular disease among diabetic patients is not true in patients who have simple glucose intolerance. Other studies have investigated the incidence in prevalence of peripheral vascular disease among patients with glucose intolerance, but not frank diabetes *(16–17)*. In all cases, the incidence of peripheral arterial disease was no different among those who have glucose intolerance compared to age-matched controls. In the Casteldaccia study *(18)* that involved 102 healthy volunteers and 102 predominately male patients with peripheral arterial disease, factors such as arterial hypertension, hypercholesteremia, hypertriglyceridemia, smoking habits, and hyperglycemia were correlated with the incidence of peripheral arterial disease . This study showed that smoking and hypercholesterolemia were the most important risk factors for the development of peripheral arterial disease. Moreover, this and other studies point to the fact that in diabetic patients the diabetes *per se* may not be as important in the development of peripheral arterial disease as the increased serum cholesterol level, which generally portends this

disease process in nondiabetic subjects *(18–20)*. In a study by Migdalis et al. *(21,22)* type 2 diabetic patients and 93 age- and sex-matched controls further supports this relationship. Subjects in this study were evaluated for risk factors associated with the development of peripheral vascular disease. Hypercholesterolemia was associated with the development of peripheral arterial disease, however, smoking was not significantly correlated with accenuated disease. Interestingly, diabetes itself clearly increased the risk for development of peripheral arterial disease. This reinforces the concept that hypercholesterolemia is an important parameter to monitor in order to help reduce progression of peripheral arterial disease in high-risk populations.

CONCLUSION

These studies all demonstrate unequivocally that hypercholesterolemia, especially in a diabetic individual, predisposes to acceleration of peripheral arterial disease. Smoking in these individuals will accelerate this process *(22)*. Lastly, presence of systolic hypertension while contributing to the development of peripheral arterial disease, in most studies has not been shown to markedly accelerate its development. Therefore, clinical studies focusing on the mechanism of arterial wall injury from low-density lipoproteins as well as growth factor homeostasis of vessel injury repair, need to be performed in high-risk individuals so that the progression of this process can be attenuated.

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5 Hyperlipidemia in Peripheral Arterial Disease

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CONTENTS

INTRODUCTION

Peripheral arterial disease (PAD) is a common manifestation of atherosclerosis affecting 18 to 20% of the population over age 75 *(1,2)*. By the late fifth decade almost 8% demonstrate significant asymptomatic disease on noninvasive testing, whereas 5% have symptoms of intermittent claudication, which is a major manifestation *(3)*. This is caused by muscle ischemia secondary to inadequate blood flow that is unable to meet oxygen demands during exercise. Intermittent claudication can lead to moderate to severe impairment in the patient's walking ability,

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and in turn can lead to disability if the patient is unable to meet the activities of daily living or those of their occupation.

PATHOGENESIS OF ATHEROSCLEROSIS

Atherosclerosis is a multifactorial disorder primarily affecting the large arteries of the carotid, coronary, and femoral beds. Clinical symptoms of atherosclerosis are manifested only late in its course. Atherosclerosis involves the deposition, modification, and cellular uptake of cholesterol derived from circulating lipoproteins *(4)*. Vascular injury to the endothelium may be the initial event in the atherogenic process. Repetitive injury of the vessel wall leads to inflammation and vessel remodeling. Various factors including hyperlipidemia and diabetes cause injury to vessel wall. Injury promotes expression of chemo-attractants in the vessel wall that leads to recruitment of inflammatory cells. Inflammation is also associated with increased levels of acute phase reactants such as C-reactive protein, plasminogen activator inhibitor (PAI)-1, and tissue plasminogen. An increase in local PAI-1 level may result in impaired fibrinolysis. Retention and oxidation of low-density lipoprotein (LDL) in vessel wall underlies many features of the atherosclerotic plaque, including reduced availability of nitric oxide and endothelial dysfunction. Oxidized LDL also increases endothelial vasoconstriction and promotes expression of adhesion molecules, cytokines, growth factors, and endothelin. Endothelin is a thrombogenic factor that may promote plaque instability and thrombotic events.

Atherosclerotic lesions with a high lipid content and a thin fibrous capsule are most likely to lead to an acute clinical event. Inflammatory cells that accumulate within the plaque produce metallo-proteinases. These metallo-proteinases degrade the extracellular matrix of the fibrous cap of the plaque, predisposing to plaque rupture. Many of these factors can be modified by lipid-lowering therapy. Lipid lowering may stabilize the fibrofatty plaque by removing lipid, thickening the fibrous cap, and increasing the collagen content of the plaque, all of which leads to decreased incidence of spontaneous fissure and rupture. Lipid lowering can also result in improvement of endothelial function.

ATHEROSCLEROSIS, DYSLIPIDEMIA, AND PERIPHERAL ARTERIAL DISEASE

Factors associated with increased risk for intermittent claudication include smoking, hypertension, hyperlipidemia, and diabetes mellitus *(5)*. Of these factors, hyperlipidemia has recently become the focus of intense study. A significant association between PAD, hyperlipidemia, and increased mortality secondary to coronary artery disease (CAD) and

cerebrovascular events has been noted. Fifty percent of patients who suffer from symptomatic PAD are dysplipidemic with total cholesterol >220 mg/dL and/or LDL >140 mg/dL *(6)*. Conversely, in a study of patients with familial hypercholestrolemia (FH), 31% had hemodynamically significant PAD localized predominantly to the femoral-popliteal vessels and 30% had associated CAD. The incidence of PAD was highest (50%) in patients with FH and CAD, underscoring the diffuse nature of the atherosclerotic vascular disease *(7)*. An increased incidence of femoral atherosclerosis has also been noted in asymptomatic patients with FH *(8)*.

Compared to patients with CAD, patients with peripheral, carotid, and aortic atherosclerosis receive less attention to lipid risk factors *(9,10)*. In one study of 299 patients with symptomatic PAD documented by angiography, only 27% had any lipid profiles done, and of these only 9% were receiving treatment for hypercholesterolemia *(9)*. Physician recognition and management of hypercholesterolemia in patients with peripheral and carotid atherosclerosis undergoing vascular surgery was evaluated in 80 patients retrospectively *(10)*. Of the 66% screened patients found to be hypercholesterolemic, only 24% received in-hospital management and only 13% received intervention at discharge. This underscores the need for education and greater awareness of surgeons and physicians involved in the management of patients with PAD.

INTERVENTION TRIALS IN ATHEROSCLEROSIS

Until the last decade, therapy for PAD was essentially limited to lifestyle recommendations such as cessation of smoking and regular physical exercise. Peripheral vasodilators were also utilized. Patients not responding to conservative treatment underwent invasive therapies such as surgery or angioplasty to achieve and maintain blood flow, as reflected by improvement in peripheral circulation and exercise capacity.

Recent advances have emphasized the importance of lipid lowering in the prevention of atherosclerosis and the management of its clinical manifestations. Stabilization and even reversal of the underlying atherosclerotic process can occur with lipid-lowering therapies. Multiple trials over the past decades have clearly shown that lowering elevated cholesterol levels reduces the progression of coronary atherosclerosis and lowers the incidence of acute coronary events. Extrapolating from these data, effective lipid reduction likely results in beneficial effects on the atherosclerotic process at other sites such as the carotid and femoral arteries, thereby reducing the need for invasive therapies.

Primary prevention trials such as Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) *(11,12)*, West of Scotland Coronary Prevention Study Group (WOSCOPS) *(13,14)*, and the

Helsinki Heart Study *(15)* have all demonstrated the benefit of lipid lowering using various hypolipidemic agents for prevention of initial cardiac events. Secondary prevention trials have unequivocally shown that lipid lowering therapy not only reduces acute coronary events and other clinical endpoints, but also results in angiographic improvement. These studies have used different classes of drugs (statins, fibrates, niacin, bile acid resins—alone and in combination) in both hyper- and normo-cholesterolemic patients with established CAD.

STATINS AND ATHEROSCLEROSIS

Statins have been used for secondary prevention of CAD with good results. Simvastatin has been evaluated in several studies including the Scandanavian Simvastatin Survival Study (4S) *(16)*, the Simvastatin/ Enalapril Coronary Atherosclerosis Trial (SCAT) *(17)*, and the Multicentre Anti-Atheroma Study (MAAS) *(18)*. In addition to lowering LDL cholesterol levels, simvastatin has been shown to cause angiographic improvement in the coronary vessels, as well as slowing the progression of diffuse and focal atherosclerosis. In addition, the drug is believed to induce positive vascular remodeling, thereby reducing the occurrence of restenosis *(19)*. The 4S study included 4444 patients with documented or symptomatic CAD. This was the first study to unequivocally demonstrate a reduction in the total CAD mortality (34% reduction in treated group with a 51% reduction in of coronary revascularization rate). Risk reduction was evident in multiple subgroups such as women, diabetics, and elderly patients (> 60 years of age). Similar results were seen in the SCAT study that evaluated normo-cholesterolemic patients with CAD, and the MAAS study that demonstrated less angiographic progression of atherosclerosis in the simvastatin treated group compared to the placebo group.

Lovastatin was used in the Canadian Coronary Atherosclerosis Intervention trial (CCAIT) *(20–22)* and the Monitored Atherosclerosis Repression Study (MARS) *(23)*. The CCAIT study *(20)* was designed to evaluate whether lovastatin therapy retards the progression or facilitates the regression of coronary atherosclerosis as assessed by serial angiograms. This study concluded that the effect of lovastatin in preventing the formation of new coronary lesions might be more important than its effect on established lesions. Subgroup analysis in women and diabetics concurred with the main analysis. The MARS was the first trial that employed serial coronary angiograms to test the effects of a single drug therapy using lovastatin more than 2 years. The results showed that there was no significant difference in lesions <50% stenosed at baseline, but for lesions >50% stenosed at baseline there was regression.

The Cholesterol and Recurrent Events (CARE) trial *(24)*, the Long Term Intervention with Pravastatin in Ischemic Disease (LIPID) study *(25)*, and the Regression Growth Evaluation Statin study (REGRESS) *(26–28)* evaluated patients with established CAD given pravastatin. CARE reported a 24% reduction in the incidence of fatal and nonfatal coronary event in the treatment group, with a 23% reduction in the need for PTCA and a 31% reduction in the incidence of stroke. There were similar findings in the LIPID study that reported a 23% decrease in the incidence of combined fatal and nonfatal coronary events. In the REGRESS study, patients with established CAD treated with pravastatin demonstrated a significant event reduction, regardless of whether they had increased or normal cholesterol levels. These data imply that the non-LDL effects of the statins may also be important for the risk reduction with this class of drugs.

Atorvastatin, one of the most potent statins available, was used in the Atorvastatin Versus Revascularization Treatments (AVERT) study *(29)*. This study randomized 341 patients with CAD to atorvastatin 80 mg/day vs angioplasty plus usual care. The mean LDL cholesterol in the atorvastatin group was 77 mg/dL compared to 119 mg/dL in the angioplasty group. Therapy with atorvastatin was associated with a significantly longer time to first ischemic event, which corresponded to a risk reduction of 36%.

The majority of late coronary events in patients post coronary artery bypass graft (CABG) relate to degeneration of saphenous vein grafts. Lipid lowering after coronary revascularization has been shown to prevent clinical events related to plaque instability, and inhibits progression of saphenous vein graft disease. In the Post Coronary Artery Bypass Graft trial, lovastatin was combined with cholestyramine to lower LDL levels to less than 85 mg/dL *(30)*. Aggressive lowering of LDL showed significantly less progression of graft atherosclerosis compared with moderate lowering of LDL. Subgroup analysis was done to evaluate the treatment effects by age, gender, and selected cardiovascular risk factors such as diabetes, hypertension, high-density lipoprotein (HDL), and triglycerides (TG) levels *(31)*. Aggressive lowering of LDL delayed progression of atherosclerosis irrespective of gender, age, and these risk factors for CAD.

FIBRATES AND ATHEROSCLEROSIS

Outcome studies employing the statins have conclusively shown that reduction of LDL translates into reduced clinical cardiovascular events. However, in patients with normal LDL or after LDL levels have been normalized, TG and HDL levels assume an important role in the progression of atherosclerosis. The importance of HDL-cholesterol as an inverse

risk factor for CAD is well known. Primary prevention trials such as the Helsinki Heart Study *(15)* using gemfibrozil therapy showed a 60% reduction in CAD events and a 34% reduction in cardiovascular deaths over a five year period in patients with low HDL levels.

Several secondary prevention trials using fibrates have evaluated the benefits of intervention in patients with normal to borderline total cholesterol and LDL levels with a low HDL level (<45 mg/dL) *(32–35)*. The BECAIT trial using bezafibrate was the first major study to explore the effect of increasing HDL and/or decreasing TG on secondary prevention of CAD. This was a double-blinded placebo-controlled trial over 5 years in young males post myocardial infarction (MI). Effect on coronary atherosclerosis was studied by serial coronary angiograms. This trial showed that progression of coronary atherosclerosis was prevented, and coronary event rate was reduced primarily by lowering TG and increasing HDL without lowering serum LDL. The benefits were particularly marked on lesions of 20 to 50% stenosis at baseline.

The Veterans Affairs High-Density Lipoprotein Intervention Trial (VAHIT) study *(36)*, was a double-blind randomized controlled trial in 2531 men with CAD whose major lipid abnormality was a low HDL-C of 40 mg/dL or less, treated with gemfibrozil during a period of 5.1 years. There was a 22% reduction in the primary end points, the risk of death from CAD or nonfatal MI, with an absolute risk reduction of 4%. The 22% reduction in major CAD was associated with a 6% increase in HDL-C, 33% decrease in TG, and no change in LDL-C levels.

These studies suggest the importance of TG-rich lipoprotein reduction and HDL raising for the retardation of atherosclerosis in mild to moderate lesions, and a role for fibric acid derivatives in secondary prevention. Analysis of data from Cholesterol Lowering Atherosclerosis Study (CLAS), the Program on the Surgical Control of Hyperlipidemias (POSCH), and MARS studies also provide evidence for the importance of TG rich lipoprotein in progression of CAD.

NIACIN AND ATHEROSCLEROSIS

Six major clinical trials have evaluated the effect of niacin on cardiovascular event reduction. The Coronary Drug Project *(37)*, the largest of these trials used niacin as monotherapy for 6 years and reported significant reduction in recurrent MI, strokes, and transient ischemic attacks (TIAs). The CLAS *(38)* and Familial Atherosclerosis Treatment Study (FATS) studies *(39)* used niacin in combination with colestipol. CLAS was the first coronary angiographic trial to unequivocally demonstrate that there was regression of CAD with aggressive lipid-lowering therapy using this class of agents when compared to a placebo group. Treatment

benefits were seen at 2 and 4 years with an 18% regression of lesions at 4 years compared to 6.4% in the placebo group, and with reduction of clinical events by 20%. In native coronary arteries, both progression of existing lesions and development of new lesions was less at two and four years. In graft vessels, treatment with colestipol-niacin did not reduce the incidence of new closures, but it did reduce formation of new lesions both at 2 and 4 years emphasizing the need for early treatment of elevated lipids after CABG. The FATS, a randomized double-blinded coronary angiogram study over 2.5 years included subjects with LDL more than 125 mg/dL. A net regression of coronary disease with marked reduction in cardiovascular events was observed.

LIPID LOWERING IN PERIPHERAL ARTERIAL DISEASE

The studies of lipid lowering in PAD are heterogeneous in terms of the type of drugs used, inclusion criteria, and outcome measures. An advantage of lipid lowering in patients with intermittent claudication, but without symptomatic CAD is that such therapy would also be expected to reduce mortality from heart disease. In addition, it might improve local symptoms such as intermittent claudication, and reduce the need for reconstructive surgery. Several studies have validated the benefits of lipid lowering on femoral atherosclerosis. These studies included symptomatic patients with intermittent claudication, as well as asymptomatic patients with critical limb ischemia identified by abnormal ankle-brachial index (ABI) or peripheral angiogram. Disease progression was assessed by ultrasound, angiography, ABI, and exercise capacity evaluated by treadmill testing. Additional end points evaluated were fatal and nonfatal clinical events and subjective measures such as symptom improvement.

The Saint Thomas's Trial was the first randomized controlled trial evaluating the effect of lipid lowering therapy on the course of femoral atherosclerosis in hyperlipidemic patients with intermittent claudication *(40)*. The patients were randomized to receive diet, cholestyramine, nicotinic acid, or clofibrate depending upon their lipoprotein phenotype. Total cholesterol (TC) decreased by 25%, LDL cholesterol by 28%, and TG by 45% following therapeutic intervention. Femoral atherosclerosis was studied by arteriography. In patients receiving lipid-lowering therapy, progression of atherosclerosis was reduced by 60% compared to the placebo group. The rate of increase in the cross-sectional area of the plaque in the treated group was one-third that of the placebo group. Change in edge irregularity was 2.5-fold greater in the placebo group, whereas twice as many arterial segments showed improvement in the therapy group.

The CLAS study *(41)* included 188 men with previous CABG and PAD, diagnosed by femoral angiograms, treated with colestipol and niacin for two years. The effect of therapy on femoral atherosclerosis was evaluated by estimating lumen abnormality measured by a computer-estimated atherosclerosis scale. Although a significant therapeutic benefit was observed in both femoral and coronary arteries, the effect was less marked on the femoral arteries. The HDL levels and the ratio of TC/HDL correlated with regression or progression of femoral atherosclerosis. Subjects who demonstrated regression of femoral atherosclerosis had higher HDL levels and a lower ratio of TC/ HDL, than subjects who had no change in femoral atherosclerosis. Of particular interest, statistical significance was obtained for HDL levels only for femoral atherosclerosis and not for coronary atherosclerosis. A significant therapy effect was found in segments with moderately severe atherosclerosis and in proximal femoral segments. The POSCH trial *(42)* was a randomized single-blind controlled trial with a mean follow-up of 10 years. Patients with established CAD were assigned to diet only vs diet and partial ileal bypass surgery. LDL reduction of 38% was noted in the surgical group with an absolute risk reduction of 10%, and relative risk reduction of 35% for fatal and nonfatal CAD events. The POSCH trial also reported a reduction in symptoms of intermittent claudication in patients with PAD in the surgical group.

The Probucol Quantitative Regression Swedish Trial *(43)* (PQRST) was a 3-year study performed to investigate the effects of probucol, cholestyramine, and diet on atherosclerosis in the femoral arteries in patients with hypercholesterolemia, with or without clinical signs and symptoms of PAD. The primary endpoint was the change in atheroma volume of the femoral artery as estimated by annual measurements of angiograms of 20 cm segments of the femoral artery. Clinical events such as MI, silent MI, new onset angina, and other events were also noted. At the end of 3 years, the lumen volume of femoral arteries, cardiovascular event rates, and ABI were the same in both groups. It has been speculated that the benefit of LDL lowering by probucol was negated by the concomitant lowering of HDL level induced by this drug.

The 4S trial *(44)*, in addition to CAD data, also reported the effects of therapy with simvastatin on noncoronary signs and symptoms during a median follow-up of 5.4 years. Patient assessment for these included a history of symptoms suggestive of intermittent claudication and a physical examination for carotid and femoral bruits that was done at baseline and followed annually. Risk of a new or worsening carotid bruit was substantially reduced by treatment, consistent with a reduction in cere-

brovascular events. Even though there was no statistically significant effect on femoral bruits, there was a 38% reduction in the incidence of new or worsening intermittent claudication.

In a nonrandomized trial Olsson et al. *(45)* evaluated the development of femoral atherosclerosis after one year of treatment with diet, nicotinic acid, and fenofibrate in 45 middle-aged men with asymptomatic hyperlipidemia. Disease progression was demonstrated in 24% of the treatment group compared to 40% in controls, whereas regression occurred in 29% and 0%, respectively.

CONCLUSIONS

Atherosclerosis is a life-long disease and measures to improve compromised blood flow by surgical methods or angioplasty is palliative. The main objective should be prevention of clinical disease by aggressive management of lipid abnormalities. The aim of treatment is to reduce LDL to <100 mg/dL, the same as for patients with prior CAD. Once lipid lowering is begun patients need regular follow-up to ascertain that the National Cholesterol Education Program (NCEP) targets are being maintained. Patients should be educated about the importance of lipid-lowering therapy to prevent further progression of atherosclerosis, especially after they undergo revascularization.

The Lipid-Treatment Assessment Project (L-TAP) *(46)* is a large epidemiological multicenter survey from a primary care setting to evaluate the percentage of dyslipidemic patients receiving lipid-lowering therapy and achieving LDL goals. Among the 4888 patients surveyed from five regions in the United States, 70% had 1 to >2 risk factors with no evidence of CAD, whereas 30% (1460 patients) had established CAD. Overall, only 38% achieved the NCEP specified LDL levels. Of particular concern was the fact that of the 1460 patients with established CAD, 82% did not reach their target LDL goal of <100 mg/dL. The reasons for not reaching therapeutic goals include patient compliance and lack of appropriate follow-up testing. In another study, up to 50% of patients on a lipid-lowering drug quit taking the drug after 1 year, with only 25% still taking their medications at the end of 2 years *(47)*. Of patients taking the drugs, a minority were taking doses sufficient to lower LDL levels to target levels.

There is now a large body of evidence available indicating the important role of hyperlipidemia in the development of PAD. There is also convincing data to indicate that patients with PAD can greatly benefit from lipid-lowering therapy.

The current challenge is to find a way to utilize lipid-lowering strategies more effectively.

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6 Hypertension and Peripheral Vascular Disease

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CONTENTS

INTRODUCTION

The majority of deaths in western societies such as the United States are attributed in large part to arteriosclerosis, or hardening and thickening of the arterial wall *(1)*. Underlying peripheral vascular disease as well as coronary artery and cerebrovascular disease is atherosclerosis, a disorder of larger arteries and one type of arteriosclerosis *(1)*. Atherosclerosis is the leading cause of death in the general population in industrialized countries *(1)*. It is well recognized that hypertension plays a major role in the pathogenesis of atherosclerotic vascular disease *(2–11)*. This has important implications from a public health perspective because of the high prevalence of hypertension and its amenability to effective treatment.

The mechanism for the pathogenesis of atherosclerosis is generally considered separate from hypertension. Hypertension, however, accelerates the atherosclerotic process in large part as a result of increased shear stress on arterial walls. This increased shear stress stimulates a variety of proliferative growth factors, e.g., platelet-derived growth factor, endothelin, and others that ultimately enhance the atherosclerotic

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process. This process is exemplified by the Charcot-Bouchard aneurysms and fibrinoid arteriolar necrosis seen in hypertensives *(5)*.

This chapter will focus on the epidemiology of peripheral vascular disease seen among hypertensive subjects. It will delineate both the incidence and prevalence of this disease, as well as elucidate risk factors for the development of peripheral vascular disease in hypertensive populations.

RISK FACTORS

The leading cause of occlusive arterial disease in the extremities in patients over the age of 40 years is atherosclerosis *(12)*. Atherosclerosis of the peripheral arterial system is most commonly seen in elderly males; the peak incidence occurs in the sixth and seventh decades of life *(12)*. The recognized risk factors for this important disease in the general population are outlined in Table 1. Risk factors include cigaret smoking, hypertension, hypercholesterolemia, hypertryglyceridemia, and diabetes as demonstrated in the landmark Framingham study *(13)*. An additional risk factor may be the presence of an abnormal fibrinogen locus genotype *(14)*. The sequelae of peripheral vascular disease occur approximately two to three times as frequently in hypertensives when compared to normotensives matched for age *(5,6,11)*. Whereas hypertension alone is an independent risk factor for peripheral vascular disease, it frequently occurs in association with other powerful predictors for development of atherosclerosis that include diabetes, obesity, and adverse lipid profiles *(4,6–9)*. Thus, hypertension may be viewed as a significant, multifaceted metabolic disturbance that has been termed *Syndrome X (15)*. It is perhaps this tendency for association with other risk factors that makes it difficult to assess the contribution of hypertension alone to the development of peripheral vascular disease.

Surprisingly, no clinical trials have specifically addressed the contribution of hypertension to the development of peripheral arterial disease. Most of the data comes from multiple regression analysis of many parameters looking for correlations, or subgroup analysis of trials designed primarily to look at coronary artery disease. However, Kannel et al. *(6)* has shown that epidemiological data analyzed in this way accurately predicts disease risk in a variety of American population samples, and in elderly as well as young coronary candidates.

There has been an enormous amount of analysis and re-analysis of Framingham data in the literature. The incidence of hypertension in the Framingham cohort increased with age in men from 3.3% at ages 30–39 to 6.2% at ages 70–79, and in women from 1.5% at ages 30–39 to 8.6%

Table 1
Major Risk Factors for Accelerated Risk
of Peripheral Vascular Disease in Hypertensive Subjects

- Smoking
- Diabetes
- Hypercholesterolemia
- Hypertriglyceridemia

at ages 70–79 *(16)*. Stokes et al. *(13)* reviewed data from 30 years of follow-up of the original Framingham study cohort of 5070 men and women aged 30–62 years who were first examined during 1948–1952, and who were at that time free of cardiovascular disease. Multiple regression analysis revealed that blood pressure is a strong and consistent predictor for the development of coronary heart disease, stroke, transient ischemic attack, and congestive heart failure, as well as peripheral vascular disease. The authors note that risk factors related to hypertension like obesity and left ventricular hypertrophy (as demonstrated on electrocardiograms [ECGs]) were independent. Moreover, heart enlargement by X-ray radiography was the best predictor of congestive heart failure. Kannel et al. *(6)* reviewed the same database and noted that the risk of peripheral vascular disease and especially coronary disease associated with hypertension, hyperlipidemia, or diabetes varies widely depending on the level of associated risk factors. Habits, such as cigarette smoking or lack of exercise, can independently affect the risk associated with any of the atherogenic traits. Analysis of the Framingham data found that at a given level of total cholesterol, risk is greatly affected by the total/high-density lipoprotein (HDL) cholesterol ratio; low HDL values were associated with greater prevalence of peripheral vascular disease, especially in diabetics.

Further analysis of 50 years of Framingham research showed that hypertension is a powerful risk factor accounting for about 35% of atherosclerotic cardiovascular disease. Hypertension was shown to predispose powerfully to all of the major atherosclerotic cardiovascular events including coronary artery disease, stroke, peripheral arterial disease, and heart failure. An analysis of risk of cardiovascular events in subjects with hypertension in persons aged 35–64 years in a 36-year follow-up of the Framingham study showed that the age-adjusted biennial rate per 1000 for peripheral arterial disease was 10 for men and 7 for women. The age-adjusted risk ratio was 2.0 for men and 3.7 for women, and the excess risk per 1000 was 5.0 for both men and women *(17)*.

Table 2
Logistic Regression Estimates and Odds Ratio
for Significant Risk Factors in the Intermittent Claudication

Risk factor	*Odds ratio (95% CI)*	*p*
Male sex	1.7 (1.3, 2.1)	.0001
Age (per 10 years)	1.5 (1.3, 1.6)	.0001
High normal blood pressure	1.3 (0.9, 1.8)	.1384
Stage 1 hypertension	1.5 (1.1, 2.0)	.0091
Stage 2 hypertension	2.2 (1.7, 3.0)	.0001
Diabetes	2.6 (2.0,3.4)	.0001
Cigarets (per 10 cigarets)	.4 (1.3, 1.5)	.0001
Cholesterol (per 40 mg/dL)	1.2 (1.1, 1.3)	.0001
CHD	2.7 (2.2, 3.4)	.0001

Murabito et al. *(18)* used the 38-year follow-up data for the original cohort in the Framingham Heart study and developed an intermittent claudication (IC) risk profile. An individual's probability of developing IC was assessed by the presence of risk factors identified on routine physical examination and laboratory analysis performed at a physician's office. The results showed that during 38 years of follow-up, 381 persons (215 men and 166 women) developed IC. In contrast to subjects free of IC, those with IC were more likely to be male, older, have a higher mean cholesterol, have a higher prevalence of stage 2 or greater hypertension, be diabetic, have coronary heart disease, and smoke a greater number of cigarets per day. In the logistic regression model, male sex, age, and smoking were associated with an approximately 1.5-fold increased risk for IC. Diabetes and stage 2 hypertension conferred a greater than two-fold increased risk, and coronary heart disease nearly tripled the risk. Table 2 shows the logistic regression estimates and odds ratio for significant risk factors in the IC profile in subjects aged 45 to 84 *(18)*.

In a review of studies describing target organ damage in African patients with essential hypertension, it was demonstrated that occlusive disease of the central and peripheral arteries is an important complication of essential hypertension. Although smoking, hypercholesterolaemia, and diabetes played an important role in the pathogenesis of central and peripheral vascular disease, this condition is more frequently seen in hypertensive than in normotensive patients *(19)*.

Some studies have demonstrated the relationship between hypertension and peripheral vascular disease by looking at higher risk populations, e.g., the elderly. Ness et al. *(20)*, in a retrospective analysis of older patients charts seen from January 1, 1998, through June 15, 1999, at an

academic, hospital-based geriatrics practice, have shown that hypertension was a significant independent risk factor for symptomatic peripheral arterial disease in the older men (odds ratio = 2.2) and women (odds ratio = 2.8). A total of 467 men (mean age 80 ± 8 years) and 1444 women (mean age 81 ± 8 years) were included in the study. In a larger study, Newman et al. *(21)* analyzed a subgroup of 1775 participants of the Systolic Hypertension in the Elderly Program (SHEP) to assess epidemiological correlates of peripheral vascular disease. The authors defined a patient as having peripheral arterial disease if the ankle–arm index was 0.9 in either leg. In this subgroup, the prevalence of peripheral vascular disease was 25% in white men, 38% in black men, 23% in white women, and 41% in black women. The authors found that even in the absence of risk factors such as smoking and diabetes, blacks had a higher prevalence of peripheral vascular disease than whites. Independent factors associated with the presence of peripheral vascular disease included age, black race, smoking, diabetes mellitus, history of myocardial infarction or angina, systolic hypertension, lower HDL cholesterol, and body mass index. However, the investigators also noted that although the prevalence of peripheral arterial disease was high in this study very few of the patients actually had symptoms of claudication. This would suggest that claudication is a very insensitive marker for peripheral vascular disease in this population.

As stated previously, peripheral vascular disease is not always symptomatic, but hypertension is still a significant risk factor for this type of disease. Hooi et al. *(22)* did a multiple logistic regression analysis of risk factors associated with the probability of asymptomatic and symptomatic peripheral arterial occlusive disease in a longitudinal study of 2327 subjects. The results showed that older age, smoking, hypertension, diabetes, hypercholesterolemia, and a sedentary lifestyle were the most important risk factors for asymptomatic peripheral arterial occlusive disease, whereas older age, smoking, hypertension, and diabetes were the most important predisposing factors for symptomatic peripheral arterial occlusive disease. Multiple logistic regression results showed hypertension was associated with the development of symptoms of IC (odds ratio = 1.5).

As is intuitive, hypertension is associated with established atherosclerotic disease. Multiple studies have shown that hypertension is present in these patients or in those known to be at high risk for atherosclerosis. Bull et al. *(2)* reviewed the cases of 232 patients receiving heart transplants at the University of Arizona at Tucson between 1979 and 1990. They found that accelerated coronary atherosclerosis occurred in 45 (19%) of the 232 patients, typically appearing within 2 years of trans-

plantation, whereas peripheral vascular disease appeared in 23 (10%) of the 232 patients usually within 3 years of transplantation. In those patients with peripheral vascular disease, 13 had occlusive disease, 9 had aneurysms, and 1 patient suffered a vertebral artery dissection. Accelerated coronary atherosclerosis afflicted 12 (52%) of the 23 patients affected by peripheral vascular disease ($p < 0.05$), and preceded the development of the peripheral vascular disease in all 12. The authors used regression analysis to ascertain risk factors predictive of the development of peripheral vascular disease after transplantation. The most powerful predictors for development of peripheral arterial disease were: (1) a pretransplant history of ischemic cardiomyopathy, and (2) posttransplant hypertension and hypertriglyceridemia ($p < 0.05$), with the presence of more than one risk factor increasing the probability of development of peripheral vascular disease. The authors suggest that aggressive medical management of hypertension and hyperlipidemia in this subpopulation may forestall or prevent the development of peripheral vascular disease after heart transplantation *(2)*.

Lee et al. *(7)* retrospectively reviewed the cases of 110 patients with peripheral vascular disease who underwent amputation between 1987 and 1990 at the Hahnemann University Hospital in Philadelphia, PA. The purpose of their review was to evaluate the prevalence of diabetes and other common predisposing factors in amputation of the lower extremities. The combination of diabetes and hypertension was present in 40 of these patients (36%). The investigators found when either diabetes or hypertension was present in a patient, hypertension not diabetes was the more common dominant underlying medical condition in patients with amputations (32 hypertensionalone patients vs 10 diabetes-alone patients). This study supports the concept that hypertension plays an important role in the development of peripheral vascular disease.

Swartbol et al. *(10)* studied 450 consecutive patients with peripheral vascular disease to evaluate risk factors associated with renal artery stenosis. Of 221 patients who had peripheral arterial disease and a renal artery lesion detected by angiography, 44 were normotensive and 177 were hypertensive. The authors concluded that hypertension secondary to renal artery stenosis was significantly correlated with peripheral vascular disease. They also noted an association with age over 70 years, smoking history, and an abnormal baseline ECG.

Not all epidemiological studies, however, confirm hypertension as a consistent risk factor for the development of peripheral vascular disease. Fowkes et al. *(4)* designed a retrospective study to assess risk factors in the general population that might be more powerful for the prediction of the development of peripheral arterial disease rather than coronary artery

disease. These investigators assessed arterial disease in the lower limbs using the World Health Organization (WHO) questionnaire designed to assess IC, the ankle-brachial pressure index, and a reactive hyperemia test. They evaluated 1592 men and women aged 55–74 years that were selected randomly in 1988 from ten general medical practices in Edinburgh, Scotland. Multiple regression of risk factors on measures of peripheral arterial disease showed associations with diabetes mellitus (but not impaired glucose tolerance), systolic blood pressure, and serum cholesterol; an inverse association with high-density lipoprotein cholesterol was identified; and only a univariate association with triglycerides was seen. The only consistent finding that emerged from multiple logistic regressions of risk factors on six separate indicators of cardiovascular disease was that smoking increased the risk of peripheral arterial disease more than heart disease. The authors concluded that diabetes and hypertension are not stronger risk factors for peripheral vascular disease than coronary disease.

Additional studies by Novo et al. *(8,9)* studied the prevalence of arterial hypertension and other risk factors in patients suffering from peripheral arterial disease in two different populations: (1) the Trabia study ($n = 835$) and (2) the Casteldaccia study ($n = 723$). In the Trabia study, the investigators observed a significantly ($p < 0.01$) greater prevalence of arterial hypertension (51.9 vs 9.8%), hypercholesterolemia (48.2 vs 21.6%), hypertriglyceridemia (53.7 vs 26.1%), smoking (64.3 vs 44.2%), and hyperglycemia (26 vs 7.9%) in patients with peripheral vascular disease than in age and sex matched controls. However, in the Casteldaccia study the most important risk factors were smoking (64.28%), hypercholesterolemia (42.86%), and hypertriglyceridemia (35.71%) in males, and obesity (60%), hypercholesterolemia (30%), and diabetes (20%) in females. In this cohort, total cholesterol levels and smoking were significantly higher in patients with peripheral arterial disease than in the general population. The authors did not find a significant association of arterial hypertension with peripheral vascular disease in the Casteldaccia study.

CONCLUSIONS

These studies generally support the concept that hypertension accelerates development of peripheral vascular disease in certain populations. The presence of other risk factors such as hypercholesterolemia, smoking, diabetes, and male gender appear to markedly accentuate the effects of hypertension on development of this process. Thus, aggressive reduction in arterial pressure and reduction or elimination of other risk factors is clearly indicated in high-risk patients.

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7 Physical Inactivity is a Risk Factor for Lower Extremity Arterial Disease and Coronary Heart Disease

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INTRODUCTION

Coronary arterial disease, carotid artery disease, and Lower Extremity Arterial Disease (LEAD) have many causes. Risk factors associated with these disease entities, which can be modified are: cigaret smoking, arterial hypertension, diabetes mellitus, various dislipidemias, i.e., hypertriglyceridemia in particular for LEAD, high low-density lipoprotein (LDL) cholesterol particularly for coronary artery disease, high lipoprotein(a) [Lp(a)], low high-density lipoprotein (HDL) cholesterol, obesity, homocysteinemia, elevated high sensitivity C-reactive protein (CRP), and physical inactivity *(1–3)*. Significant progress has been made to lower the trend for cigaret smoking in the United States and other industrialized countries through educational programs for the public, reviews by the press, legislative action at all three levels of government (local, state, and federal), as well as landmark decisions by the judiciary. The pioneering work of the American Heart Association in initiating smoking cessation programs was closely followed by the American Lung Association, the American Cancer Society, the World Health Organization *(4)*, and more recently by the European Union.

Not much progress has been made with regards to obesity and physical inactivity as risk factors for cardiovascular disease (CVD). Since 1970, obesity in the United States has become a public health problem and continues to grow. The concept that sedentary lifestyle may lead to an increase in prevalence of CVD, especially myocardial infarction (MI) and sudden cardiac death, has been recognized by cardiovascular physicians and all health care providers *(5)*.

Data relating to the level of regular exercise and risk for all CVDs have been derived from a variety of sources, including annual patient studies, observational studies, prospective studies, and retrospective studies *(6–9)*. The risk for CVD and LEAD in particular increases with sedentary lifestyle as opposed to a physically active lifestyle *(10–12)*. No study has been executed so far that provides biological evidence of the cause or causes, biochemical link or links, between exercise status and cardiovascular morbidity and mortality or the specific pathophysiology of physical inactivity. The basic biological progression of atherosclerosis at the molecular level continues to be studied and is gradually being established *(13)*. The existing observational studies can only provide inferences and associations between the predictive value for the development of coronary artery disease, LEAD, and physical inactivity *(12)*.

For the past 50 years, more than 50 published studies have reported the protective effect of regular exercise on the development and deceleration of progression of CVD. Physically active cardiovascular patients

can derive significant benefits in improving the clinical manifestations of coronary heart disease and of LEAD *(12,14,15)*. Regular physical exercise is important in secondary and tertiary prevention of coronary heart disease end points, like a second MI or premature and sudden cardiac death *(16,17)*. Prospective *(18)* and retrospective *(19)* studies have examined the significance of physical activity on the job and during leisure time of many groups of men and a smaller number of women. Physical activity was classified based on work category, lifestyle questionnaires, interviews, and other epidemiological methods.

The presence of coronary heart disease was established by examining death certificates, hospital records, emergency room data, physicians' records, and medical evaluations or interviews with spouses or close associates. Activity levels ranged from high metabolic expenditures to minimal physical energy expenditure. There has been no uniform methodological approach to study physical inactivity as a risk factor for CVD. Most studies of sedentary lifestyle and CVD were conducted during the latter half of the previous century. There has been great variability of methodologies and data collection techniques. Nonetheless, these studies lead to certain specific suggestions. The scope of this chapter is not to present and analyze each study on physical inactivity and cardiovascular health. A comprehensive review of the scientific reports on the primary preventing effect of physical activity leads to the following conclusions:

1. Physically active people develop less coronary heart disease than their inactive counterparts *(20–22)*, with all other variables remaining equal.
2. Whenever physically active people do develop coronary heart disease, they do so at a later age with less severe clinical manifestations *(8,16,17)*.
3. Most studies show a consistent finding: being physically active does not increase an individual's overall risk for CVD.

The statistical significance of many published reports varies with some studies demonstrating a highly significant beneficial effect of exercise *(16)*, and other studies showing a favorable trend, but a statistically insignificant value. A few studies showed no difference in coronary heart disease prevalence among physically active compared to physically inactive individuals.

It is a fallacy to believe that individuals with risk factors for cardiovascular events like smoking, hypertension, or hyperlipidemia can neutralize or minimize their adverse effects by exercise. In the Seven Countries Study *(23,24)*, Finland had the highest overall prevalence of coronary heart disease. The Finnish population consumes large amounts of animal fat. Finnish lumberjacks are an example of physically active people in whom the risk for coronary heart disease remains high *(25)*. The approach to prevent coronary heart disease should be multifaceted

and continuous. Simply put, one should modify and optimize all the modifiable known risk factors for CVD and exercise regularly.

There are several studies in the literature that show a reduction of the risk for cardiovascular events in the more physically active individuals compared to the least physically active people; between those who exercise moderately and regularly and those who do almost nothing physically *(8,9,16,26)*. However, there is no gradient for physical exercise. There is no threshold of exercise over which there is greater benefit. In fact, there is a very small difference in risk when moderately active people are compared with very athletic individuals. On the other hand, published data show significant benefit in preventing coronary heart disease events with regular physical activities at relatively low intensity *(6,8,9,16,26)*. Examples of moderate activity exercise include brisk walking on a horizontal level, walking upstairs, lifting and carrying light objects, light and heavy gardening, and participating in active games and sports *(27)*. The greatest cardiovascular and overall physical, biological, and psychological benefit is derived from large muscle aerobic activity, which can substantially increase cardiac output with small increases in systolic blood pressure *(28)*. This large muscle aerobic activity, commercially named *cardiovascular* is in contrast to heavy lifting or isometric exercise. Heavy lifting significantly increases systolic blood pressure with a relatively small increase in cardiac output. The benefit from heavy isometric exercise is small and prospectively may have a negative effect as a result of repeated elevations of the systemic arterial pressure.

A frequently asked question is about physical activity on the job. "At work I walk all over the place and carry heavy objects at times." It is true that the initial observations, which established an association between physical activity and CVD, were based on job description, e.g., the London, England bus drivers as compared to the conductors who walk on both decks of the two-decker buses issuing tickets *(29,30)*. Similar studies have been done with truck drivers and cab drivers. It is methodologically appropriate and objective to classify people as physically inactive or active, according to the job or job description instead of questionnaires or interviews describing past physical activities. The prevalence of coronary artery disease is lower among physically active individuals by the mere job or profession, which calls for walking on a flat level for hours or climbing stairs or walking up and down hills, as many farmers and rural mailmen do. Studies performed by analyzing on-the-job activities show a protective gradient only for high level of workload. Physically active people by job necessity appear to have a decreased coronary mortality, provided that all the other risk factors remain similar or the same *(31)*; lifting and carrying objects, walking for

a long distance, working farms, heavy labor workers, cyclist couriers, and others. These observations of on-the-job activity found the same benefit for high-intensity physical activity performed in short bursts and lower energy expenditure job activities performed during the work day for longer periods of time, e.g., railroad workers, postal workers, and others with a physically comparable workload. However, there may be a selection bias in favor of the healthier individuals who can perform physically demanding activities on the job compared to someone who is less healthy and prefers or is assigned to a desk job.

DOES ON-THE-JOB PHYSICAL ACTIVITY PROVIDE PROTECTION?

Low intensity activity on a regular basis has been reported to be inversely associated with ischemic electrocardiographic (ECG) changes. Specifically, the prevalence of ischemic changes on the resting ECG is inversely related to regular on-the-job physical activity. Job-related physical activity included walking to work among 8948 civil service workers in London, England *(32)*. Civil servants who walked for 20 minutes or more to work on a regular basis had one-third fewer abnormalities on their resting ECG, compared to their colleagues who drove to work or took the train or bus. This inverse relationship between ECG abnormalities and regular on job physical activity could not be accounted for by differences in age, civil service grade, smoking, serum cholesterol, or glucose tolerance. The employees who walked regularly to work were also less overweight.

In England, the relation of leisure-time activity to cardiovascular mortality has been studied among middle-age employees of the civil service using a 2-day activity record procedure *(33)*. A self-kept 48-hour record of physical activities was completed every Monday for the preceding weekend. There was a significant inverse relationship between activities requiring a peak energy expenditure of 7.5 k/cal per minute for 30 minutes or longer each day, and mortality from coronary heart disease. This protective effect of vigorous activity was not related to plasma cholesterol, blood pressure, cigaret smoking, or obesity. The benefit of vigorous activity was apparent in all age groups of participants, ranging from 40 to 69 years *(33)*. Another study evaluated the relationship between past physical activity habits and cardiovascular health among Harvard University alumni *(34)*. Five hundred seventy-two first MI cases were analyzed among 16,936 men between 1962 and 1972. The charts of 1413 deceased alumni between 1962 and 1978 were also examined. These analyses showed that regular post-graduation exercise of those

who did not participate in sports as college students predicted low risk for coronary heart disease. Sedentary alumni who were former athletes were at higher risk for cardiovascular events. Sedentary students who became physically active later in life gained cardiovascular protection and acquired a low risk for coronary heart disease and its complications. The results of this study are similar to several reports of job-related activity and risk for coronary heart disease; more physically active jobs early in someone's career followed by years of sedentary work resulted in higher coronary risk than the physically active individuals who continued to be physically active throughout their careers *(34)*.

Cardiovascular mortality and physical activity was studied in a cohort of 636 healthy Finnish men age 45 to 64 years followed for 20 years *(35)*. Approximately 39% of this cohort was classified as physically active at the onset of the study in 1964. By 1984, there were 287 deaths, 106 as a result of coronary heart disease. In the time period covering the first two-thirds of the follow-up, men with high physical activity record had lower risk of death than did men with practically no physical activity. During the last third of the study, the two survival curves gradually converged. Of the men who died, those engaged in high physical activity lived 2.1 years longer ($p = 0.002$) than those with no physical activity after adjusting for age, smoking, blood pressure, serum cholesterol, and body mass index (BMI). This significant difference was primarily a result of fewer cardiovascular deaths among the highly active group.

PHYSICAL INACTIVITY IS AN INDEPENDENT RISK FACTOR FOR THE DEVELOPMENT OF CARDIOVASCULAR DISEASE, CORONARY DISEASE, AND LOWER EXTREMITY ARTERIAL DISEASE

Regular physical activity is an independent factor in delaying sudden (premature) cardiac death among middle-age men. The relation of self-selected leisure-time physical activity to first major coronary event and overall mortality was studied in 12,138 middle age men who participated in the Multiple Risk Factor Intervention Trial (MRFIT) *(36)*. The basic methodology of the MRFIT study was a questionnaire requesting the leisure-time physical activity for the preceding year quantified in minutes per day. The study participants were categorized as low, moderate, or high, according to the leisure-time physical activity performed. The combined rate of fatal coronary heart disease, sudden cardiac death, and all-cause mortality in the moderate category was 64%, whereas the same rate in the low leisure-time physical activity group was 73% ($p < 0.01$). Interestingly, the mortality rates of subjects with high leisure-time physi-

cal activity were similar to that of subjects with moderate leisure-time physical activity. The combined fatal and nonfatal major cardiac events were 20% lower in the high leisure-time physical activity compared to those subjects in the low leisure-time category ($p < 0.05$). Statistical adjustments for other baseline risk factors were made *(36)*. Leisure-time physical activity has a modest inverse relationship to coronary heart disease and overall mortality in middle-age men *(36)*. In a study in Finland *(37)*, physical activity at work and during leisure-time was studied utilizing a questionnaire methodology. The study population consisted of 3978 men, age 30 to 59 years and 3688 women, age 35 to 59 years. The follow-up for each study participant was 7 years. Low physical activity at work was associated with an increased risk for MI, stroke, and overall mortality in both men and women even after controlling for age, serum cholesterol, diastolic blood pressure, smoking, and body weight. In this prospective study, the relative risk for MI was 1.5 (95% confidence interval 1.2–2) for men and 2.4 (95% confidence interval 1.5–3.7) for women. Men and women at highest risk for cardiovascular and overall mortality reported no vigorous exercise during work or any vigorous leisure-time exercise. Those at lowest risk reported vigorous exercise at work and off work (*see* Table 1).

WHAT IS THE RELATION OF PHYSICAL FITNESS TO FUTURE CARDIOVASCULAR EVENTS OR TOTAL MORTALITY?

This question was addressed by the Lipid Research Clinics' prevalence survey in the study of 4276 individuals age 30 to 69 years who were followed for an average of 8.5 years *(39,40)*. Baseline assessment of individuals in this study included an analysis of the conventional coronary risk factors and treadmill exercise testing. The performance during maximal exercise was used as a measure of physical fitness. Men who had clinical evidence of CVD at baseline ($n = 649$) had their records analyzed separately. Among the remaining 3106 men, 45 deaths occurred from cardiovascular causes. After adjustment for age and known cardiovascular risk factors, a lower level of physical fitness was found to be associated with overall CVD, a higher coronary mortality, and death rate from all causes. The relative risk for cardiovascular mortality for the least-fit apparently healthy men was compared with the most fit healthy men according to their exercise capacity. The relative risk for overall cardiovascular mortality was 3.6 (95% confidence interval 1.6–5.6) ($p = 0.0004$). For death resulting from coronary heart disease the relative risk was 2.8 (95% confidence interval 1.3-6.1) ($p = 0.007$).

Table 1
Risk Ratios of Major End Points (and 95% Confidence Intervals) by Tertile of Total Leisure-Time Physical Activities in Men in MRFIT*

Tertile of leisure-time physical activities			
End points†	*1*	*2*	*3*
		Age-Adjusted Risk Ratios	
CHD death	1.00	0.63‡ (0.43–0.86)	0.64‡ (0.47–0.88)
Sudden death	1.00	0.63‡ (0.42–0.93)	0.65§ (0.44–0.96)
Fatal/nonfatal MI	1.00	0.88 (0.75–1.04)	0.81‡ (0.68–0.95)
All-cause deaths	1.00	0.71‡ (0.57–0.88)	0.83‖ (0.67–1.01)
		Risk Ratios Adjusted by Proportional Hazards Regression¶	
CHD death	1.00	0.64‡ (0.47–0.88)	0.67§ (0.49–0.92)
Sudden death	1.00	0.64‡ (0.43–0.96)	0.67§ (0.45–1.00)
Fatal/nonfatal MI	1.00	0.90 (0.76–1.06)	0.83§ (0.70–0.99)
All-cause deaths	1.00	0.73‡ (0.59–0.91)	0.87 (0.70–1.07)

*MRFIT indicates Multiple Risk Factor Intervention Trial.
†CHD, coronary heart disease; MI, myocardial infarction.
‡$p < .01$.
§$p < .05$.
‖$p < .07$.
¶Regression of end points of age (years), levels of diastolic blood pressure (mmHg), total cholesterol (mmol/L [mg/dL]), number of cigarets per day, and treatment group (1. special intervention; and 2. usual care).
From Leon et al. *(38)*.

Highly significant associations were also seen for vasculopathic men. A low level of physical fitness in vasculopathic men was associated with a higher risk of death, particularly from cardiovascular events *(39,40)*. This higher risk for death in the sedentary patients is a risk factor, separate and independent of the known risk factors for coronary artery disease and LEAD. It should be emphasized that physical fitness in this study was at least a regular walk on the treadmill; there was a close relationship of physical characteristics and life habits to treadmill exercise capacity *(41)*.

In a separate study, the Cooper Clinic (Dallas, TX) *(42)* investigators studied the relationship of physical fitness, as measured by maximal treadmill performance, to all-cause and cause-specific mortality in 10,224 men and 3120 women who completed comprehensive medical examinations. The average follow-up was slightly more than 8 years. There were a total of 110,482 person-years of observation. During this

time, 240 men and 43 women died. Age-adjusted all-cause mortality rates declined across physical fitness quintiles from 64.0 per 10,000 person-years in the least-fit men to 18.6 per 10,000 person-years in the most-fit men. Corresponding values for women were 39.5 per 10,000 person-years to 8.5 per 10,000 person-years. These statistical inferences were validated after adjustment for age, smoking, cholesterol levels, systolic blood pressure, fasting blood glucose level, and family history for coronary artery disease *(42)*.

HIGHER LEVELS OF PHYSICAL FITNESS DELAY ALL-CAUSE MORTALITY AND CARDIOVASCULAR MORTALITY IN PARTICULAR

The relation of maximal oxygen uptake (measured during maximal exercise on a stationary bicycle) to cardiovascular mortality was studied in 2014 Norwegian men 40 to 59 years of age at the beginning of the study, with a follow-up period of 16 years *(9)*. The relative risk of death from any cause in men in the fourth (highest) fitness quartile was compared with that in men in the first (lowest) quartile. The relative risk for total mortality in the highest fitness quartile was 0.54 after adjustment for age, smoking, serum lipid levels, blood pressure, resting heart rate, vital capacity, BMI, and glucose tolerance. Total mortality was similar among subjects in the three lowest quartiles. The adjusted relative risk of death from cardiovascular events in the highest quartile compared to the lowest was 0.41 ($p = 0.013$). The relative risks for the third and second quartiles (compared with the lowest) were 0.45 ($p = 0.026$) and 0.59 ($p = 0.15$), respectively *(9)*.

The least fit and the least active have the highest rate for cardiovascular morbidity and mortality. Moderate increases in physical fitness and activity such as a daily brisk walk for half an hour, are associated with a significant reduction in risk. There is a small dose–response relation at higher levels of activity and fitness. However, the magnitude of the benefit declines rapidly as fitness levels increase *(43)*.

DOES MODERATE DAILY EXERCISE HAVE AN IMPACT ON SECONDARY PREVENTION OF CORONARY HEART DISEASE?

In addition to studies that have compared morbidity and mortality rates in physically active and inactive cardiac patients, controlled studies show that patients with a history of MI randomly assigned to exercise and control groups demonstrate a trend of lower mortality in the more

physically active post-MI patients *(44)*. Benefit apparently derived from an increase in caloric expenditure of no more that 300–400 kcal per session three to four times per week at a moderate intensity (60 to 75% of maximal exertion or functional aerobic capacity) *(44)*.

Randomized clinical trials of physical rehabilitation after MI tend to demonstrate lower mortality in treated (rehabilitated) patients. Meta-analyses of studies performed in the early 1980s showed a lower mortality in those patients who participated in cardiac rehabilitation as compared to those who did not *(45,46)*.

PROPOSED BIOLOGICAL MECHANISMS BY WHICH EXERCISE MAY CONTRIBUTE TO THE PRIMARY OR SECONDARY PREVENTION OF CORONARY HEART DISEASE

Exercise can:

- Maintain or increase myocardial oxygen supply *(47,48)*
- Delay progression of coronary atherosclerosis *(49–51)*
  - Improve lipoprotein profile (increase HDL/LDL-cholesterol ratio) *(52)*
  - Improve carbohydrate metabolism (increase insulin sensitivity) (probable)
  - Decrease platelet aggregation and increase fibrinolysis *(53)*
  - Decrease adiposity *(51,54,55)*
- Increase coronary collateral vessel formation
- Increase epicardial artery diameter *(51,54,55)*
- Increase coronary blood flow (myocardial perfusion) or distribution
- Decrease myocardial work and oxygen demand *(56–58)*
- Decrease heart rate at rest and submaximal exercise *(59)*
- Decrease systolic and mean systemic arterial pressure following regular submaximal exercise *(60,61)*
- Increase cardiac output during submaximal exercise *(62,63)*
- Decrease circulating plasma catecholamine levels (decrease sympathetic tone) at rest and during submaximal exercise *(64)*
- Increase stroke volume at rest and during submaximal and maximal exercise *(65,66)*
- Increase ejection fraction at rest and during exercise *(67)*
- Increase intrinsic myocardial contractility *(68)*
- Increase left ventricular myocardial function by decreasing afterload
- Increase electrical stability of the myocardium *(38,51,55,69)*
- Decrease regional ischemia following a regular program of submaximal exercise *(51,55,69)*
- Increase ventricular fibrillation threshold *(38,51,55,69)*

Exercise also:

- Improves carbohydrate metabolism *(70)*
- Improves fat metabolism *(71)*
- Improves muscle endurance *(72)*
- Enhances endorphin release *(70)*
- Improves self-esteem *(72)*

We should make every effort in prescribing the appropriate diet and motivate every patient to become physically active. In a broad sense, regular physical activity is of paramount importance for every patient. It has short-term benefits and long-term dividends, both biologically and psychologically. The mission of every health care provider is to prolong life and improve the quality of life.

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8 Pathogenesis of Atherosclerotic Vascular Disease

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INTRODUCTION

Epidemiological studies have identified risk factors for coronary heart disease (CHD) and its underlying pathology: atherosclerosis. Genetic and environmental factors interact to shape an individual's age-related risk of atherosclerosis *(1,2)*. In the Framingham Heart Study, substantial proportion of the variability in carotid intima-media thickness (IMT) is explained by genetic factors *(3)*. The role of gene polymorphism has been reviewed in ref. *4*.

Natural History

Intrauterine undernutrition leads to changes of fetal growth and metabolism, and programs later development of CHD risk factors: hypertension, impaired glucose, and cholesterol metabolism are affected by intrauterine growth *(5)*; even hypercholesterolemia during pregnancy determines long-term susceptibility of children to atherosclerosis *(6)*. Body fat is a good indicator for future coronary artery disease (CAD) in 9- to 10-year-old boys and girls *(7)*.

In 3000 autopsies of persons aged 15–34 whose deaths were caused by either accident, homicide, or suicide, the extent of fatty streaks and raised lesions in the right coronary artery (RCA) and abdominal aorta positively correlated with high-density lipoprotein cholesterol (HDL-C), hypertension, impaired glucose tolerance (IGT), and obesity. Because they also were associated negatively with HDL-C and positively with smoking for atherosclerosis of the abdominal aorta, controlling risk factors should start in adolescence *(8)*.

It was suggested that for lesions starting in young people the role of endothelial dysfunction, decreased nitric oxide (NO), and increased adhesive molecules predominate, whereas for lesions starting in older people the role of metabolic changes of the endothelium and alteration in the extracellular matrix (ECM) predominate *(9)*.

Risk Factors

In a community-based study in Taiwan, hypertension was found to strongly influence carotid atherosclerosis *(10)*. In addition, the fasting insulin and insulin/glucose ratio are independent risk factors for the accelerated development of CAD *(11,12)*. However, it should be noted that the administration of exogenous insulin or the sulphonylureas, which are insulin secretagogues, did not increase cardiovascular disease (CVD) especially exogenous insulin. Thus, insulin may not be the primary culprit implicated in the increased mortality from CHD *(13)*.

Microalbuminuria in healthy subjects is associated with atherosclerotic risk factors such as increased systolic and diastolic blood pressure (BP) decreased Apo A1 and HDL-C levels *(12)*. Minor derangements of renal function are associated with an increase in CVD risk factors and promote progression of atherosclerosis (reviewed in ref. *14*). However, in a separate study in contrast to BP, body mass index (BMI), and triglycerides (TGs), there was no relation between urinary albumin excretion and flow-mediated vasodilation in apparently healthy subjects. This suggests that the presence of atherogenic risk factors precedes the development of endothelial dysfunction in microalbuminuric but otherwise healthy subjects *(15)*.

A summary of risk factors associated with the development of CHD from atherosclerosis is summarized in Table 1. Those with the strongest relationship with CHD are low-density lipoprotein-cholesterol (LDL-C), total cholesterol (TC), and the total cholesterol/ high-density lipoprotein cholesterol (TC/HDL-C) ratio *(1)*.

RHEOLOGY

Patients with hypertension have increased common carotid artery IMT and lower mean shear stress with a negative correlation between shear stress and IMT in hypertensives. In the general population, decreased shear stress may contribute to atherosclerosis *(16)*. It was suggested that the ubiquitous atherosclerotic changes in fetal and pediatric subjects when serum cholesterol levels are normal demonstrate the importance of repetitive hemodynamic stresses, rather than hypercholesterolemia, in atherosclerosis (reviewed in ref. *17*).

Local blood flow conditions modulate the production of vasoactive substances, NO and endothelin (ET)-1, by human umbilical vein endothelial cells (HUVEC) *(18)* and shear stress upregulates monocyte chemotactic factor (MCP)-1, interleukin (IL)-8, ET-1, and connective tissue growth factor (CTGF) *(19)* and induces collagen XII expression, which may stabilize the vascular structure in HUVEC *(20)*.

Laminar flow exposure leads to activation of antioxidant genes in endothelial cell (EC) *(21)*and promotes EC survival and quiescence and the secretion of substances that promote vasodilation and anticoagulation *(22)*. Flow induces an increase in oxidative stress in EC, which is dependent on the pulsatile nature of flow *(23)*.

In rabbits, exposure of the arterial wall to low wall shear stress may activate ECs increasing intercellular permeability, which in turn increases the vulnerability of these regions to atherosclerosis *(24)*.

Table 1
Some Factors Associated With Development of Atherosclerosis

LDL-cholersterol*
Total cholesterol*
Total cholesterol/HDL*
Smoking*
Increased triglycerides
Hypertension
Obesity
Diabetes
Left ventricular hypertrophy
Hyperinsulinemia
Increased fibrinogen
Herpes infection
Lp(a)
Homocysteine
Chlamydia pneumoniae
Hypothyroidism
CRP
PAF-AH
LPL
Phospholipase aII
Low wall shear stress

*Indicates the strongest correlation with atherosclerosis development.

Also, the role of L-selectin in various situations of leukocytes recruitment could be affected, among other reasons, by wall shear *(25)*. L-selectin is a leukocyte adhesion molecule that is rapidly shed after leukocyte activation so that it appears to be decreased in CAD *(26)*.

Pulsatile oscillatory shear stress induces a procoagulant phenotype of human EC by increasing tissue factor mRNA and protein expression and activity *(27)*.

BLOOD CELLS, VASCULAR INJURY, AND THROMBOSIS

Vascular injury and thrombosis are common initial events in atherosclerosis (reviewed in ref. *28)*. Intrinsic pathway enhances the thrombogenicity of atherosclerotic lesions after the removal of the endothelial layer and exposure of smooth muscle cells (SMCs) and macrophages to blood flow *(29)*. Studies on plasma levels of tissue factor pathway inhibitor (TFPI) in patients with various diseases suggest that TFPI (a Kunitz-type protease inhibitor that inhibits the initial reactions of blood

coagulation) may be a marker of EC dysfunction *(30)*. Patients suffering from proven peripheral artery disease (PAD) have higher plasma levels of tissue factor (TF) and vascular endothelial growth factor (VEGF) compared with controls, with a significant correlation between the two. This suggests a link between the hypercoagulable state in PAD and the process of angiogenesis *(31)*.

Plasminogen activator inhibitor (PAI)-1 plasma levels depend on gene regulation, insulin resistance, diabetes mellitus (DM), hypertriglyceridemia, and activated renin angiotensin system through a receptor-dependent mechanism (reviewed in ref. *32*). In Apo E –/– PAI-1 –/– mice, loss of PAI-1 promoted the growth of advanced atherosclerotic plaques as a result of enhanced ECM deposition. Also, plaques exhibited collagen fiber disorganization and degradation; therefore, though PAI-1 may promote plaque growth caused by its antifibrinolytic properties, PAI-1 has a protective role by limiting plaque growth and preventing abnormal matrix remodeling *(33)*.

Annexin (AN) II is a coreceptor on ECs for plasminogen and tissue plasminogen activator (tPA). Recombinant AN II enhanced plasmin generation on HUVECs in vitro, reduced thrombus formation in a rat carotid artery thrombus model *(34)*, and increased urokinase plasminogen activator (uPA) expression in atherosclerotic arteries contribute to intimal growth and constrictive remodeling leading to lumen loss (although increased uPA expression in EC decreases intravascular thrombosis) *(35)*.

Tissue transglutaminase (tTG), a family of enzymes catalyzing the formation of stable covalent crosslinks between proteins, are upregulated by thrombin in HUVEC and have a role in the stabilization of atherosclerotic plaques *(36)*.

In a cross-sectional study, the presence and extent of atherosclerosis, as measured by ultrasound, correlated positively with plasma fibrinogen *(37)*. Plasma fibrin D-dimer levels are strongly and independently associated with the presence of CAD in patients with stable angina *(38)*. However, there is doubt about its etiologic contribution because elevated plasma fibrinogen concentrations in Apo E*3-Leiden transgenic mice do not affect the progression of diet-induced atherosclerotic lesions *(39)*. γ A/γ fibrinogen is a fibrinogen isoform that constitute 15% of total plasma fibrinogen. It contains an additional binding site for factor XIII and active thrombin, and forms fibrin clots that are resistant to fibrinolysis in vitro. This isoform is not associated with age or gender, and was higher in CAD patients than controls independent of total fibrinogen levels (which rises with age and are higher in women) *(40)*.

Mast cell (MC), the high basal cardiac and serum histamine in Apo E k/o mice, along with the high number of cardiac mast cells, suggest possible ongoing cardiac mast cell activation that may participate in atherosclerosis *(41)*.

Activated MCs may participate in the weakening and rupture of atherosclerotic plaques by causing the loss of matrix-synthesizing SMC by inhibiting the proliferation of SMCs in vitro and reducing their ability to produce collagen. Chymase, a neutral serine protease secreted by activated mast cells, can also inhibit SMC-mediated collagen synthesis, and moreover cause degradation of the collagen matrix by activating latent interstitial collagenase (matrix metalloproteinase [MMP]-1). Furthermore, chymase can induce SMC apoptosis (reviewed in ref. *42*).

MC chymases also degrade Apo E and apo angiotensin II (AII) and inhibit the apoprotein-mediated removal of macrophage cholesterol in Apo A1 knockout (KO) mice *(43)*. Chymase and angiotensin converting enzyme (ACE) regulate AII production in distinct tissue compartments *(44)*.

Platelets produce NO, which is increased by the dietary supplementation of L-arginine to hypercholesterolemic rabbits. This effect is associated with reduced platelet aggregation *(45)*. Platelet activation is increased in patients with CAD *(46)*. Hypercholesterolemia primes human platelets for recruitment to lesion-prone sites via endothelial von Willebrand factor (vWF), platelet GP1bα, and platelet p-selectin before lesions are detectable *(47)*.

Platelet factor 4 (PF4), a cationic protein released by activated platelets, inhibits the catabolism of LDL. Retention of LDL complexes on cell surfaces may facilitate pro-atherogenic modification *(48)*, such as promoting oxidized oxLDL formation, binding to oxLDL directly, increasing oxLDL binding to vascular cells and macrophages, and increasing the amount of oxLDL esterified by macrophages *(49)*.

Activated platelets stimulate MCP-1 and intercellular adhesion molecule (ICAM)-1 of HUVECs *(50)*. Circulating activated platelets bind to leukocytes, preferentially monocytes, to form platelet-monocyte/leukocyte aggregates that interact with atherosclerotic lesions leading to the delivery of the platelet-derived chemokines and platelet factor 4 (CXCL4) to the monocyte surface and endothelium of atherosclerotic arteries *(51)*.

Receptors for extracellular nucleotides, the P2 receptors, have been recognized as fundamental modulators of leukocytes, platelets, SMCs and EC, P2 receptors mediate chemotaxis, cytokine secretion, NO generation, platelet aggregation, and cell proliferation in response to accumulation of nucleotides into the extracellular milieu. Clinical trials have

shown the benefit of antagonists of the adenosine diphosphate (ADP) platelet receptor(s) in the prevention of vascular accidents in patients with atherosclerosis (reviewed in ref. *52*).

Activated macrophages stimulate angiogenesis that can further recruit inflammatory cells and more angiogenesis. Plaque angiogenesis promotes the growth of atheromas and the angiogenesis inhibitor, angiostatin, reduces plaque angiogenesis and inhibits atherosclerosis *(53)*.

THE ENDOTHELIUM

Endothelial dysfunction is an independent predictor of cardiovascular events (reviewed in ref. *54*); different risk factors cause endothelial dysfunction probably through increased oxidative stress and/or inflammation (reviewed in ref. *55*).

The endothelium has a strong role in atherosclerosis. The specific EC markers, soluble E-selectin, vWF, and soluble thrombomodulin were examined; patients with symptomatic PAD have increased vWF (as a marker of generalized atherosclerosis) and soluble thrombomodulin (as a marker of disease extent), but not soluble E-selectin *(56)*.

NO is a vasodilator and inhibits vascular SMC (VSMC) proliferation, leukocyte adherence to endothelium, and platelet adhesion and aggregation *(57)*.

There are three different forms of NO synthase (NOS):

1. Endothelial NOS (eNOS) is responsible for producing NO in the vascular wall (reviewed in ref. *58*). In rat aortic EC, acute exposure to both estradiol and insulin-like growth factor (IGF)-1 was associated with an increase in eNOS activity *(59)*. The presence of abnormal ECM in the vessel wall could be responsible for decreased NO synthesis *(60)*, however, chronic over expression of eNOS accelerates atherosclerosis under hypercholesterolemia. eNOS dysfunction, demonstrated by lower NO production relative to eNOS expression and enhanced superoxide production in the endothelium, appears to play important roles in the progression of atherosclerosis in Apo E KO/eNOS transgenic mice *(61)*.
2. Inducible NOS (iNOS) expression and the resultant increased NO production are associated with atherosclerotic lesions *(62)*. iNOS activity and gene transcription in VSMC was increased following exposure to IGF-I which was reduced by AII pretreatment *(63)*.
3. Neuronal NOS (nNOS) plays important roles in suppressing arteriosclerotic vascular lesions *(64)*.

In patients with atheromas levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS, are increased and correlated with endothelial dysfunction (reviewed in ref. *57*). Dimethylarginines

are the result of the degradation of methylated proteins. ADMA is metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH); DDAH activity and/or expression may therefore contribute to the pathogenesis of endothelial dysfunction in various diseases (reviewed in ref. *65*).

L-arginine supplementation does not affect lesion formation in western type diet-fed Apo E KO mice, but it negates the protective effect of iNOS gene deficiency in Apo E/iNOS double KO mice suggesting that L-arginine supplementation may paradoxically contribute to, rather than reduce, lesion formation by mechanisms that involve lipid oxidation, peroxynitrite formation, and NOS uncoupling *(66)*.

Serum levels of soluble adhesion molecules in patients with stable angina are comparable to those of healthy persons. Unstable angina is characterized by significant elevation of sP-selectin and soluble vascular cell adhesion molecule (sVCAM)-1 serum levels *(67)*.

In ECs, oxLDL suppressed basic fibroblast growth factor (bFGF) expression and DNA synthesis, so atherosclerosis was associated with impaired bFGF-dependent EC growth *(68)*. VEGF serum levels were increased in CAD and PAD compared with controls. Flt-1 is VEGF receptor, soluble Flt-1 was lower in patients with PAD, but not CAD, compared with controls *(69)*. VEGF promotes neovascular growth as is oxidative stress: in human and rat VSMC, reactive oxygen species (ROS) enhance neovascularization and VEGF protein and mRNA expression *(70)*. VEGF, in cholesterol-fed mice doubly deficient in Apo E/Apo B100, increased macrophage in bone marrow and blood, and increased plaque area and plaque macrophage and EC content. The same results hold true for rabbits *(71)*.

EC synthesize and secrete proteoglycans *(72)*. Accumulation of proteoglycans in the intimal atherosclerotic lesions may predispose to further lipid accumulation, calcification, and thrombosis. One of the proteoglycans, heparan sulfate, interacts with antithrombin III giving ECs a nonthrombogenic surface. A threshold concentration for LDL may exist above, which there is an exponential irreversible increase in EC permeability that was associated with a decrease in basement membrane-associated heparan sulfate proteoglycan (HSPG) content. This increased permeability could be induced by antiserum against the core protein of HSPG *(73)*.

oxLDL, oxidative stress, and AII can induce EC apoptosis that can compromise vasoregulation, increased SMC proliferation, SMC migration, and blood coagulation (reviewed in ref. *74*). Progressive telomere shortening in vivo has been observed in regions susceptible to athero-

sclerosis, vascular EC with senescence-associated phenotypes are present in human atherosclerotic lesions and EC senescence induced by telomere shortening showed increased ICAM-1 expression and decreased eNOS activity, contributing to atherogenesis *(75)*. Ageing in the liver is associated with pseudocapilarization of the sinusoidal endothelium that results in impaired clearance of chylomicron remnants, postprandial hypertriglyceridemia, and atherosclerosis (there is loss of pores which normally allows passage of chylomicron remnants for uptake and metabolism by hepatocytes) (reviewed in ref. *76*).

THE ROLE OF INSULIN RESISTANCE AND HYPERURICEMIA

Insulin stimulates Ca-ATPase, Na-K-ATPase, and PI3K and decreases calcium influx into VSMC. Therefore, insulin resistance causes increased intracellular ionized calcium and reduced intracellular Mg ion concentration that has been associated with vasospasm, increased vascular reactivity, increased intracellular calcium concentration, the formation of pro-inflammatory agents, oxygen radicals, platelet aggregation, decreased cardiac bioenergetics, cardiac failure, lipoprotein oxidation, gender-related modulation of NO, increased vWF and oxygen free radical activity reflecting endothelial dysfunction and oxidative stress, and changes in membrane fatty acid saturation *(11,77)*.

However, postprandial hyperinsulinemia is independently associated with CAD irrespective of fasting glucose, postprandial glucose, and fasting insulin levels in nondiabetic women with clustering of factors of the metabolic syndrome *(78)*.

Insulin stimulates tumor necrosis factor (TNF)-α production in macrophages *(79)*; TNF-α could play a role in the development of insulin resistance in humans, both in muscle and in vascular tissue *(80)*.

The adipocyte-derived hormone, adiponectin, accumulates in the injured artery from the plasma and suppresses endothelial inflammatory response and VSMC proliferation, as well as macrophage-to-foam cell transformation in vitro. In Apo E KO mice, adiponectin treatment led to inhibition of lesion formation with smaller lipid droplets in the lesions, and suppressed mRNA of VCAM-1 and class A scavenger receptor (SR). Adiponectin migrates to foam cells in the fatty streak lesions *(81)*.

Hyperuricemia, related to decreased renal blood flow, accompanies nephrosclerosis, predates proteinuria, and follow left ventricular hypertrophy. In the UKPDS, macrovascular and microvascular complications were reduced by BP and blood glucose control *(82)*.

THE ROLE OF DIETARY CONSTITUENTS

Polyunsaturated Fatty Acids

In a cross-sectional study, a higher intake of linoleic or linolenic acid was inversely related to the prevalence odds ratio of CAD in humans *(83)* Polyunsaturated fatty acids (PUFA) have anti-atherosclerotic effects that appear to be caused by several mechanisms:

1) Modification of the arachidonic acid (AA) cascade *(84)*.
2) Reduction of monocyte production of platelet activating factor (PAF) *(85)*.
3) Inhibition of coagulation *(86)*.
4) Reduction in synthesis and action of peptide mediators of cell proliferation including IL-1, TNF *(87)* and platelet-derived growth factor (PDGF) *(88)*.
5) Increased formation and/or release of endothelium dependent relaxing factor (EDRF), i.e., NO *(89)*.
6) Reduction in erythrocyte aggregation and deformability *(90)*.
7) Reduction in endothelial-monocyte adhesion (reviewed in ref. *91*).
8) ω-3 fatty acids in fish oil replace prostaglandin (PG)I2 and PGA2 with PGI3 and PGA3 favoring vasodilation and suppression of VSMC growth *(72)*.
9) Conjugated linoleic acid reduces severity of atherosclerosis, reduces body fat, and enhances lean body mass *(92)*.

Thus, there are a number of potential mechanisms by which PUFA are anti-atherosclerotic. However, in a group of selected patients with documented CAD, ω-3 PUFA, given for 2 years did not demonstrate an effect on slowing the progression of atherosclerosis of the carotid arteries as measured by ultrasound *(93)*.

Monounsaturated Fatty Acids

The preventive effects of a monounsaturated fatty acid (MUFA)-rich diet on atherosclerosis may be explained by the enhancement of HDL-C levels and impairement of LDL-C levels, LDL susceptibility to oxidation, cellular oxidative stress, thrombogenicity, and atheroma plaque formation (reviewed in ref. *94*).

Olive oil has an effect on lipid oxidation—inpatients with PAD, antioxidants present in extra virgin olive oil protect LDL against oxidation and decrease LDL uptake by macrophages compared to refined olive *(95)*.

Olive oil has an effect on inflammation—olive oil decreases expression of VCAM-1 and interferes with activation of nuclear factor κB (NFκB) *(96)*.

In rabbits, supplementing saturated fat diet with olive oil improved lipid profile, reduced vascular thrombogenecity and platelet activation with less severe morphological lesions of the endothelium and the vascular wall *(97)*.

However, in Watanabe heritable hyperlipidemic (WHHL) rabbits, fish oil decreased blood lipids (cholesterol and triacylglycerol levels of intermediate density lipoprotein [DL] and very low-density lipoprotein [VLDL]) and atherosclerosis compared with olive oil, although LDL oxidation was higher with fish oil *(98)*. Also, depending on the underlying genotype, dietary MUFA and carbohydrates can actually increase atherosclerosis susceptibility *(99)*.

Saturated Fats, Hydrogenated Fats/Trans–Polyunsaturated Fatty Acids, and Oxidized Fats

Postprandial hypertriglyceridemia increases oxidative stress and further deteriorates endothelial function even in patients with CAD *(100)*. In humans, consumption of a diet high in hydrogenated fat increases production of inflammatory cytokines without affecting cellular immunity *(101)*.

Oxidized fat and lipid oxidation products are present in human foods, are absorbed, and appear in blood circulation and can have deleterious CVD effects in humans (reviewed in ref. *102*).

Paradoxically, linoleic acid, although an n-6 PUFA, has an atherogenetic role through oxidative stress and cytokine mediated inflammatory response on the endothelium (reviewed in ref. *103*). Corn oil (an n-6 PUFA source) increased oxidative stress and induced endothelial damage *(104)* On the other side, the incorporation of palm olein oil containing 38% palmitic acid, a saturated fatty acid, into a moderate fat, moderate cholesterol diet of nonhuman primate has antiatherogenic activity independent of cholesterolemic effects *(105)*.

A high antioxidant vitamin status may help prevent the initiation and progression of early atherosclerotic lesion in man *(106)*. Other dietary constituents that have an atheroprotective effect include low glycemic index, low-fat, high-protein diet *(107)*, tomatoes (reviewed in refs. *108* and *109*), and dietary soy isoflavone *(110)*.

OBESITY

The role of obesity has been reviewed *(111)*. Central fat distribution may contribute to atherogenesis, in part, because of associated alterations in insulin and lipoprotein levels *(112)*, and because of the adverse effect on endothelium as suggested by the association of obesity with

increased vWF levels *(113)*. Also, central obesity is associated with increased PAI-1 levels, increased fibrinogen levels, increased blood viscosity, and hypertension. It is also associated with increased uric acid levels through increased platelet aggregation and adhesion, increased blood viscosity, and propensity to coagulation *(11)*. In a cross-sectional study, central fat mass in elderly women is associated with atherogenic tendencies and peripheral fat mass exhibits an independent dominant antiatherogenic effect *(114)*. Fat cells from people with central adiposity have greater metabolic activity compared to normal cells. This is exemplified by an increase in lipolysis and release of free fatty acids (FFA) that may interfere with insulin clearance and exacerbate hypertriglyceridemia *(115)*. Moreover, plasma insulin concentration inversely correlates with HDL-C and directly correlates with TG concentrations in plasma *(116)*.

In a case control study, there was some trending supporting evidence that, in the absence of DM, homeostatic glycemic control (HbA1C) was a risk factor for atherosclerosis *(117)*. Also, subjects from the general population with mild to moderate hyperglycemia following oral glucose load, but not in the fasting state, showed increased CVD risk (reviewed in ref. *118)*.

In men with atherogenic dyslipemia of the insulin resistance syndrome, plasma C-reactive protein (CRP) levels showed positive correlations with body fat mass, waist girth, visceral adipose tissue, and insulin levels (measured in the fasting state and after a 75-g oral glucose load), but not with plasma lipoprotein levels *(119)*.

However, in a cross-sectional study, insulin sensitivity and hyperinsulinemia did not play a role in femoral atherosclerosis, unlike lipid profile and BP in a follow-up setting *(120)*.

ALCOHOL

Alcohol effects are mixed: in the Rottingham study *(121)* there was an inverse correlation between alcohol consumption and PAD in nonsmoking men and women. In a prospective study, the association between regular alcohol intake and *early* atherosclerosis was J-shaped: the beneficial effects appeared to be through inhibition of the injurious actions of high levels of LDL-C. The association between regular alcohol intake and *advanced* atherosclerosis was U-shaped *(122)*. The adverse and beneficial effects of alcohol on CVA are mediated by atherogenic and antiatherogenic properties. The relation between carotid artery disease and alcohol was U-shaped, approximately one-fourth of the atherosclerosis risk caused by severe alcohol consumption was mediated by

the risk profile associated with drinking, whereas the apparent beneficial effect of low alcohol intake emerged independent of conventional risk attributes *(123)*. In female rabbits, alcohol intake representing 20 to 30%, but not 10%, of caloric intake increased VLDL and LDL, but not HDL, and decreased LDL receptor mRNA. It also increased lesion area and aortic cholesterols *(124)*.

Red wine strongly inhibits ET-1 synthesis *(125)*; phenolic acids in wine prevent LDL oxidation in the arterial wall *(126)*, increases eNOS expression in HUVEC *(127)*, inhibits PDGF-β receptor activation *(128)*, and inhibits the proliferation and DNA synthesis in cultured rat VSMC *(129)*. However, alcohol is a complementary component of phenolics in the benefits of red wine in hypercholesterolemic golden Syrian hamsters *(130)*.

SMOKING

The role of smoking has been reviewed in ref. *131*. Smoking has several contributing effects to atherosclerosis: in a prospective study, a direct association was found between atherosclerotic changes and number of cigarets smoked per day. This risk was reduced, but not eliminated up to 10 years after cessation of smoking *(132)*.

Even passive smoking has been shown to increase experimental atherosclerosis *(133)*. Exposure of healthy nonsmoking subjects to passive smoking, in a follow-up study, caused an acute decrease in serum ascorbic acid levels and antioxidant defense, decreased LDL capacity to resist oxidation, increased serum levels of lipid peroxidation end products, and LDL isolated from these subjects was taken up by cultured macrophages at an increased rate *(134)*. Secondhand smoke also caused endothelial dysfunction and increased adrenergic responsiveness (which was abolished by inhibition of NOS and removal of endothelium) and atherosclerosis in hypercholesterolemic rabbits. These effects were mitigated by L-arginine supplementation *(135)*.

There is intersubject variability in cigaret-induced pathologies that is partly mediated by genetic variants, for example, eNOS intron 4 rare allele homozygotes are more likely to have myocardial infarction (MI) if they smoke (reviewed in ref. *136*). Also, there is a synergistic effect between the Apo E allele epsilon 4 and smoking on carotid atherosclerosis in the Family Heart Study *(137)*.

Smoking increased isolated blood monocytes adhesion to unstimulated HUVEC, mediated by integrin CD11b/CD18, along with decreased plasma vitamin C levels *(138)*. In addition, the proathrogenic effect of cigaret smoking are mediated in part by the chronic infection found in smokers *(139)*.

Both secondhand smoke and hypercholesterolemia were associated with significantly increased mitochondrial DNA damage, protein nitration, and increased atherosclerotic lesion formation *(140)*.

HOMOCYSTEINE

The role of homocysteine (Hcy) in lipid oxidation has been addressed and reviewed *(141)*. In males, moderate increase in Hcy levels in a case control study was an independent risk factor for cerebral infarction and MI and may predict the severity of the disease; levels were increased in 20 to 30% of patients with premature atherosclerosis. Also, in the presence of other traditional risk factors Hcy may have a permissive role in the endothelial damage even within the normal range, and this role may be related to free radical generating systems *(142)*.

However, in a prospective study, atherosclerosis risk in communities (ARIC), there was doubt about a relation between CHD and Hcy and no association between CHD and C677T mutation of methylenetetrahydrofolate reductase gene or three mutations of cystathionine β-synthase gene. However, it was possible that vitamin B_6 offered an independent protection *(143)*, and recently a review concluded that there is insufficient evidence to regard Hcy levels as a causative factor in atherosclerosis *(144)*.

In Apo E null mice increased Hcy by a diet enriched with methionine but depleted in folate, B_6, and B_{12} increased atherosclerotic lesion area and complexity and enhanced expression of receptor for advanced glycation endproducts (AGE), VCAM-1, TF, and MMP-9 in the vasculature. These effects were suppressed in parallel with decreased plasma Hcy levels upon dietary supplementation with folate, B_6, and B_{12} *(145)*. Also, methionine load increased vWF in patients with arterial or venous occlusive disease with or without hyperhomocysteinemia, suggesting endothelial dysfunction *(146)*.

Hyperhomocysteinemia, in coronary arteries increased TNF-α expression, which enhanced oxidative stress ultimately impairing flow-induced dilation that can be reversed by superoxide dismutase (SOD) *(147)*. Also, in EC Hcy inhibits dimethylarginine dimethylaminohydrolase (DDAH) enzyme activity by direct interaction (enzyme that degrades asymetric dimethylarginine [ADMA]), causing ADMA to accumulate and inhibit NO synthesis, which might explain how Hcy impairs NO dependent vasodilation *(148)*.

HUVEC converts Hcy to thiolactone (a byproduct of Hcy auto-oxidation). Folic acid inhibits this conversion; protein homocysteinylation (indirect incorporation of Hcy into protein) increases with increasing

Hcy concentration and decreases with increasing folic acid and HDL in EC cultures. All this suggests a role in vascular disease *(149)*. Hcy thiolactone combines with native LDL to form oxLDL, which is taken up by intimal macrophages to form foam cells (reviewed in ref. *150*).

Hcy increased arterial SMC growth and collagen production. The addition of aquacobalamin controlled these effects *(151)*. Diet-induced hyperhomocysteinemia in Apo E deficient mice promotes early atherosclerosis and plaque fibrosis, but does not weaken collagen or induce plaque rupture *(152)*.

In human platelets, Hcy diminished NO production through decreased uptake of L-arginine without any effect in NOS activity *(153)*.

INFLAMMATION

Inflammation and its mediators have received a lot of attention as a contributing factor to atherosclerosis.

In 81-year-old subjects, high plasma TNF-α was associated with atherosclerosis but not with TC, LDL, and BMI, and was weakly correlated with TG, leukocytes, CRP, and low HDL/TC ratio *(154)*. The presence of antinuclear antibodies is substantially more prevalent among subjects with severe coronary atherosclerosis than those with normal coronary arteries *(155)*. On the other hand, in a double-blind randomized placebo controlled cross over trial of healthy men, acute systemic inflammation augmented local forearm tPA release suggesting it can invoke a protective response *(156)*.

Cell Adhesion/Chemotaxis

Leukocyte count is associated with aortic arch plaque thickness, and is specifically correlated with aortic arch plaque thickness equal to or more than 4 mm in stroke-free subjects *(157)*. In WHHL rabbits, leukocyte adherence to intact arterial endothelium is one of the geneses and promoters of atherosclerosis *(158)*.

Chemokines contribute to leukocyte recruitment, angiogenesis, and proliferation and migration of SMC into atherosclerotic plaques; in addition, leukocytes and EC are an important source of chemokines and many atherosclerotic risk factors increase chemokine expression. Furthermore, interactions between cells such as leukocytes and EC amplify chemokine release that may contribute to sustained chemokine generation in inflammatory conditions (reviewed in ref. *159*). Fractalkine (FK; now also called CX3CL1) is a unique chemokine that functions not only as a soluble chemo-attractant but also as a transmembrane-anchored adhesion receptor, and is expressed on activated ECs. The fractalkine

receptor, CX3CR1, is expressed on cytotoxic effector lymphocytes, including natural killer (NK) cells and cytotoxic T-lymphocytes and on macrophages. Soluble fractalkine causes migration of NK cells, cytotoxic T-lymphocytes, and macrophages, whereas the membrane-bound form captures and enhances the subsequent migration of these cells in response to secondary stimulation with other chemokines. Furthermore, stimulation through membrane-bound fractalkine activates NK cells, leading to increased cytotoxicity and interferon-γ production (reviewed in ref. *160)*. CX3CR1–/– mice have a significant reduction in macrophage recruitment to the vessel wall and decreased atherosclerotic lesion formation *(161)*.

oxLDL, but not LDL, upregulates CXCR2 expression in monocytes and promotes the chemotaxis and adhesion of monocytes *(162)*.

E- and P-Selectins, play important roles in early and advanced stages of atherosclerosis: in E- and P-selectin double (LDL receptor deficient) KO mice on an atherogenic diet, lesions were smaller but contained the same number of macrophages than control animals (LDL receptor deficient) *(163)*.

MCP-1 and chemokine receptor 2 (CCR2) play important roles in monocyte recruitment: MCP-1 expression is increased in human atherosclerotic plaques, vascular endothelium, and VSMC exposed to minimally modified lipids. In Apo E deficient mice that lack CCR2, lesion formation was decreased with no effects on lipids *(164)*; on the other hand, MCP-1 expression by leukocytes (macrophages) increases macrophage number and oxidized lipid accumulation and atherosclerosis progression in Apo E deficient MCP-1 transgenic mice, with no effects on plasma lipoprotein profile *(165)*. However, local MCP-1 overexpression at the vessel is not sufficient in rabbits, and activation by other factors induced by hypercholesterolemia is required *(166)*.

ICAM-1 and lymphocyte function-associated protein (LFA)-1 pathway are involved in monocyte-endothelium interaction during a cholesterol-rich diet. In rats on high-cholesterol diet, ICAM-1 expression was increased on aortic EC, which was associated with increased monocyte adherence (more than 85% of adherent macrophages exhibited LFA-1 antigen and injecting the animals with ICAM-1 monoclonal antibody and LFA-1 monoclonal antibody reduced mostly LFA-1 positive macrophages) *(167)*. In clinically healthy middle-aged men, levels of soluble ICAM-1 (sICAM-1), but not sVCAM-1 or E-selectin, were associated with subclinical atherosclerosis and inflammatory variables *(168)*.

TNF-α is produced by macrophages and promotes inflammatory reactions, including induction of vascular adhesion molecules and

macrophage recruitment and proliferation. However, in mice TNF receptor p55 protects against atherosclerosis because its absence was associated with increased scavenger receptor activity *(169)*. Additionally, in individuals younger than 70 years of age in a multi-ethnic urban population, relative elevation of TNF receptor levels, but not TNF levels, was associated with carotid atherosclerosis *(170)*.

Monocyte Activation

The role of monocytes has been reviewed in ref. *171*; Neopterin, a marker of macrophage activation, was higher in patients with unstable angina compared with patients with stable angina independent of *C. pneumoniae* seropositivity *(172)*.

The interaction of monocyte with nonenzymatically glycated matrix protein in the vessel wall may result in a faster rate of induction of monocyte/macrophage (M/M) differentiation leading to foam cell formation *(173)*. During the initiation of lesion development, foam cells derive mainly from circulating precursors rather than resident macrophages *(174)*. Macrophages in humans induce human VSMC apoptosis ultimately promoting directly plaque rupture *(175)*. Cyclo-oxygenase (COX)-2 is upregulated in activated M/M, LDL receptor –/– mice null for macrophage COX-2 developed less atherosclerosis than LDL receptor –/– mice, even though COX-2 contributes to the production of prostacyclin (reviewed in ref. *176*).

Immune Reactions

Immune reactions can modulate atherosclerosis development (reviewed in ref. *177)*; nonspecific stimulation of the innate immune system by endotoxin accelerates cholesterol-induced atherosclerosis in rabbits *(178)*.

Complement components have been isolated from lesions and lesion severity is decreased in complement-deficient animals; complement-mediated release of MCP-1 from human VSMC may be important in monocyte recruitment *(179)*.

Increased auto-antibodies against oxLDL was associated with CAD *(180)*. in Apo E deficient mice, lipopolysaccharide (LPS) injection caused an increase in antibodies against oxLDL, an increase in atherosclerotic lesion size, and increased IL-4-producing NK T cells *(181)*. Anti-phosphorylcholine (PC) antibodies have reactivity against oxLDL and other oxidation-specific structures containing PC antigen; cells undergoing apoptosis express a range of oxidation-specific neoself PC determinent *(182)*. However, in another study there was no significant association between circulating antibody titers to malondialdehyde-modified (MDA)-LDL and coronary atherosclerosis *(183)*. Human-

derived anti-oxLDL auto-antibody blocks oxLDL uptake by macrophages and localizes to atherosclerotic lesions in vivo *(184)*, In Apo E knockout (KO) mice, splenectomy aggrevated atherosclerosis, and transfer of spleen cells reduced disease development (specifically B-cells). This protection was demonstrated on nonsplenectomized mice, and was associated with an increase in antibodies to oxLDL. In protected mice, few CD4+ T-cells were found. Therefore, it is suggested that B-cell associated protective immunity develop during atherosclerosis and reduces disease progression *(185)*.

Elevated IgA anti-β2-glycoprotein 1 (an antigen of autoimmune anticardiolipin antibodies) and lgG antiheat shock protein (Hsp) 60/65 antibodies are associated with increased risk of ischemic stroke independent of other risk factors *(186)*. The antigen-antibody complex target oxLDL, so the complexes are involved in oxLDL uptake into macrophages (reviewed in ref. *187*).

Other Inflammatory Mediators

In vitro studies demonstrated that ligation of the immune mediator, CD40, on atheroma-associated cell types mediates expression of cytokines, chemokines, growth factors, MMPs, and procoagulants (reviewed in ref. *188*). CD40 and CD40 ligand are expressed in cells in atherosclerotic lesions: in LDL receptor deficient hypercholesterolemic mice, CD40 ligand antibody limited atherosclerotic lesions with less lipid content, fewer macrophage and T-lymphocytes, and decreased VCAM-1 expression in the lesions *(189)*.

Interferon-γ can induce arteriosclerotic changes in the absence of immunocytes by potentiating VSMC mitogenesis *(190)*. In Apo E –/– mice, interferon-γ deficiency decreased atherosclerosis, in males only, by decreasing T-lymphocyte presence and cell activation without influencing serum cholesterol concentration *(191)*.

The expression of proinflammatory ILs and their receptors has been demonstrated in atheromatous tissue, and serum levels of several ILs have been found to correlate positively with arterial coronary disease. Several ILs have been involved in vitro with upregulation of adhesion molecules on EC, activation of macrophages, and SMC proliferation. Some ILs are proatherogenic and others are anti-atherogenic (reviewed in ref. *192*).

Factors secreted by adipose tissue especially visceral adipose tissue (adipokines) increase with increased adiposity and regulate a number of factors that contribute to the development of atherosclerosis (reviewed in ref. *193*).

Synthetic peroxisome proliferator-activated receptors (PPARs) agonist in clinical use such as thiazolidinediones (TZDs) may limit inflammatory response *(194)*. Also, PPARγ, but not PPARα, activators repress interferon-γ-induced MHC-II expression and subsequent inhibition of T-lymphocyte activation in atheroma-associated cells *(195)*.

The role of atherogenic lipoproteins on inflammation has been reviewed in ref. *196*. PAF (a proinflammatory phospholipid) metabolism is independent of cellular cholesterol content, and foam cells and macrophages produce transiently PAF upon phagocytosis at inflammatory sites in intima *(197)*. On the other hand, in HUVEC Apo E, secreted locally at lesion sites by macrophages may be anti-inflammatory *(198)*.

Given the lipoprotein binding and complement activation of CRP (an acute phase response protein) and its localization in atherosclerotic vessels, there is a strong likelihood that CRP may be involved in the atherosclerotic process (reviewed in ref. *199)*. Human coronary artery SMCs, but not HUVECs, could produce CRP in response to inflammatory cytokines *(200)*. In human saphenous vein EC, CRP increases ET-1 and IL-6 production leading to increased MCP-1 production and native LDL uptake by macrophages *(201)*. In human aortic endothelial cells (HAEC), CRP induced PAI-1 expression and activity *(202)*; CRP may contribute to foam cell formation in atherosclerotic lesion by causing the aggregation of LDL molecules that are then taken up by macrophages *(203)*. However, CRP level was not related to the extent or presence of coronary atherosclerosis in another study *(204)*.

The role of NFκB has been reviewed in ref. *205*. NFκB is a key regulator of inflammation, immune responses, cell survival, and cell proliferation; inhibition of the NFκB pathway in macrophages leads to more severe atherosclerosis in low density lipoprotein receptor (LDLR) deficient mice, possibly by affecting the pro- and anti-inflammatory balance that controls the development of atherosclerosis (reduced production of LPS-stimulated TNF, and reduction in IL-10) *(206)*.

Endothelin-converting enzyme (ECE)-1 activates ET-1, both are abundantly present in human arteries and at different stages of atherosclerotic plaque evolution. The upregulation of the ECE-1/ET-1 system is closely linked to the presence of chronic inflammation and is present in very early stages of plaque evolution *(207)*. ET system is upregulated by LDL and oxLDL in cultured human coronary SMC and human monocyte-derived macrophages *(208)*.

Also, dendritic cells (DC) accumulate in atherosclerotic lesions (reviewed in ref. *209*); oxLDL can activate DC but high concentrations increase DC apoptosis *(210)*. Direct contact between activated DC that

over-express HSP70 and T-cells might be responsible for T-cell activation, and might facilitate the presentation of lipid antigen to T-cells directly within the human arterial wall in early intimal lesions *(211)*.

INFECTIONS

The first inflammatory stage of atherosclerosis starts early in life with more severe lesions developing only if classical risk factors, especially cholesterol, remain present. Immune responses mounted against antigens cross-react with homologous host proteins in a form of molecular mimicry, for example, HSP are secreted by *C. pneumoniae*, *H. pylori*, mammalian vascular cells exposed to stress such as CVD risk factors, and cells within atherosclerotic plaques In addition, serum titers of anti-HSP antibodies are correlated positively with the future risk of CHD, and purified anti-HSP antibodies lyse stressed human EC and macrophages in vitro. Furthermore, immunization with HSP exacerbate athersclerosis in animal models (reviewed in refs. *212,213*). However, there is molecular mimicry between epitopes of oxLDL and *Streptococcus pneumonia* in LDLR –/– mice: pneumococcal immunization led to increased IgM levels against oxLDL and decreased the extent of atherosclerosis *(214)*.

Risk factors for atherosclerosis evoke HSP over-expression on EC, macrophage, and SMC; HSP could be a soluble factor in the blood, which is positively correlated with atherosclerosis in humans (normally it is localized intracellularly) Soluble HSP binds to the toll-like receptor (TLR)-4/CD14 complex initiating an immune response, including the production of pro-inflammatory cytokines by macrophages and adhesion molecules in EC via NFκB activation. Titers of HSP antibodies are increased in patients with atherosclerosis, and T-lymphocytes specifically responding to HSP have been found in atherosclerotic plaques (reviewed in ref. *215*). In addition, TLR-4, the receptor for bacterial LPS, recognizes cellular fibronectin and HSP 60 and is expressed in human adventitial fibroblasts and macrophages, the activation of adventitial TLR-4 augmented neointima formation in a mouse model *(216)*. Also, pro-inflammatory bacterial peptidoglycan (a functional LPS analog) and its co-receptor, toll-like receptor-2, have been observed in atherosclerotic arteries in association with the vulnerable plaque phenotype *(217)*. Pathogens interfere with macrophage cholesterol metabolism through inhibition of the liver X receptors (LXR) signaling pathway: activation of TLR 3 and 4 by microbial ligands blocks the induction of LXR target genes including ATP-binding cassete transporter class A1 (ABCA1) in cultured macrophages, as well as in aortic tissue in vivo As

a consequence of these transcriptional effects, TLR3/4 ligands strongly inhibit cholesterol efflux from macrophages *(218)*.

The pronounced expression of IL-6 and IL-8 by virus-infected monocytes may induce local or systemic inflammation, which may be associated with plaque rupture. These processes may be accelerated by the lack of the production of the anti-inflammatory cytokine IL-10 *(219)*.

In vitro and animal model studies have suggested that infectious agents and their products can activate the coagulation cascade enzymatically or by upregulating TF. Alternatively, some microbes can directly trigger platelet aggregation (reviewed in ref. *220*).

The role of *C. pneumoniae* has been reviewed in ref. *221*. Also, there is a significant association between infectious burden and the extent of atherosclerosis *(222)*; the IgG antibody response to multiple pathogens is an independent risk factor for endothelial dysfunction and the presence and severity of CVD *(223)*.

PATHOLOGY OF ATHEROMA

The atherosclerotic process begins, according to the response-to-injury-hypothesis, with a structural or functional injury to the endothelium resulting in increased permeability of the endothelial barrier to blood cells, hormones, and lipoproteins *(224–226)*. The elastic lamina, secreted by and subjacent to endothelium, provides a barrier to the entry of macromolecules and cells and its absence is associated with atherosclerosis *(227)*. Platelets aggregate at the site of injury and release growth factors and chemo-attractants that subsequently stimulate proliferation of VSMC. Migration of these cells and macrophages to the subintimal region subsequently occurs, the place where the atherosclerotic process develops *(11,224–226)*.

Half the infants in the first 6 months of life have foam cells in susceptible segments of the coronary arteries Later on, fewer children have them, but at puberty they accumulate and preatheromas develop and contain lipid droplets and dead cell remnants (extracellular lipids) and macrophages *(228)*. Atheromas have a lipid core in which increased extracellular lipids displace SMC and ECM and then calcium granules appear in some SMCs and among the extracellular lipid of the core, lipid core and inflammation may be an underlying cause of lesion rupture, which makes intervention important at this young age *(228)*.

This lipid core incites inflammation and in conjunction with the thin fibrous cap this constitutes a vulnerable plaque, rupture of such vulnerable plaques account for the majority of CHD death, particularly sudden death. The early reduction with CHD death with HMG-CoA reductase

inhibitors is caused by a reduction in lipid core *(229)*. Large necrotic cores, fibrous caps < 65 μ, and numerous macrophages within the cap likely indicate instability and rupture of thin cap atheromas. Speckled pattern of calcification predicts vulnerability to rupture (reviewed in ref. *230*). Coronary artery plaques with positive remodeling (outward expansion of the vessel wall) have a higher lipid content and macrophage count, both markers of plaque vulnerability compared with lesions with negative remodeling (vessel shrinkage). These results may explain why plaque rupture is often apparent at sites with only modest luminal stenoses (but marked positive remodeling) *(231)*. Also, hemodynamic forces may play a crucial role in the pathogenesis of plaque disruption *(232)*. Plaques that develop into end-stage *culprit plaques* are distinct from *stable plaques* by location and early lesion morphology, suggesting distinct lesion development and progression pathways; also, polygenic hypertension accelerates coronary plaque progression and complication *(233)*.

The atherosclerotic process changes the cellular responsiveness and function of the vessel as a result of changes in VSMCs (reviewed in ref. *234*) This can be understood by knowing the phenotypic make-up of the VSMC. There are two major phenotypes of arterial VSMCs *(224,225)*: (1) the *contractile phenotype* found in arterial media and made up of myofilaments; it is responsible for contraction and relaxation of the vasculature; and (2) the *synthetic VSMC* phenotype, which is found in the intima during the atherosclerotic process following the migration of contractile VSMC from the media.

Several factors are considered important in the transformation to a synthetic phenotype:

1. Fas ligand expression increased SMC in intima leading to acceleration of atherosclerotic lesion growth *(235)*.
2. Calcium deficiency in chick embryos accelerates cell proliferation, decelerates sarcomeric protein expression in vitro, and induces atherosclerosis *(236)*.
3. LDL acts as a growth factor promoting VSMC growth (reviewed in ref. *237*).
4. Lp(a), in transgenic rabbits, promotes SMC proliferation and dedifferentiation possibly because of impaired fibrinolytic activity *(238)*.
5. Unsaturated lysophosphatic acid (LPA), but not saturated LPA, specifically induces VSMC differentiation *(239)*.
6. In a rat SMC line, overexpression of LR 11, a member of LDL receptor family and highly expressed by VSMC of hyperplastic intima but not media, induced enhanced migration and invasion activities of intimal SMCs in vitro *(240)*.

7. gp38k (CHI3L1) is a secreted heparin-binding glycoprotein whose expression in vitro is associated with VSMC migration and invasion into the underlying gelatinous matrix *(241)*.
8. Activator protein (AP)-1 activation is crucial for the mediation of VSMC proliferation in response to balloon injury to the rat carotid artery *(242)*.
9. Activation of extracellular signal-regulated kinases (ERKs), protein kinase C, tyrosine kinase, and p38 MAPK is essential for pressure-induced DNA synthesis in rat VSMC *(243)*.
10. The demonstration of microsatellite instability and loss of heterozygosity in SMCs of human plaques suggests that genomic destabilization may play a pivotal role in atherosclerotic mechanisms. Furthermore, the use of accepted biomarkers of carcinogenic exposure-such as DNA adducts and cytogenetic endpoints recently has provided evidence consistent with the view that somatic cell alterations are critical in atherogenic process (reviewed in ref. *244*).
11. SMCs develop hypomethylation in vitro during transformation from a contractile to a synthetic phenotype. Genomic hypomethylation occurs during atherogenesis in human, mouse, and rabbit lesions, and it correlates with increased transcriptional activity. Also, methyltransferase is expressed in atherosclerotic lesion and hypomethylation is present in advanced lesions and may reflect cellular proliferation and gene expression in atherosclerotic lesions *(245)*.
12. Extracellular uridine 5'-triphosphate (UTP) induces mitogenic activation of SMCs through binding to P2Y2 nucleotide receptors. P2Y2 receptor mRNA is upregulated in intimal lesions of rat aorta, and has a role in the development of intimal hyperplasia in collared rabbit carotid artery *(246)*.
13. Polyamines such as putrescine, spermidine, and spermine are involved in the transition of migrated VSMC into a synthetic phenotype *(226)*.
14. Smooth muscle associated protein 2 was upregulated during neointima formation in a rat carotid endarterectomy model *(247)*.

On the other hand, other factors are important in establishing the contractile phenotype:

1. The expression of the major receptor protein for NO signaling in smooth muscle, cGMP-dependent protein kinase, is important to establish contractility in rat aortic SMC in culture *(248)*.
2. The natriuretic peptides (NPs), atrial natriuretic factor (ANF), and B and C type NPs (BNP and CNP) have potent antiproliferative and antimigratory effects on VSMCs. NPs and their receptors (NPRs) mRNAs are present in human coronary arteries *(249)*.
3. The TR3 orphan receptor, the mitogen-induced nuclear orphan receptor (MINOR), and the nuclear receptor of T-cells (NOT) are a subfamily of

transcription factors belonging to the nuclear receptor superfamily, and are induced in activated SMCs. There is expression of TR3, MINOR, and NOT in neointimal SMCs, whereas there is no expression detected in medial SMCs; TR3 orphan receptor inhibits SMC proliferation and vascular lesion formation *(250)*.

These synthetic cells proliferate, take up LDL-C and synthesize abnormally large amounts of collagen, elastin, and proteoglycans *(225,226)*. Hypoxia increases LDL uptake and enhances lipid accumulation in arterial SMC *(251)*. Thus, VSMC that are contractile in the media become phenotypically different upon migrating to the intima.

Human aortic SMC gained macrophage scavenger receptor and macrophage phenotype supporting possible transformation of SMC into foam cells: CD36 is a scavenger receptor class B for oxLDL and plays an important role in foam cell formation from monocytes (reviewed in ref. *252*). PPARγ was expressed in CD 36 positive cells, PPARγ ligand upregulated CD36 expression in CD36 positive cells only *(253)*; absence of CD36, in an atherogenic Apo E null backgroud, resulted in a 70% decrease in total lesion area in western diet-fed mice. oxLDL can stimulate its own uptake by induction of CD36 gene expression that involved activation of PPARγ (reviewed in ref. *254*).

Bone marrow cells have the potential to give rise to vascular progenitor cells that hone in on damaged vessels and differentiate into SMC and EC *(255)* However, progressive progenitor cell deficits, the effect of concomitant vascular aging, may contribute to the development of atherosclerosis *(256)*, because the age-dependent failure of the bone marrow to produce vascular progenitor cells responsible for arterial repair drives atherosclerosis and its thromboembolic complications (reviewed in ref. *257*).

GROWTH FACTORS, CYTOKINES, AND MITOGENESIS

A number of mitogens play an important role in the atherosclerotic process *(258)*. Table 2 summarizes some of the more important mitogens implicated in the atherosclerotic process. The contribution of each factor will be briefly discussed in the context of the atherosclerotic process.

Angiotensin II

AII has the following effects:

1. In Apo E-deficient mice, AII stimulates macrophage cholesterol biosynthesis through AT1 receptor followed by induction of HMG CoA

Table 2
Some Mitogens and Cytokines Associated With Development of Atherosclerosis

Platelet derived growth factor
Insulin-like growth factors
Epidermal growth factor
Transformation growth factor-β
Fibroblast growth factor
Interleukin-1
Endothelin-1
Tumor necrosis factor-α
MCP-1
VMAP-1
Selectins
ICAM-1
VCAM-1
LFA-1
CD40 and CD40L
ECM (heparan sulfate)
Tissue factor
PAI-1
vWF
VEGF

reductase gene expression, inhibition of AII activity attenuate atherosclerosis *(259)*.

2. In mouse peritoneal macrophages, All atherogenicity may be related, at least in part, to its inhibitory effect on macrophage cholesterol efflux mediated through AT1 and not the ABC1 transporter, thus leading to cellular cholesterol accumulation *(260)*.
3. AII stimulates macrophage uptake of oxLDL, mediated by IL-6 in Apo E deficient mice *(261)*.
4. AII inhibits insulin signaling through AT1 receptor and bradykinins/NO dependent blood flow *(11)*.
5. AII stimulates expression of platelet-derived growth factor (PDGF)-A chain mRNA and secretion of PDGF-like molecules, and vascular SMC hypertrophy *(262)*.
6. AII activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enhancing SMC growth and proliferation, stimulating inflammatory proteins, and modulating matrix remodeling *(263)*.
7. AII, through activation of NF-κB-mediated pro-inflammatory genes, promotes vascular inflammation, leading to acceleration of atheroscle-

rosis and induction of aneurysm in Apo E-KO mice. Downregulation of PPAR-α and -γ by AII may diminish the anti-inflammatory potential of PPARs, thus contributing to enhanced vascular inflammation *(264)*.

8. In Apo E-KO mice, AII treatment accelerated atherosclerosis in the carotid artery, increased blood pressure, increased arterial stiffening, increased pulse wave velocity, and decreased arterial elasticity. These functional changes were correlated with morphological and biochemical changes as demonstrated by an increase in collagen content, a decrease in elastin content, and breaks in the internal elastic lamina in the aortic wall. In addition, endothelium-independent vasorelaxation to sodium nitroprusside was impaired *(265)*.
9. MMPs have no role in AII-induced atherosclerosis but MMPs have a role in AII-induced abdominal aortic aneurysm (AAA) formation in mice *(266)*.

AT1 receptor is responsible for most of the pathophysiologic actions of AII. Estrogen deficiency and hypercholesterolemia increases AT1 receptor expression. Both AT1 receptor antagonist and ACE inhibitors improve endothelial dysfunction; AT2 causes antiproliferation and counteracts the cell growth induced by AT1 activation (reviewed in refs. *267* and *268*). Hypoxia and hyperglycemia upregulates AT(1) receptor expression in cultured VSMC, suggesting a mechanism for enhanced AII-induced VSMC proliferation and the development of atherosclerosis in diabetes *(269)*. Whereas antagonism of AT1 receptors totally prevented the formation of aneurysms, antagonism of AT2 receptors promoted a large increase in the severity of AII-induced vascular pathology with no change in plasma lipid concentrations and only transient and modest increases in blood pressure *(270)*.

Also, mineralocorticoid receptor antagonism improves endothelial function and reduces O2*-generation in diet-induced atherosclerosis *(271)*. In Apo E –/– mice, administration of the selective aldosterone blocker, eplerenone, significantly reduced oxidative stress and atherosclerosis progression *(272)*.

In rabbits, vascular ACE activity was significantly increased by atherogenic diet with significant correlation between vascular ACE activity and plaque area, however, contractile responses of the femoral arteries to AI and AII in the atherogenic diet-fed animals were not different from those of the normal diet-fed animals *(273)*. ACE inhibition improves vascular compliance, NO production, vascular relaxation, plasma markers of relaxation, oxidative stress, and thrombosis (reviewed in ref. *274*). On the other hand, hypercholesterolemia in rabbits resulted in atherosclerosis, loss of endothelium-dependent relaxation, increased AT1 in aortic tissues with enhanced constrictor response to AII *(275)*.

Platelet-Derived Growth Factor

PDGF may also interact with other growth factors, such as IGF-I to enhance VSMC proliferation and migration *(276)*. Infusion of PDGF into rats subjected to carotid injury produces a two- to threefold increase in medial VSMC proliferation, but a 20-fold increase in intimal thickening and VSMC migration from media to intima within a week following injury *(277)*. However, in Apo E null mice elimination of PDGF-β from circulating cells or blockade of PDGF receptors transiently delays, but does not appear sufficient to prevent smooth muscle accumulation in advanced lesions of atherosclerosis *(278)*.

VSMCs isolated from intimal lesions after balloon catherization synthesize significantly greater amounts of PDGF than VSMC isolated from normal media. PDGF receptor activity also increases when VSMC change to a synthetic phenotype. Both PDGF and epidermal growth factor (EGF) then interact to further promote migration of VSMC to the intima and subsequent proliferation of these migrated cells *(279)*. Also, PDGF-β is capable of inducing MMP-3 expression *(280)*.

IGF-I, IGF-II and insulin appear to have an important role in the pathogenesis of atherosclerosis *(281)*. IGF-I has been shown to stimulate 3H thymidine incorporation by VSMC. IGF-I is an autocrine/paracrine peptide, which is produced by VSMC and cardiomyocytes under the control of stretch, AII, PDGF, and growth hormone (GH) *(282,283)*. Arterial injury is accompanied by a rapid and long lasting induction of VSMC IGF-I mRNA expression *(281)*. EC produce IGFs, and EC dysfunction may lead to increased release of IGFs, which, in turn, may promote neointimal VSMC proliferation *(281)*. They also stimulate proliferation of EC *(281)*; and IGF-I promotes vascular inflammation in EC by potentiating c-jun and NFκB activation by TNF-α and enhancing TNF-α-mediated adhesion molecule expression *(284)*.

Platelets express both IGF-I, and IGF-II, the expression being localized to the α-granules. Platelets also have IGF-I receptors, and platelet adherence and degranulation (activation) leads to the release of IGF *(281)*. Macrophage precursors also have IGF-I receptors and IGF stimulates the proliferation of these cells and their conversion into multinucleated cells *(281)*. VSMC express receptors for IGF-I, IGF-II, and insulin *(281,285)*. PDGF and IGF-I have synergistic effects on expression of the proto-oncogene c-*myc* in cultured bovine VSMC and in promoting cell growth *(281)*. Although insulin does not increase the mitogenic effect of IGF-I, the mitogenic response of insulin is mediated, in part, through an IGF-I receptor (281). Insulin has been shown to increase IGF gene expression in aortic VSMC *(281)*. Further, in insulin-deficient diabetic

rats, aortic IGF-I mRNA abundance is significantly reduced compared to that in nondiabetic rats *(285)*. Infusions of insulin into the aorta resulted in a twofold increase in IGF-I mRNA in aorta indicating that hyperinsulinemia might play its role in atherogenesis, in part, through enhanced expression of IGF-I in vessel wall *(285)*.

Insulin alone or with PDGF does not appear to have a significant effect on VSMC migration *(285,286)*. However, VSMC migration induced by the cyclooxygenase product 12-HETE is increased in relation to the concentration and duration of exposure to insulin *(287)*; this effect is augmented by increasing glucose concentration *(286)*.

IGFs, lipoproteins, and insulin are abundant in plasma under normal conditions. Thus, it is possible that these factors are also important in vivo *(11,286,288,289)*; this concept was examined by taking platelet extract from humans and exposing it to rat aortic VSMCs. It caused proliferation of these cells, an effect that was more pronounced when the extract from diabetic vs normal subjects was used *(290)*. Moreover, this increased proliferative effect by the platelet extract from diabetic patients was markedly reduced after treatment to normalize plasma glucose. This adds further support to the notion that either hyperosmolarity or some factor other than insulin contributes to the development of the atherosclerotic process. The AGE-receptor for AGE interaction in VSMC, in addition to growth factors induced by AGE, contributes to the stimulatory effects of diabetic sera on VSMC proliferation that can accelerate atherosclerosis *(291)*.

However, several studies in nondiabetics suggest that lowered circulating IGF-I levels account for a poor outcome of CVD. Also, genetically determined lowered IGF-I levels increase the risk of MI with type 2 DM. It is possible that the premature and progressive decline in serum IGF-I bioactivity in ageing patients with type 2 DM is an important pathophysiological abnormality because it contributes to elevated glucose and lipid levels and the progression and poor outcome of CVD (reviewed in ref. *292)*. Individuals without ischemic heart disease (IHD) but with low circulating IGF-I levels and high IGF binding protein (BP)-3 levels have significantly increased risk of developing IHD during a 15-year follow-up period *(293)*.

Homozygosity for a disrupted IGF-II allele in mice lacking apolipoprotein E results in aortic lesions that are approximately 80% smaller and contain approximately 50% less proliferating cells compared with mice lacking only apolipoprotein E; and targeted expression of an IGF-II transgene in SMC, but not the mere elevation of circulating levels of the peptide, causes per se aortic focal intimal thickenings. These observa-

tions provide a direct evidence for an atherogenic activity of IGF-II in vivo *(294)*.

Epidermal Growth Factor

EGF has been shown to be secreted by platelets, and to stimulate proliferation of VSMC in culture *(262)*. The mitogenic effects of EGF are calcium dependent and are similar to other mitogens such as IGF-I *(262)*. The VSMC proliferative effects of EGF are potentiated by insulin, suggesting that factors such as hypertension and hyperinsulinemia may be synergistic in promoting the atherogenic process.

Heparin-binding EGF-like growth factor (HB-EGF), produced locally by vascular macrophages and SMCs, has been suggested to induce the migration and proliferation of VSMCs. HB-EGF mRNA is abundantly expressed in human adipose tissue. Also, plasma HB-EGF levels increase in parallel with fat accumulation in humans, and the subjects with CAD have higher plasma HB-EGF levels, associated with fat accumulation *(295)*.

Transforming Growth Factor β

Transforming growth factor (TGF)-β is produced by many cells including VSMC *(296)*, EC *(296)*, macrophages *(297)*, T-lymphocytes *(296)*, and platelets *(298)*. Thus, it may have a modulatory role in the atherosclerotic process. Inhibition of TGF-β signaling accelerates the development of atherosclerotic lesion in Apo E deficient mice, and favors the development of lesions with increased inflammatory component and decreased collagen content *(299)*. However, in another study that was associated with a decrease in plaque area *(300)*, TGF-β has been shown to decrease proliferation of VSMCs despite induction of cellular hypertrophy *(301)*. However, TGF-β can stimulate VSMC growth *(225)*. Also, native LDL can stimulate SMC proliferation with increased protein kinase C activity (both are inhibited by α-tocopherol through a mechanism unrelated to its radical scavenging properties) through inhibition of TGF-β secretion by SMC; α-tocopherol increases TGF-β release *(302)*. Its net effect on VSMC growth depends, in part, on its ability to stimulate formation of appropriate ECM proteins *(225,296)*. TGF-β stimulates expression of PDGF-A chain mRNA and secretion of PDGF-like molecules *(279)*; Thus, TGF-β has a regulatory role rather than an initiating role of primary events.

Fibroblast growth factors (FGF) type 1 and 2 are expressed in EC and VSMC *(225,303)*. FGF plays an important role in control of VSMC replication whenever cell injury has occurred *(225)*. FGF stimulates growth in quiescent VSMC in culture *(225)*.

ET-1 also acts as a growth factor for VSMC. This effect is more pronounced in the presence of insulin *(304,305)*. Norepinephrine and histamine increase EC proliferation and increase VSMC proliferation and migration *(262)*. Epinephrine also stimulates proliferation of VSMC and α agonists stimulate PDGF-A chain gene expression *(262)*.

Cytokines such as IL-I and TNF-α are produced by macrophages *(306,307)*. Both of these cytokines inhibit EC growth and stimulate VSMC growth, the opposite mitogenic effects correlate with reduction and increase in FGF receptor number displayed by EC and SMC, respectively *(308)* However, TNF-α markedly decreased IGF-I mRNA and increased IGFBP-3 mRNA and protein in rat aortic VSMCs leading to a reduction in bioactive IGF-I thereby decreasing VSMC viability and promoting plaque instability; whereas IGF-I prevents apoptosis, promotes matrix formation, and can decrease TNF-α and IL-1β-induced proteoglycan degradation *(309)*. Also, IL-I promotes growth of VSMC via induction of synthesis of PDGF with no effect on intracellular Ca^{2+} *(310)*.

Another cytokine, IL-6, induces an increase in VSMC thymidine uptake and proliferation *(311)*. IL-6 also stimulates PDGF production and the VSMC proliferative effects of this cytokine are inhibited by PDGF antibody (as measured by thymidine uptake) *(311)*. These results indicate that IL-6 has an autocrine function through stimulation of PDGF production *(311)*.

Another cytokine, smooth muscle-derived growth factor (SDGF), has been shown to be distinct from competent and progression growth factors and to stimulate different pathways in VSMC *(312)*. Thus, cytokines have a profound and complex effect on VSMC proliferation, and thus the atherogenic process.

Stress and the Sympathetic Nervous System

In the ARIC study, clinical atherosclerosis was associated with low income and low education *(313)*. Also, there was a correlation between cerebrovascular disease and anger and aggression *(314)*. Chronic psychological risk factors (hostility and low socioeconomic factors) are important at early disease stages. Episodic factors (depression and exhaustion) are involved in the transition from stable to unstable atherosclerotic plaques, and acute psychological triggers (mental stress and anger) can promote myocardial ischemia and plaque rupture (reviewed in ref. *315)*. It has been proposed that postprandial insulin resistance along with increased levels of cortisol and catecholamines, specifically

stress, play a major role in the development of early occlusive atherosclerosis (reviewed in ref. *316*).

Stress induced a heightened state of CVD reactivity, injured endothelium and induction of adhesion molecules on EC to which recruited inflammatory cells adhere and translocate to the arterial wall. An acute phase response is also engendered and stress induces an atherosclerotic lipid profile with oxidation of lipids and, if chronic, a hypercoagulable state (reviewed in ref. *317*). The prolonged endothelial dysfunction induced by mental stress is prevented by selective endothelin-A receptor antagonism *(318)*, whereas leisure time physical activity had a positive effect and TV watching time a negative effect on HDL, BMI, waist girth, waist-hip ratio, and subscapular and triceps skinfold thickness (the reverse effect on TG) but not with carotid artery IMT *(319)*.

Sex Hormones Effects

Sex hormones have different influences on atherosclerosis (reviewed in ref. *320*). The incidence of CHD in women younger than 55 years of age is one-third that of men, but becomes closer to that of men by age 75. Also, mortality from CHD is higher in women. In summary, estrogens have the following effects: inhibition of L-type calcium currents, increased HDL, decreased LDL, decreased LDL oxidation, decreased VSMC growth, and increased NO production by platelets, EC, VSMC, and myocardiocytes (reviewed in ref. *321*). The presence of CAD in postmenopausal women is independently associated with altered cholesterol metabolism, as reflected by low synthesis and inefficient elimination of cholesterol *(322)*. Also, among premenopausal women undergoing coronary angiography for suspected myocardial ischemia, disruption of ovulatory cycling characterized by hypoestrogenemia of hypothalamic origin appears to be associated with angiographic CAD *(323)*.

Postmenopausal hormone replacement therapy (HRT) is associated with lower total number of atherosclerotic plaques and less severe atherosclerotic lesions, which may be associated with the effect of HRT on LDL-C oxidation *(324)*.

HRT has a negative modulatory effect on E-selectin, VCAM-1, ICAM-1, MCP-1, and TNF-α, and an inconsistent effect on IL-6, and a stimulatory effect on TGF-β (reviewed in ref. *325*). In rabbits basal and hypercholesterolemia-induced increases in MCP-1 protein are modulated by estrogens *(326)*. Also, the anti-atherogenic effects of estrogens may be mediated by inhibition of Ang II-induced leukocyte recruitment through endothelial NO and prostacyclin release in vivo *(327)*.

In an animal model of atherosclerosis E2 restores FasL expression, which is suppressed by atherogenic levels of serum cholesterol *(328)*. High levels of Hcy diminish with estrogen (reviewed in ref. *329*). In HUVEC, E2 caused a decrease in expression of the NADPH oxidase subunit gp91phox, up-regulated eNOS expression, and inhibited the increase in adhesion molecule and chemokine expression in cells exposed to cyclic strain. Cyclic strain enhanced endothelial O2 formation, thereby offsetting the inhibitory effect of NO on the expression of these gene products *(330)*.

Estrone (E1) and E2 are produced in SMC of the human aortic wall, and their production is mediated by aromatase and 17β-hydroxysteroid dehydrogenase type I (17β-HSD I), respectively. Also, aromatase overexpression, possibly as a result of alternative splicing, may play some role in the development of atherosclerosis (the levels of aromatase mRNA were significantly higher in female cases than in male cases and higher in the specimens with fibroatheroma than other lesions *[331]*). Estrone sulfate (E1S) is a major circulating plasma estrogen that is converted into the biologically active estrogen, E1, by steroid sulfatase (STS). E1 is also sulfated and reverted into E1S by estrogen sulfotransferase (EST). STS expression levels were found to be significantly higher in the VSMCs obtained from female aortas with mild atherosclerotic changes than in those with severe atherosclerotic changes, and in male aortas regardless of atherosclerotic changes. EST expression levels in the VSMCs of these aortas, however, were significantly higher in female aortas with severe atherosclerotic changes and in male aortas than in female aortas with mild atherosclerotic changes. IL-1β markedly inhibited the expression of STS mRNA and enzyme activity, but stimulated the expression of EST mRNA and enzyme activity. In addition, IL-1β also reduced E2 production from E1S and E1 in VSMCs *(332)*.

Progesterone is an inhibitor of human VSMC proliferation induced by hyperglycemia and hyperinsulinemia in vitro *(333)*. However, in The Heart and Estrogen/Progestin Replacement Study (HERS) carotid B-mode ultrasound examinations substudy, there were no significant treatment effects on carotid IMT *(334)*. Also, in the Los Angeles Atherosclerosis Study the common carotid artery IMT was significantly related to years since bilateral oophorectomy, even though 90% of this group had a history of HRT. This finding which conflicts with a concept that such therapy reverses the adverse effect of bilateral oophorectomy on CHD *(335)*. HRT significantly increased antibodies against Hsp 65 and against LDL with a low degree of oxidative modification. The hormone-mediated immune response may trigger an inflammatory response within the vessel wall and potentially increase plaque burden *(336)*. Also, oral

contraceptives increase the risk of developing CAD in users with CVD risk factors (reviewed in ref. *337*). In addition, estradiol promotes osteoblastic differentiation and calcification on vascular cells (arterial calcification increases the risk of MI) *(338)*.

Men have higher androgen receptors than women in macrophages and dihydrotestosterone causes an increase in macrophage CE content in men but not in women *(339)*. Also, men have enhanced oxidative stress compared to women *(340)*.

However, in ARIC higher total testosterone (T) and sex hormone-binding globulin (SHBG) were found to be inversely related to carotid atherosclerosis in postmenopausal women not using HRT *(341)*. In elderly males, total T levels were inversely independently associated with anti-oxLDL antibodies *(342)*; and, hypotestosteronemia may accelerate the development of atherosclerosis and increase the risk for CHD in obese men in a cross-sectional study *(343)*, T appears to suppress activation of pro-inflammatory cytokines, and men with low T levels are at increased risk of CAD (reviewed in ref. *344)*.

Dehydroepiandrosterone sulfate (DHEA-S) is inversely associated with cardiovascular death in males but not females. DHEA-S correlated positively with HDL-C and negatively with LDL-C, and the mean atherogenic index in a cross-sectional study in men and women *(345)*. An early cardiovascular involvement was detected in severely obese normotensive premenopausal women with hyperinsulinaemia and low DHEA-S, even in the absence of other well-known CVD risk factors *(346)*.

LIPOPROTEINS

Low-Density Lipoprotein

LDL-C is known to cause numerous cellular changes that contribute to the atherosclerotic process through the subendothelial retention of Apo B 100 containing lipoproteins, followed by biologic responses to the retained material (reviewed in ref. *347*). One of the first alterations in EC function induced by LDL-C is an attenuation of endothelial-dependent vasodilation. This reduced responsiveness to vasodilation occurs before any clinical evidence of atherosclerosis. Studies in isolated vessels from normal animals demonstrate a reduction in endothelial-dependent vasodilation within minutes of exposure to oxLDL *(348,349)*.

Lipoprotein Uptake

Lipid homeostasis is dependent on the interaction between receptor mediated and enzymatic reactions that regulate cholesterol and triglycerides. Receptor mediated lipoprotein uptake occurs through different mechanisms:

1. Macrophages express LDL receptors that recognize Apo B- and Apo E-containing lipoproteins. LDL receptors are down regulated by intracellular cholesterol *(350)*. In mice, macrophage LDL receptor is partly responsible for lipid accumulation in foam cells when VLDL is increased under dietary stress, but not with extreme LDL accumulation *(351)*.
2. Scavenger receptors (SR) (reviewed in ref. *352*) recognize modified lipoproteins such as acetylated LDL, oxLDL, or malondialdehyde LDL as well as other negatively charged substances in a nonregulated way *(350,353,354)*. This results in a massive lipid accumulation. Deletion of the SR class A in inbred mouse strain macrophage has no impact on plasma lipids, but causes a reduction in atherosclerotic lesion areas *(355)*, whereas SR class A overexpression in Kupffer cells of the liver of Apo E deficient mice may scavenge atherogenic particles more efficiently from the blood compartment, and thus may cause decreased VLDL-C levels and thus have a protective effect *(356)*.
3. Fc receptor, under certain conditions, can mediate the uptake of lipoprotein-antibody complexes, which results in lipid accumulation *(350)*.
4. The receptor for AGE mediates uptake of glycated lipoprotein (RAGE) *(354)*.
5. Scavenger receptor for phosphatidylserine and oxidized lipoproteins (SR-PSOX) in humans may be involved in oxLDL uptake, and subsequent foam cell transformation in macrophages in vivo *(357)*.
6. Apo B48, a new receptor which binds and internalizes TG-rich lipoproteins, was identified in human M/M. It is colocalized in human atherosclerotic lesion foam cells *(358)*.

Mice with double KO (dKO) to LDLr related protein 5 and Apo E have severe hypercholesterolemia, and advanced atherosclerosis compaired to Apo E KO mice *(359)*. LDLr related protein-1 mediates suppression of SMC migration induced by PDGF, and prevents atherosclerosis by controlling PDGF receptor activation by forming a complex with PDGF receptor *(360)*.

Nonreceptor mediated uptake of lipoprotein by macrophages can also occur by phagocytosis or through secretory enzymes released by macrophages *(350)*. The most important of these enzymes is lipoprotein lipase (LPL), which hydrolyzes TG to FFA. FFAs can be taken up by macrophages and re-esterified. This results in a marked increase in TG accumulation *(361,362)*. In inbred mouse strains, susceptibility to atherosclerosis is associated with high macrophage LPL secretion and mRNA levels *(363)*. In transgenic mice, smooth muscle-derived LPL translocates to the endothelium; increased LPL-mediated FFA loading initiates vascular dysfunction via PKC-mediated activation of endothelial NADPH oxidase despite normal lipoprotein levels *(364)*. However,

LPL in post-heparin plasma is unrelated to the presence or extent of CAD *(365)*, and transgenic rabbits overexpressing LPL are protected against diet-induced hypercholesterolemia and atherosclerosis *(366)*.

In addition to LPL, macrophages secrete oxygen-free radicals, proteases, and Apo E, all of which affect lipoprotein accumulation *(350,353)*. In the ARIC study, Apo E epsilon 2/3 was associated with carotid atherosclerosis, probably through delayed clearance of TG-rich lipoproteins *(367)*. Apo E-deficient mice develop hyperlipidemia and atherosclerosis, and the lipoprotein oxidation occurs in macrophage-rich areas in early lesions. However, in necrotic areas of advanced lesions oxidation occurs diffusely in the extracellular areas *(368)*. Physiologic levels of Apo E in the vessel wall are antiatherogenic in conditions of severe hyperlipidemia, and can affect later stages of plaque development *(369)*.

The enhancement of receptor-mediated-TG-rich lipoprotein uptake is multifactorial. Two very important factors include *conformational changes* in apoproteins resulting in increased affinity for LDL receptor *(362)*, and *loss of Apo C (361)*. Apo C1 acts by interfering with Apo E-mediated lipoprotein uptake, but Apo C1 also has actions independent of Apo E *(370)*.

Sphingomyelin (SM) correlate with the severity of CAD; SMase yields ceramide, ceramide levels are increased in atherosclerotic plaques and in LDL isolated from these lesions. LDL showed SMase activity that could be involved in nonreceptor-mediated endocytotic entry of LDL into cells. Also, LDL aggregetion seems to involve ceramide because ceramide has a tendency for self-aggregation (reviewed in ref. *371*). Ceramide produces oxidative stress in human EC, thereby reducing bioactive NO (*372*).

Lipoprotein Modification

Lipoprotein modification takes place in VSMC, EC, and macrophages.One such modification consists of *peroxidation* of PUFA in LDL, a process that can be inhibited by vitamin E *(373)*. In another cross-sectional study, circulating oxLDL was associated with carotid and femoral artery IMT and plaque occurrence and with TNF-α and CRP *(374)*.

15-Lipooxygenase is important in early human atherosclerosis, whereas in advanced lesions the enzyme is silent and its products, that were accumulated earlier, may be decomposed or superimposed by large amounts of nonenzymatic lipid peroxidation products *(375)*. There is an important in vivo role of vascular 12/15 lipooxygenase in VSMC growth, migration, and matrix responses associated with atherosclerosis *(376)*.

Advanced atheromas (in eroded or ruptured plaques) contain myeloperoxide (MPO), which produces the pro-oxidant species hypochlorous acid (HOCl). Granulocyte macrophage colony-stimulating factor (GMCSF), but not macrophage colony-stimulating factor (MCSF), regulates the ability of macrophages to express MPO. Also, pro-inflammatory stimuli, such as CD40 ligand, LysoPC, or cholesterol crystals induce release of MPO *(377)*. The generation of tyrosyl radicals by MPO allows activated phagocytes to damage both proteins and lipids (reviewed in ref. *378)*. However, MPO deficient LDL-receptor deficient mice on a high-fat, high-cholesterol diet had larger atherosclerotic lesions, so MPO was protective in these mice *(379)*.

It is hypothesized that superoxide produced by neutrophils causes further lipid peroxidation of native LDL and produces oxLDL, which is the source of ceroid pigment in human foam cells *(380)*. There is a vicious cycle of increased NADPH oxidase-dependent superoxide anion formation, augmented generation and uptake of oxLDL, and further potentiation of oxidative stress by oxLDL itself, AII, and ET-1 in EC (reviewed in ref. *381)*. ET-1 stimulated uptake of oxLDL in HUVEC and induces lectin-like oxLDL receptor-1 (LOX-1) via ET-1 receptor B. ET-1 receptor blockade reduces the formation of atherosclerotic lesions in experimental studies *(382)*. Mildly oxidized LDL induces NADPH oxidase expression and superoxide anion formation in HUVEC *(383)*. p47phox(–/–) SMCs had diminished superoxide production and a decreased proliferative response to growth factors compared with wild-type cells supporting the hypothesis that superoxide generation in general, and NADPH oxidase in particular, have a requisite role in atherosclerotic lesion formation *(384)*.

In WHHL rabbits, aortic accumulation of hydroxide of CE and TG is not required nor is α-tocopherol depleted during atherosclerosis *(385)*. It was suggested that the atherogenecity of oxLDL in cultured humans aortic SMC is a result of LDL aggregation and not oxidation *(386)*. PC hydroxyalkenals, a class of oxidized PC, are present in vivo and possess multiple functions characteristic of oxLDL and 4-hydroxynonenal *(387)*.

Copper-zinc superoxide dismutase (CuZn-SOD) inhibits cell-mediated oxidation of LDL, but transgenic mice overexpressing CuZn-SOD might have increased atherosclerotic lesion area *(388)*. The role of copper in atherosclerosis is difficult to predict and the studies are conflicting: copper is an intrinsic constituent of SOD and ceruloplasmin and a component of Lysyl oxidase (the enzyme involved in collagen synthesis, a major component of ECM). Also, copper ions catalyze oxidative modification of LDL in vitro and possibly in vivo (reviewed in ref. *389)*.

Enzymatically modified LDL (E-LDL): combined treatment with trypsin, cholesterol esterase, and neuraminidase transforms LDL, but not HDL or VLDL to particles with properties akin to those of lipid extracted from atherosclerotic lesions. Triple enzyme treatment disrupts the ordered and uniform structure of LDL particles, and gives rise to the formation of inhomogeneous lipid droplets 10–200 nm in diameter with a pronounced net negative charge, but lacking significant amounts of oxidized lipid *(390)*.

LDL modification by group X secretory phospholipase A2 (sPLA2-X) in the arterial vessels is one of the mechanisms responsible for the generation of atherogenic lipoprotein particles. sPLA2-X was found to induce potent hydrolysis of PC in LDL leading to the production of large amounts of unsaturated FA and lyso-PC, which contrasted with little, if any, lipolytic modification of LDL by the classic types of group IB and IIA secretory PLA2s. Treatment with sPLA2-X caused an increase in the negative charge of LDL with little modification of Apo B in contrast to the excessive aggregation and fragmentation of Apo B in oxLDL *(391)*.

The Influences of Modified Lipoproteins

The oxidized fatty acid fragments and sterols diffuse out of LDL into adjacent cells to exert chemotaxis, and the trapping of monocytes into the atherosclerotic lesion as macrophages. oxLDL also alters gene expression for and secretion of growth factors and cytokines by macrophages and EC *(115)*.

Influences on Endothelial Cells

1. oxLDL activation of LOX-1 (the receptor for oxLDL identified on EC) causes activation of NFκB through increased ROS in EC, followed by induction of adhesion molecules and endothelial apoptosis. LOX-1 expression is induced in vitro by many inflammatory cytokines, oxidative stress, hemodynamic stimuli, and oxLDL. In vivo, the expression is induced by hypertension, hyperlipidemia, and diabetes, and is accumulated in the atherosclerotic lesions. Besides oxLDL, LOX-1 can recognize apoptotic/aged cells, activated platelets, and bacteria (reviewed in ref. *392*).
2. Incubation with oxLDL enhances EC production of colony stimulating factors *(115)*. However, MCSF prevents atherosclerosis progression in WHHL rabbits by increasing net CE hydrolysis *(393)*.
3. oxLDL is a chemo-attractant to monocytes, induces monocyte-binding protein, and stimulates the production of MCP-I by EC *(115)*. Oxidized membrane vesicles and apoptotic blebs stimulate EC to specifically bind monocytes, with oxidized phospholipids being the active principle

and responsible for biological activity in MVs and apoptotic blebs *(394)*. LDL, IDL, chylomicrons, and postprandial remnant particles, but not VLDL when mildly oxidized by fibroblasts overexpressing 15-lipooxygenase, increased monocyte chemotaxis and adhesion to EC, although chylomicron and VLDL had an increased 18:1/18:2 ratio (and should be less susceptible to oxidation). Only chylomicrons had no PAF-acyl hydrolase (PAF-AH). Similarly, Japanese subjects with decreased PAF-AH activity stimulated adhesion more. This activity resided in the lipid fraction and could be blocked by PAF receptor antagonist; so the above activity is related to 18:1/18:2 ratio and PAF-AH activity, and that phospholipids such as PAF are generated during lipoprotein oxidation *(395)*. In addition, in Japanese patients with vascular occlusive disease a missense mutation G994T in PAF-AH was significantly more frequent than in controls. Patients with the mutation had more risk factors of CAD and stroke than controls with normal genotypes. Also, within the normal genotype subgroups plasma PAF-AH activity was higher in patients with vascular occlusive disease than in controls *(396)*. In HAEC, phospholipid peroxidation generate free saturated and unsaturated aldehydes and esterified aldehydes (core aldehydes) (aldose reductase may be involved in preventing inflammation and diminishing oxidative stress early in atherosclerosis due to its ability to reduce lipid- derived aldehydes) *(397)*. The binding of CE core aldehydes to LDL might represent the process common to the oxidative modification of lipoprotein *(398)*.

Influences on Thrombogenecity

Vascular thrombogenicity is induced by progressive LDL oxidation, and alterations of the antioxidant/oxidant balance of LDL particle in favor of the antioxidant tone are protective against the thrombotic response triggered by oxidative stress *(399)*. oxLDL may promote expression of CD40 and CD40L in human atheromas. Platelet-enriched plasma of mice deficient in CD40L showed markedly delayed fibrin clot formation *(400)*. oxLDL induces surface tissue factor pathway and dysregulates fibrinolysis with a net increase in the inhibitory rate (reviewed in ref. *401)*.

Effects of Diabetes on LDL Oxidation

Diseases that enhance LDL oxidation include diabetes and hypertriglyceridemia *(115)*. Hyperglycemia increases LDL oxidation, in part, through an increase in glycation products, which subsequently enhances free radical production in stimulated inflammatory cells *(115)*. Insulin and IGF-I cause an upregulation of LDL receptor and down regulation of HDL receptor *(281)*. Insulin increases uptake and esterification of

LDL-C by VSMC (*3*). LDL from diabetic patients is more atherogenic and more likely to be bound (by affinity chromatography on Ricinus communis agglutinin-agarose). Bound LDL has lower sialic acid content and higher fructosyl lysine level, and induces cholesterol accumulation in cultured cells and has lower neutral lipids. Desialylated LDL has a low neutral carbohydrate level, decreased content of major lipids, small size, high density, increased electronegative charge, less phospholipids, and unesterified cholesterol on their surface, with resultant altered tertiary Apo B structure with more exposure of proteoglycans-binding regions and high affinity binding of small dense LDL to arterial proteoglycans. Secretary phospholipase A2 (PLA2) in arterial tissue or plasma reduces phospholipid content in the surface monolayer LDL leading to the formation of small, dense LDL with an enhanced tendency to interact with proteoglycans. The circulating level of secretary phospholipase A2-IIA is an independent risk factor for CAD. Transgenic mice expressing human group IIA secretory phospholipid A2 spontaneously develop atherosclerotic lesions with lower HDL-C and higher LDL/VLDL-C. Also, group IIAs PLA2 can contribute to atherosclerotic lesion development through a mechanism independent of systemic lipoprotein metabolism *(402)* However, in mice, endogenous mouse secretory phospholipase AII gene does not significantly affect HDL or atherosclerosis *(403*; reviewed in ref. *404*). Incubation of LDL with serum induces desialylation, through enzymes close to sialyltransferase, leading to the above changes in addition to loss of α-tocopherol from LDL, increased LDL susceptibility to oxidation, and accumulation of cholesterol covalently bound to Apo B, a marker of lipoperoxidation. Therefore, in diabetics LDL is smaller, denser, electronegative, desialylated, and glycated *(405–407;* and reviewed in ref. *408*). However, a different study concluded that asialylated LDL has little value as a risk factor for coronary atherosclerosis in CAD patients *(409)*. Large numbers of small Apo B 100-containing lipoproteins are far more atherogenic than lower numbers of large Apo B 100-containing lipoproteins despite nearly identical cholesterol levels (reviewed in ref. *410*). Thus, both hyperinsulinemia and increased oxidation of LDL-C likely contribute to the accelerated atherosclerosis of diabetes mellitus.

The role of Apo B containing lipoproteins other than LDL has been reviewed in ref. *411*. βVLDL enhances iNOS expression and nitrite accumulation in IL-1β-stimulated VSMC *(412)*. In rat aortic SMC, remnant lipoproteins transactivate EGF receptor via PKC and the shedding of membrane-bound soluble heparin-binding EGF-like growth factor from SMCs, resulting in SMC proliferation *(413)*.

Oxidative Stress

Oxidative stress includes, in addition to LDL oxidation, the cellular production of ROS. Protein thiol groups are important targets for post-translational protein modification by ROS (reviewed in ref. *414*), and advanced oxidation protein products were found to be an independent risk factor for CAD *(415)*. ROS function through several mechanisms including xanthine oxidase, NAD(P)H oxidases, and NOS. Also, oscillatory shear is a potent stimulus of superoxide production (reviewed in ref. *416*). Oxidative stress in humans with CAD is exacerbated by a reduction of vascular extracellular SOD (reviewed in ref. *417*).

8-isoprostane (8-epiPGF[2α]) is a marker of antioxidant deficiency and oxidative stress status in vivo. High plasma levels of 8-epiPGF(2α) are associated with the extent and severity of CAD, and with the occurrence of different atherosclerotic risk factors *(418)*. However, the elevated levels of another biomarker of oxidative stress, thiobarbituric acid reaction substances (TBARS), were associated with increased risk of CVD prevalence, but not after adjusting for glucose *(419)*.

Oxidative DNA damage and repair increase significantly in human atherosclerotic plaque *(420)*. In human aortic VSMC, oxidative stress increases ET-1 generation and autocrine ET-1 activity in VSMC, contributing to endothelial dysfunction *(421)*. α 1 antitrypsin (AT) is produced and oxidized by macrophages, then attached to LDL in the intimal layer of the arterial wall. Although oxidized AT-LDL complex that escapes into the blood stream can be cleared by hepatocytes, the remaining can be taken up by macrophages and contribute to lipid accumulation in arterial wall cells as the early stage of atherosclerosis *(422)*.

There is increased reactive carbonyl compound (RCO) with attendant protein modification (carbonyl stress) in atherosclerosis carbonyl stress might be derived from hyperglycemia (and lipedemia), oxidative stress, and/or impaired detoxification of RCO *(423)*.

Antioxidant Defenses

1. Thioredoxin, a thiol-disulfide oxidoreductase, is possibly involved in antioxidant protection in human coronary arteries *(424)*.
2. Exercise-induced plasma oxidative stress could be responsible for the prevention of atherosclerosis by stimulating arterial antioxidant response. In addition, vitamin E could be deleterious in exercisers by inhibiting antioxidant enzyme buildup in the arterial wall in LDL receptor –/– male mice fed an atherogenic diet *(425)*.
3. In humans, a greater activity of antioxidant enzyme in intracranial arteries may contribute to their greater resistance to atherosclerosis.

With increasing age intracranial arteries respond with accelerated atherogenesis when their antioxidant protection decreases, relatively more than that of extracranial arteries *(426)*.

4. Uncoupling protein 2 (UCP2) regulates the production of ROS in macrophages, and it has a protective role against atherosclerosis *(427)*.
5. The p66(Shc–/–) mouse is the unique genetic model of increased resistance to oxidative stress and prolonged life span in mammals *(428)*.

Other Harmful Factors

Phospholipid transfer protein (PLTP) (carrier protein that shuttles between lipoproteins to redistribute lipids) deficiency in mice is associated with decreased atherosclerosis despite decreased HDL levels. Two mechanisms are involved: decreased Apo B-containing lipoprotein production and levels, and increased antioxidation potential. Human studies indicated that PLTP activity positively correlated with aging, obesity, DM, and CAD (reviewed in ref. *429*). PLTP mRNA protein expression and activity was increased by cholesterol loading of macrophages. PLTP increased HDL binding to biglycan, suggesting a role in lipoprotein retention on ECM *(430)*.

Acyl CoA:cholesterol acyltransferase (ACAT) in Apo E/LDLr double KO mice, may be responsible for lipid accumulation in atherosclerotic lesions, and its inhibition diminishes the lipid deposition via a direct effect on macrophages in the arterial wall *(431)*. In atherosclerotic plaque, the ability of macrophage foam cell transformation may be augmented by the dual expressions of ACAT1 and ACAT2 *(432)*. ACAT2 deficiency, in Apo E deficient mice, results in decreased CE synthesis in the small intestine and the liver. ACAT2-derived CE has a crucial role in the development of atherosclerosis in mice and suggest that TG-rich Apo B-containing lipoproteins are not as atherogenic as those containing CE *(433)*.

Bile salt dependent lipase, also known as carboxyl ester lipase, secreted by acinar cells of the pancreas and associated with LDL in blood, locates with SMC in atherosclerotic lesions and triggers SMC proliferation *(434)*. It may also play a role in lipoprotein metabolism and oxLDL-induced atherosclerosis (reviewed in ref. *435*).

Clusterin/apolipoprotein J (Apo J), a conserved secreted glycoprotein, has been associated with normal and premature senescence. Its serum levels are increased with age and in CHD patients *(436)*.

Other Protective Factors

Hepatic lipase (HL) is synthesized de novo by macrophages in addition to the liver *(437)*. Low HL activity in humans is associated with

CAD, and the presence of T allele at position –514 in the HL promoter that leads to reduced promoter activity was associated with lower HL activity and higher CAD extent *(438)*. Increased hydrolysis of CE by the overexpression of hormone sensitive lipase leads to complete elimination of CE from foam cells by increasing efflux and decreasing influx of cholesterol *(439)*.

TGF-β1 inhibited the activity and expression of two key components needed for VLDL-remnants uptake, LPL and LDL receptor. TGF-β1 inhibited CE mass induced by oxVLDL-remnants in part by decreasing the expression of scavenger receptor type AI/II and CD36. Furthermore, TGF-β1 enhanced cholesterol efflux through upregulation of the ATP-binding cassette (ABC) transporters ABCA1 and ABCG1 *(440)*.

Iron

In a prospective study, serum ferritin was one of the strongest risk predictors of atherosclerotic progression. The mechanism appeared to be through modification of LDL-C atherogenic potential. Lowering iron stores was beneficial during the follow-up period, also, LDL-C and ferritin were synergistic. This study could explain gender differences in atherosclerosis *(441)*. However, there are conflicting data. In a case control study, ARIC, no association could be found between body iron stores and early atherosclerosis *(442)*. In New Zealand White rabbits on a chow diet containing 1% (wt/wt) cholesterol, iron overload decreased atherosclerosis secondary to decreased cholesterol levels. Iron overload and deficiency had no effect on LDL oxidation susceptibility, and iron deficiency had no effect on atherosclerosis *(443)*.

Lipoprotein(a)

The role of lipoprotein(a) [Lp(a)] in the atherosclerotic process has been extensively reviewed *(444,445)*. Lp(a) is an LDL-like particle with Apo B100 and Apo (a) components, the latter similar to plasmin *(445)*. A summary of the effects of Lp(a) on the atherosclerotic process is summarized in Table 3.

A cross-sectional study, ARIC, suggest that Lp(a) is a risk factor for preclinical and clinical atherosclerosis *(446)*. However, it was found to be an independent factor associated with thickening of common carotid arteries only in severely hypercholesterolemic patients, and not in patients with moderately elevated or normal levels of blood cholesterol *(447)*. Also, Lp(a) transgenic mice develop lipid lesions in aorta more frequently than nontransgenic animals even on a low-fat diet *(448)*.

Table 3
Some Effects of Lp(a) on Factors in the Development of Atherosclerosis

1. Interferes with clot lysis mechanisms
   - Down regulates plasmin
   - Facilitates matrix lipoprotein deposition
   - Increases plasminogen activator inhibitor (PAI)-1
2. Increase plaque formation
   - Facilitates macrophage migration into VSMC
   - Promotes intimal deposition of Apo B
   - Inhibits TGF-β activity

Normal vasculature does not contain Lp(a), however, the vascular content of Lp(a) increases in a variety of inflammatory conditions *(445)*; large amounts of Lp(a) are found in atherosclerotic lesions. In early atherosclerotic lesions in humans and animals, there is a dramatic deposition of Lp(a) on the thickened intimal endothelial surface *(445)*. Whereas LDL in atherosclerotic arteries is exclusively blood-derived, the accumulation of Lp(a) within the artery may be caused in part to in situ production of Apo A within the vessel wall *(449)*.

Lp(a) can be modified by oxidative events and by the actions of lipolytic and proteolytic enzymes in the generation of products that exhibit atherothrombogenic potential (reviewed in ref. *450*); oxidized Lp(a) is more potent than native Lp(a) in stimulating human VSMC growth *(451)*. Small size Apo A isoforms may represent a cardiovascular risk factor by themselves or with plasma Lp(a) concentrations (reviewed in ref. *450*).

The Effects of Lp(a)

1. Lp(a) stimulates VCAM-1 and E-selectin production by cultured human coronary artery EC *(452)*.
2. Lp(a) in tissues may promote VSMC migration by down regulating plasmin generation at the cell surface, and thereby inhibit latent TGF-β activation *(445)*. Thus, Lp(a) may indirectly increase VSMC migration as TGF-β normally inhibits this process *(445)*;
3. Lysosomal acid lipase (LAL) expression is suppressed in monocytes from patients with Lp(a) hyperlipidemia and by purified Lp(a). IL-6 secretion was also induced by purified Lp(a) *(453)*.
4. Apo (a) is produced in situ in the vessel wall. Lp(a) binds and inactivates TFPI and induces PAI type 2 expression in monocytes (reviewed in ref. *454*). Apo A has antithrombolytic potential as a result of its plasmino-

gen-like properties at the endothelial and subendothelial intima: (1) At the endothelial surface, high plasma levels of Lp(a) can interfere with plasminogen-plasmin conversion and clot lysis *(455)*, and (2) Lp(a) can traverse endothelium and accumulate in intima as lipid poor Apo B100-Apo A complex or free Apo A *(445)*. High Lp(a) plasma levels and increased endothelial permeability increases the transfer of Lp(a) to the intima *(444)*. Once in the intima Lp(a) can complex with glycosaminoglycans, proteoglycans, or fibrin. Lp(a) can aggregate and also bind to glucosaminoglycans, fibrin, and fibronectin, and gets retained in the ECM and phagocytosed by macrophage. Plasmin enhances the binding of Lp(a) to immobilized fibrinogen and fibrin leading to increased incorporation of fibrin into the vessel wall *(445)*.

5. Lp(a) levels are related to HLA class 2 in males with early CAD, and it is suggested that an autoimmune process may occur in patients with inherited high Lp(a) levels with certain HLA class 2 genotypes *(456)*. Lp(a) also becomes incorporated into macrophages in the intima leading to formation of foam cells *(457)*.

Thus, Lp(a) appears to be an important factor in promoting atherogenesis under certain conditions, however, Lp(a) has potential regenerative and antineoplastic properties that might paradoxically counterbalance its atherothrombogenicity as attested by the compatibility between raised plasma Lp(a) levels and longevity (reviewed in ref. *458*).

High-Density Lipoprotein

HDL, in contrast to LDL, is thought to protect against development of atherosclerosis. The effects of HDL are summarized in Table 4. A prospective study in Japanese-American men (the Honolulu Heart Program) has shown that the risk of atherosclerosis is increased in subjects with low HDL-C and high TG when TC is borderline high or high but not when TC is desirable, independent of other risk factors *(459)*. Increasing HDL-C levels in Apo E KO mice retards progression of advanced atherosclerotic lesions and remodels them to a more stable appearing phenotype *(460)*.

Decreased Apo A1 appears to be a major component of the dyslipidemic serum profile in patients with atherosclerotic occlusive disease of the lower extremities *(461)*. Despite similar TC and HDL-C levels, A1 HDL is more anti-atherogenic than A1/A2 HDL in transgenic mice *(462)*. This is because lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester trasfer protein (CETP) are present mainly in Lp A1 and because Lp A1:A2 can inhibit Lp A1-promoted cholesterol efflux. Also, mice overexpressing Apo A1 have more cholesterol efflux and are more protected against atherosclerosis mediated through decreased foam cell

Table 4
Some Effects of HDL-Cholesterol on Atherosclerotic Process

Reverse cholesterol transport*
Inhibits VSMC mitogens and matrix formation
Stimulates endothelial cell repair
Stimulates arterial endothelial cell PGI2 synthesis
Facilitates metabolism of triglyceride rich lipoproteins
Faciliates fibrinolysis

*Protects LDL from oxidation.

formation in vivo, and mice overexpressing Apo A2 have the opposite effects *(463,464)*. Apo A1 expression from macrophages protects against atherosclerosis without affecting plasma Apo A1 and HDL-C levels *(465)*.

Transgenic mice expressing CETP had worse atherosclerosis as a result of CETP-induced alterations in the lipoprotein profile *(466)*. Also, humans with heterozygous CETP deficiency and HDL-C > 60 mg/dL have a reduced risk of CHD (reviewed in ref. *467*). However, CETP inhibition alone did not have an anti atherogenic effect in a rabbit model of severe hypercholesterolemia, suggesting a relatively minor effect of raising HDL (through CETP inhibition) as compared with a decrease in non-HDL-C or TG levels *(468)*. On the other hand, in humans CETP is expressed in macrophages in the atherosclerotic lesions and may possess an antiatherogenic function to remove cholesterol from the cells *(469)*.

LCAT deficiency in LDLr –/– mice and Apo E –/– mice fed an atherogenic diet, resulted in aortic cholesterol deposition likely caused by a reduction in plasma HDL, increased saturation of CE in apo B lipoproteins, and in the Apo E –/– background, increased plasma Apo B lipoprotein concentration *(470)*. LCAT-deficient mice are associated with an increase in oxidative stress that is paradoxically reversed in a hyperlipidemic background possibly caused by the redistribution of paraoxonase (PON) to the non-HDL fraction. This may in part contribute to the reduced atherosclerosis seen in Apo E –/– xLCAT –/– mice (this could explain the surprising finding that LCAT-deficient subjects have severe hypoalphalipoproteinemia yet are not prone to premature CHD) *(471)*.

Apo AII alters reverse cholesterol transport, but also may inhibit LCAT activity (deleterious effect), CETP activity (beneficial effect), may increase hepatic lipase activity (beneficial effect), and may inhibit the hepatic cholesteryl uptake from HDL (deleterious effect) (reviewed in ref. *472*). There is a positive association of Apo AII with FFA and

VLDL-TG plasma concentration. Apo AII transgenic mice have increased concentration of Apo B-containing lipoproteins and increased atherosclerotic susceptibility. They also have impairment of two functions: reverse cholesterol transport and protection of LDL oxidative modification (reviewed in ref. *473*).

ATP-binding cassette transporter class A1 (ABCA1) transmembrane protein is crucial for efficient efflux of cellular cholesterol and HDL maturation. Mutations in ABCA1 are the cause of Tangier disease characterized by near absent HDL (reviewed in ref. *474*). ABCA1 might regulate intestinal cholesterol absorption. Unsaturated FA can reduce ABCA1 gene activity by enhancing its degradation, where liver X receptors (LXR) agonists significantly increase expression of ABCA1 gene. Preliminary evidence suggests that increasing ABCA1 gene expression may be beneficial in the prevention of diet-induced atherosclerosis (reviewed in ref. *475*). Early in free cholesterol (FC) loading of macrophages, there is an increased degradation of ABCA1 with consequent decreased ABCA1-mediated cholesterol and phospholipid efflux. Also, in advanced atherosclerosis foam cells accumulate FC contributing to foam cell death and lesional necrosis *(476)*.

Individuals with ABCA1 mutations had lower amounts of cholesterol efflux, lower HDL-C, and greater IMT than controls *(477)*. Apo E –/– mice overexpressing human ABCA1 developed smaller less complex lesions, had increased efflux of cholesterol from macrophages, their HDL particles were significantly better acceptor of cholesterol, and had increased phospholipid levels, which correlated significantly with their ability to enhance cholesterol efflux *(478)*. Additionally, in LDLr — and Apo E –/– mice complete absence of ABCA1 has a major impact on plasma lipoprotein homeostasis, and the proposed anti-atherogenic effect resulting from ABCA1 deficiency is compensated by a less atherogenic profile. However, in ABCA1 deficiency in macrophages only the anti-atherogenic properties of ABCA1 are demonstrable independent of plasma lipids and HDL levels *(479)*.

Scavenger receptor class B type 1 (SR B1) facilitates the efflux of cholesterol in peripheral tissues to HDL and mediates the selective uptake of CE from HDL in the liver. SR B1 deficiency in mice is associated with deregulation of cholesterol homeostasis in the arterial wall, resulting in increased susceptibility to atherosclerosis with increased expression of inflammatory markers and lipid deposition in the aorta *(480)*. Inflammatory mediators down regulate SR B1 in macrophages *(481)*.

HDL, obtained from monkeys, is susceptible to oxidation and oxidized HDL caused a decrease in free cholesterol efflux, an increase in

LDL uptake by macrophages, a decrease in monocyte antioxidant enzymes, and an increase in the number of monocytes adherent to endothelium *(482)*. PON is an esterase associated with HDL that destroys oxidized lipids by hydrolyzing LDL-associated phospholipids and CE hydroperoxides. Low CHD risk is associated with polymorphism of PON-1 (reviewed in ref. *483*). PON-1 activity and concentration were significantly lower in subjects with CHD than in control subjects *(484)*. In inbred mice on atherogenic diet that are either susceptible or resistant to atherosclerosis, loss of HDL ability to protect against LDL oxidation is associated with decreased serum PON levels, and, in recombinant inbred mice derived from the parental strains, low PON mRNA levels segregated with aortic lesion development *(485)*. In addition, a pro-atherosclerotic diet reduced PON activity in transgenic rabbits over-expressing Apo A1. However, the areas covered by lesions were similar to the control group *(486)*. Human PON-1 transgenic mice (dietary or Apo E null mouse models) had significantly reduced atherosclerotic lesions, and HDL isolated from these mice protected against LDL oxidation *(487)*. Also, PON-11 inhibits macrophage cholesterol biosynthesis and atherogenesis in mice *(488)*. In mice, increased macrophage PON-2 expression under oxidative stress could represent a selective cellular response to reduce oxidative burden that may lead to attenuation of macrophage foam cell formation *(489)*. Other factors that protect LDL against oxidation, and thus shown to attenuate progression or prevent the atherosclerotic process include antioxidants such as vitamins C and E and the trace element magnesium *(490,491)*. These antioxidants may have a particularly important prophylactic role in diabetic patients who are especially prone to LDL oxidation.

Premature atherosclerosis can occur in patients with familial chylomicronemia resulting from mutation in the LPL gene *(492)*. Approximately 1 in 20 male patients with atherosclerosis have a Asn291Ser mutation in the human LPL gene that is associated with decreased LPL activity and HDL levels (the relative frequency of this mutation is higher in patients with lower HDL levels) *(493)*.

sPLA(2)-X (involved in the pathogenesis of atherosclerosis via potent lipolysis of LDL leading to macrophage foam cell formation) as well as group V secretory PLA(2) (sPLA(2)-V) [another group of sPLA(2) that can potently hydrolyze phosphatidylcholine (PC)] also possess potent hydrolytic potency for PC in HDL linked to the production of a large amount of unsaturated fatty acids and LysoPC. Modification with sPLA(2)-X or -V resulted in significant decrease in the capacity of HDL to cause cellular cholesterol efflux from lipid-loaded macrophages *(494)*.

Endothelial lipase is synthesized and functions at the endothelium, and has primarily phospholipase A1 and TG lipase activities. No endothelial lipase activity was detected under basal condition, however, its expression is highly regulated by cytokines and physical forces. It is a major determinant of HDL concentration, structure, and metabolism in mice and a major determinant of HDL concentration in humans: its expression in animals leads to decreased HDL-C levels, so it might play a role in atherosclerosis development (reviewed in refs. *495–497*).

Diet-induced reduction in plasma HDL shows a physiological and a genetic correlation with repression of cholesterol-7-α-hydroxylase, the liver specific enzyme that regulates the conversion of cholesterol into bile acids. Constitutively expressing this enzyme in mice prevented them from developing atherosclerosis and developing decreased HDL-C levels *(498)*.

Effects of HDLs

HDL inhibits monocyte chemotaxis, inhibits the adhesion of monocyte and blood cell to vascular endothelium, inhibits endothelial dysfunction and apoptosis, inhibits LDL oxidation, inhibits complement activation, reduces platelet aggregability and coagulation, inhibits platelet activation, and inhibits factor X activation. HDL helps maintain endothelial integrity, facilitate vascular relaxation, stimulates the proliferation of EC and SMC, stimulates the synthesis of prostacyclin and natriuretic peptide C in EC, stimulates protein C and S activation, and may favor fibrinolysis. These functions are exerted by different components of HDL, this complexity emphasizes that changes in HDL functioning rather than plasma HDL-C levels determine the anti-atherogenicity of therapeutic alterations of HDL metabolism (reviewed in refs. *499* and *500*).

Other Factors Involved in HDL Metabolism

LXR are nuclear receptors activated by oxysterols. Their activation may protect against tissue cholesterol overload (reviewed in ref. *501*). LXR and their ligands are negative regulators of macrophage inflammatory gene expression, in addition to being established mediators of lipid-inducible gene expression (reviewed in ref. *502*). The elimination of macrophage LXR activity causes aberrant regulation of cholesterol transporter expression, lipid accumulation in macrophages, splenomegaly, and increased atherosclerosis, suggesting that LXRs are endogenous inhibitors of atherogenesis *(503)*. Similar results were found in mice depleted for LXRα and -β with impaired TG metabolism, increased LDL-C, and decreased HDL-C *(504)*. Additionally, LXR activation induces the expression of PLTP in the liver and in macrophages. PLTP is highly expressed in macrophages within human atherosclerotic lesions

(505). Also, LXRα and LXRβ inhibit basal and cytokine-inducible expression of MMP-9 in murine peritoneal macrophages *(506)*.

PPARs enhance cholesterol efflux and stimulate critical steps of the reverse cholesterol transport pathway (reviewed in ref. *507*). PUFA and activating PPAR increase hepatic cholesterol uptake. PPARγ induces expression of SR B1 in rat hepatocytes, liver EC, and Kuppfer cells *(508)*. PPARα activation in human macrophages and foam cells results in an enhanced availability of free cholesterol for efflux through the ABCA1 pathway by reducing cholesterol esterification rates and ACAT1 activity *(509)*.

PPARγ may have an anti-atherosclerotic action by inhibiting thromboxane receptor gene expression in VSMC *(510)*. PDGF-induced PPARγ expression in human aortic VSMC; this PDGF-induced PPAR expression might provide a feedback mechanism by which PPARγ inhibits PDGF-induced VSMC proliferation and migration *(511)*.

However, PPARα and γ increase human macrophage LPL secretion (vascular wall LPL is proatherogenic) *(512)*. Also, PPARΔ controls the inflammatory status of the macrophage; deletion of PPARΔ from foam cells increased the availability of inflammatory suppressors, which in turn reduced atherosclerotic lesion area by more than 50% *(513)*.

Fatty-acid binding proteins (FABP) are involved in fatty acid metabolism and cellular lipid transport. Adipocyte FABP (aP2) is also expressed in macrophages; bone marrow transplantation from Apo E –/–, aP2 –/– mice to Apo E–/– mice led to the development of smaller atherosclerotic lesions in recipient mice without differences in cholesterol, glucose, or insulin in plasma *(514)*.

oxLDL-mediated increases in aP2 gene expression accelerate CE accumulation, which is important in converting macrophages to foam cells. aP2 is detected in foam cells in active atherosclerotic lesions. The induction of aP2 expression by oxLDL involves activation of PPARγ by components of oxLDL that also function as PPARγ ligands suggesting that PPARγ agonists, i.e., TZD, might exacerbate atherosclerosis *(515)*.

EXTRACELLULAR MATRIX

Production of ECM is regulated by a number of growth factors:

1. In Apo E deficient mice there is an important role for leukocyte-derived osteopontin (OPN) in mediating AII-accelerated atherosclerosis and aneurysm formation *(516)*.
2. Human vessels susceptible to atherosclerosis showed increased accumulation of subendothelial proteoglycans (caused by increased synthesis and not to decreased degradation) mediated by TGF-β *(517)*.

Several ECM components have an influence on the atherosclerotic process. OPN is a soluble secreted phosphoprotein that binds with high affinity to several integrins, and it has been found at the site of atherosclerotic lesions. In OPN transgenic mice, the expression of OPN induces medial thickening without injury and neointimal formation after injury *(518)*. OPN is atherogenic and macrophages expressing OPN can be easily activated and thus promote atheromatous lesions if a high fat diet is consumed in mice *(519)*.

Other ECM components include connexin43 (Cx43) *(520)*, caveolin (Cav)-1 *(521)*, collagen VIII *(522)*, lumican protein *(523)*, and galactosaminoglycans *(524)*.

ECM–Lipoproteins Interaction

Regions of blood vessels that accumulate proteoglycans have a high propensity to accumulate lipid, particularly in areas associated with endothelial regrowth *(72)*. LDL-proteoglycan complexes have been isolated from different regions of ECM within atherosclerotic vessels.

Lipids influence the proteoglycan content of the vascular wall and proteoglycans, and, consequently, influence lipid deposition in VSMC and macrophages *(72)*. Proteoglycans accomplish this by altering the charge of lipids, decreasing degradation of LDL, and increasing CE synthesis by macrophages *(72)*. Endothelial-derived proteoglycans bind to and modify LDL so that it becomes more negatively charged, allowing greater recognition by macrophages and incorporation to form foam cells. ECM proteoglycans bind and retain specific positively charged domains on Apo B and Apo E lipoproteins with resultant modifications altering their interaction with ECM molecules *(525)*. The interaction between atherogenic lipoproteins and proteoglycans involves an ionic interaction between basic amino acids in Apo B 100 and negatively charged sulphate groups on the proteoglycans. Mice expressing proteoglycan binding-defective LDL developed significantly less atherosclerosis than mice expressing wild type control LDL, therefore, subendothelial retention of Apo B100-containing lipoproteins is an early step in atherosclerosis *(526)*. The interaction of ECM with Apo B100 lipoproteins causes structural modification in lipoproteins that increase their susceptibility to proteases, phospholipases, and free radical-mediated processes *(527)*.

oxLDL, growth factors, and cytokines influence proteoglycan structure, rendering them more likely to bind and retain lipoproteins *(525)*. In vitro, elevated levels of nonesterified fatty acids (NEFA) alters the matrix of EC basement membrane, making them more permeable to macromolecules. NEFA causes changes in the expression of genes controlling

the proteoglycan composition from human arterial SMC, causing formation of a matrix with high affinity for LDL (reviewed in ref. *527*).

Extracellular Matrix Degradation

Vascular remodeling, defined as any enduring change in the size and/or composition of an adult blood vessel, allows adaptation and repair. On the other hand, inappropriate remodeling, including its absence, underlies the pathogenesis of major cardiovascular diseases such as atherosclerosis and restenosis. Because degradation of ECM scaffold enables reshaping of tissue, participation of specialized enzymes called MMPs has become the object of intense recent interest in relation to physiological (*good*) and pathological (*bad*) vascular remodeling. The major drivers of vascular remodeling, hemodynamics, injury, inflammation, and oxidative stress regulate MMP expression and activity. Alternatively, nonspecific MMP inhibition seems to oppose remodeling as suggested by the inhibition of intimal thickening and outward arterial remodeling. MMP-related genetic variation may contribute to heterogeneity in the presentation and natural history of atherosclerosis (reviewed in ref. *528*). In vitro evidence suggests that MMP activity may facilitate atherosclerosis, plaque destabilization, and platelet aggregation (reviewed in ref. *529*).

Degradation of ECM can influence plaque rupture. MMPs were implicated in this process through imbalance with endogenous inhibitors. Tissue factor pathway inhibitor (TFPI)-2 is one such inhibitor whose expression, in contrast to the classic tissue inhibitor of MMPs (TIMPs), correlated negatively with MMP level in human atheromas. TFPI-2 colocalized with SMC in the normal media and fibrous cap, but weakly at the site of plaque rupture (macrophage enriched shoulder region). Human phagocytes, an abundant source of MMP, lost their ability to express this inhibitor during their differentiation in vitro *(530)*.

Lipoproteins can influence matrix-degrading enzymes, thus influencing plaque stability *(525)*. LDL increases the secretion of MMP-1 and collagen 1 mRNA in cultured human skin fibroblasts, thus LDL affects tissue remodeling *(531)*. However, Apo E KO mice expressing MMP-1 specifically in the macrophage and on atherogenic diet, demonstrated decreased lesion size without plaque rupture (lesions were less extensive and immature with fewer cellular layers, and diminished contents of fibrillar collagen) *(532)*.

MMP-9 appears to be involved, not only in degradation, but also in reorganization of a collagenous matrix, both facets being essential for the outcome of arterial remodeling *(533)*. Serum MMP-9 is elevated in patients with severe coronary stenosis compared with controls (arterial inflammation is reflected in increased serum concentration of MMP-9)

(534). Leukocyte-derived MMP-9 is associated with aortic wall degeneration and aneurysm formation *(535)*.

Increased expression of several cathepsins in atherosclerotic lesions suggests that these proteases may participate in the remodeling of ECM associated with the atherosclerotic process *(536)*. Human atherosclerotic lesions overexpress cathepsin S (lysosomal cysteine protease) and have low levels of the endogenous cathepsin S inhibitor, cystatin C, compared with normal arteries. LDL receptor –/– cathepsin S –/– mice developed less atherosclerosis than LDL receptor –/– mice *(537)*.

Plasmin, generated by macrophage-secreted u-PA, activates pro-MMP-3 produced by accumulated macrophages. MMP-3 activity may then contribute to a reduction of plaque size, possibly by degradation of matrix components, and promote aneurysm formation by degradation of the elastica lamina *(538)*.

COX-2 and prostaglandin E synthase (PGES) are colocalized in symptomatic lesions. Synthesis of COX-2 and PGES by activated macrophages is associated with acute ischemic syndromes, possibly through MMP-induced plaque rupture *(539)*.

The Role of Extracellular Matrix in Vascular Calcification and Ossification

Proteoglycans accumulate in intimal lesions of large and small vessels in atherosclerosis, and may enhance the calcification associated with increasing complexity of the atherosclerotic lesion. In bovine aortic media, arterial calcification can be regulated by matrix composition (increased in the presence of collagen 1 and fibronectin and decreased in the presence of collagen IV) through interaction with α 5 integrins *(540)*.

Bone formation tends to occur in heavily calcified carotid lesions devoid of ulceration and hemorrhage; patients with extensive calcification of the carotid plaques are less likely to have symptomatic disease *(541)*. When human atherosclerotic plaques demonstrated calcification or bone formation, bone morphogenetic protein (BMP)-2, BMP-4, osteopontin, and osteonectin were upregulated. Interestingly, this upregulation was associated with a sustained immunoreactivity of matrix Gla protein, osteocalcin, and bone sialoprotein *(542)*. Osteoprotegerin (OPG) serum levels are associated with the severity of CAD and are increased in elderly men and patients with diabetes mellitus, thus increased OPG serum levels may reflect advanced cardiovascular disease in men *(543)*. Endochondral ossification is another possible mechanism by which calcification of vascular tissue may occur in oophorectomized aged female Sprague Dawley rats *(544)*.

Lp(a) accelerates advanced atherosclerotic lesion formation and may play an important role in vascular calcification through increased alkaline phosphatase activity and enhanced calcium accumulation in cultured rabbit aortic SMC *(545)*.

HDL reduces osteoblastic differentiation and calcification of vascular cells. HDL also inhibited the osteogenic activity that was induced by IL-1β, IL-6, and oxLDL. Oxidation of HDL renders it pro-osteogenic *(546)*.

Macrophage TNF-α and oncostatin M, only when applied together, increased alkaline phosphatase activities and in vitro calcification in human VSMC in the presence of IFN-γ and 1α,25-dihydroxyvitamin D3 *(547)*. Thus, proteoglycans and other extracellular substances contribute significantly to the progression of the atherosclerotic lesion.

PHYSIOLOGICAL ANTAGONISTS OF ATHEROSCLEROSIS

Macrophages synthesize and release growth factors, cytokines, adhesive glycoproteins, prostaglandins, and leukotrienes. One of the prostaglandins PGI2 has significant antiatherosclerotic properties. PGI2 synthesis is reduced in human, rabbit, and rat atherosclerotic blood vessels *(72)*. DM reduces PGI2 synthesis in rats, an effect that is additive in the presence of an increased blood cholesterol *(11)*. Other factors that decrease vascular eicosanoid synthesis include smoking, aging, and viral infections. This might be related, in part, to decreased arachidonic acid availability for synthesis of PGI2 *(72)*. These eicosanoids, particularly PGI2 and PGE2, normally hydrolyze cellular CE forming free cholesterol, which is more readily removed from the cell *(72)*. HDL induces PGI2 production in vascular EC and VSMC, which contributes to HDL-mediated cholesterol efflux *(72)*. Also, endogenous PGE2 may modulate inflammation during atherogenesis *(548)*.

Cholesterol enriched VSMC (foam cells) synthesize less eicosanoid, and thus are not as responsive to the cholesterol efflux effects of HDL. EC synthesize PGI2 in response to thrombin, bradykinin, leukotrienes, kallikreins, immune complexes, complement complexes, histamine, serotonin, and AII *(72)*. Thus, EC themselves can modulate the production of factors that impact on the atherosclerotic process.

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9 Lower Extremity Arterial Disease Co-Existing With Coronary Artery Disease

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CONTENTS

INTRODUCTION

The most common risk factor for both disease entities is age; older than 40 years of age for men and older than 50 years of age for women. As the population ages, the prevalence of coronary artery disease (CAD), lower extremity arterial disease (LEAD), carotid disease, and overall cardiovascular disease increases (Fig. 1).

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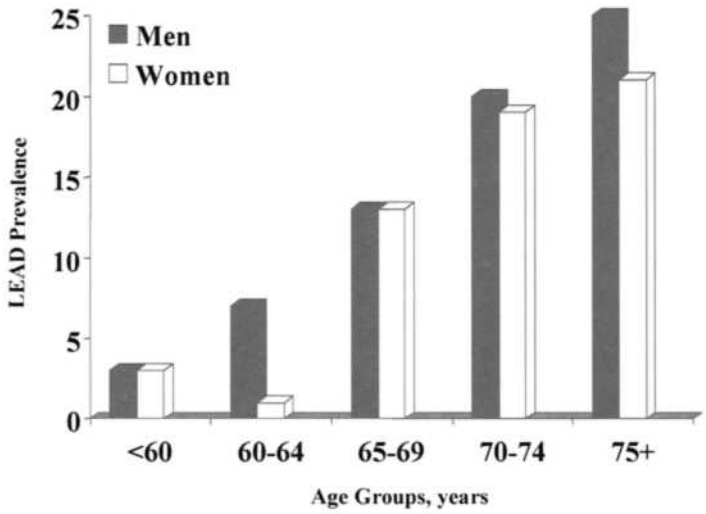

Fig. 1. The increased prevalence of LEAD in men and women with advancing age. (Reprinted from ref. *36* with permission from the Society for Vascular Surgery and the American Association for Vascular Surgery.)

RISK FACTORS OF LEAD CO-EXISTING WITH CORONARY ARTERY DISEASE OTHER THAN OVER 40 YEARS OF AGE

- Obesity, as measured by the body mass index (BMI), is a risk factor for both moderate and severe LEAD and coronary heart disease (CHD) *(1,2)*. It should be noted that approximately 90% of obese patients with moderate LEAD *(1)* are claudication free.
- Cigaret smoking as measured by the number of packs/years is the most prevalent risk factor for CAD and severe LEAD *(3)*. Of patients with severe LEAD, 72.2% are asymptomatic *(1)*.
- Significant associations ($p < 0.05$) exist between:
- Elevated serum triglycerides and LEAD *(4)*.
  - Systolic and diastolic hypertension and LEAD *(4)*.
  - In men, elevated fasting blood glucose is a significant risk factor for LEAD *(4–6)*.
- Low serum values of high-density lipoprotein cholesterol (HDL-C) are an important risk factor for CAD and severe LEAD *(4,7,8)*. In men, low blood levels of HDL are found in very symptomatic patients with disabling intermittent claudication.
- Physical inactivity causes low HDL levels and low HDL is a risk factor for symptomatic LEAD and for CHD *(9)*.

Table 1
Risk Factors for LEAD Co-Exisitng With CAD

Modifiable
• Smoking
• Diabetes
• Obesity
• Hypertension
• Dyslipidemia
• Hyperhomocysteinemia
Nonmodifiable
• Advanced age
• Male gender
• Postmenopausal
• Positive family history

- Diabetes mellitus is the strongest risk factor for LEAD *(4,10)* in tandem with cigaret smoking *(3,4)*. Elevated low-density lipoprotein cholesterol (LDL-C) alone is not a separate risk factor for LEAD, but it is for CAD *(11)* (Table 1).
- The combination of hypertriglyceridemia with low HDL and decreased LDL-C particle size is a dislipidemia known as *pattern B* dislipidemia. In pattern B dislipidemia the most important role in atherogenesis leading to LEAD is played by the high level of triglycerides *(4,12)*. Another type of dislipidemia is encountered in syndrome X, characterized by high triglycerides and low HDL. Aggression (aggressive personality) has also been associated with LEAD and CAD *(12)*.
- In (dis)metabolic syndrome X (Table 2) insulin resistance plays a pivotal role for the development of diabetes mellitus, hypertriglyceridemia, and LEAD.

Other risk factors for both LEAD and CAD are:

- Apolipoprotein B level; taken as a single measurement, the apolipoprotein B level alerts the clinician on the number of potentially atherogenic particles *(13)*.
- High lipoprotein (a) [Lp(a)] *(14–17)*. There is no effective pharmacologic agent to significantly reduce the high Lp(a) level; niacin has a modest effect—an effective strategy is to keep the LDL-C very low. This approach markedly reduces the atherogenic effects of elevated Lp(a) *(18)*. Lp(a) is a lipid particle similar to LDL with an additional attached glycoprotein apoprotein (a), which has been associated with premature atherosclerosis and thrombosis *(19)*. A trial comparing the effects of statin therapy alone vs combination therapy with apheresis and statin to lower Lp(a) showed that Lp(a) is an independent risk factor

Table 2
Diagnostic Criteria for Metabolic Syndrome

Feature	*Criterion**
Abdominal girth	Waist circumference
Men	>102 cm (40 in)
Women	>88 cm (35 in)
Fasting plasma HDL-C	
Men	<40 mg/dL (1.03 mmol/L)
Women	<50 mg/dL (1.29 mmol/L)
Fasting plasma triglycerides	≥150 mg/dL (1.69 mmol/L)
Fasting blood glucose	≥110 mg/dL (6.1 mmol/L)
Blood pressure	≥130/85 mm Hg

HDL-C, high-density lipoprotein cholesterol.
*A diagnosis of metabolic syndrome is made if a patient has three or more of the criteria listed.
Adapted from Adult Treatment Panel III *(11)*.

for the development of LEAD *(20)*. Combination treatment of apheresis and statin resulted in a significant reduction in serum Lp(a) compared to statin therapy alone, which lowered LDL-C, but not Lp(a). After 2 years, there was a significant reduction in the number of new peripheral arterial stenoses measured by duplex ultrasonography in the patients receiving apheresis *(21)* combined with statin therapy compared with the group on statin alone. Elevated Lp(a) calls for an aggressive reduction of LDL-C as low as possible in patients with LEAD and CAD.

- Elevated serum homocysteine *(22–26)*. High serum homocysteine is an independent risk factor for LEAD; it also increases cardiovascular mortality *(22)*. Homocysteine causes the formation of reactive oxygen species, and thus promotes endothelial dysfunction, proliferation of smooth muscle cells of the arterial wall, and accelerates atherosclerosis *(22)*.
- Elevated fibrinogen *(27–29)*. There is laboratory variability in fibrinogen assessment and, on the other hand, fibrinogen is an acute phase reactant. Smoke cessation decreases fibrinogen levels. It is also worth emphasizing that vigorous physical exercise promotes fibrinolytic activity.
- Fibrinogen genotype *(30,31)*.
- High blood viscosity *(32)*.
- Inflammatory processes, associated with elevated biomarkers, like high sensitivity C-reactive protein (CRP) and interleukin (IL)-6 that induces the expression and release of CRP *(33–35)*. Indeed, inflammation characterizes all phases of atherosclerosis

The prevalence of LEAD coexisting with CAD depends on the populations screened and the methodology used. Intermittent claudication alone, which is a clinical diagnosis based on symptoms, cannot be relied upon for the accurate detection of LEAD. Noninvasive testing is essential—like the ankle–brachial index (ABI), which is a simple, accurate, inexpensive, and a noninvasive method. The prevalence of LEAD increases with age; from less than 3% below the age of 60 years to more than 20% at age 75 and older *(36)*. The majority of patients over the age of 75 with LEAD are claudication free. Overall, risk factors for LEAD are very similar to those for CAD. Cigaret smoking, diabetes mellitus, and/or insulin resistance are not only risk factors, but also prognosticators for the development of LEAD. There is a great deal of correlation between survival and LEAD severity *(37,38)*.

The ABI can be used not only to diagnose accurately, simply, and noninvasively LEAD, but also assesses prognosis *(39)*. The worst prognosis was found in patients with ABI of less than 0.4 *(40)*. There is a close correlation between LEAD severity and mortality during a 10-year period *(41)*. The ABI used to diagnose LEAD or make a prognosis for survival is the lower of the two, left or right ankle. The severity of LEAD is closely associated with the risk for myocardial infarction (MI), ischemic stroke, and cardiovascular mortality in both symptomatic and asymptomatic patients. The lower the ABI, the higher the risk for cardiovascular events *(37,38)*. More than 50% of patients diagnosed to have LEAD by low ABI have no typical symptoms *(37,38)*. In the Systolic Hypertension in the Elderly Program (SHEP) study *(42,43)*, an adjusted relative risk of 2.5 was found when cardiovascular disease morbidity and mortality were examined in subjects with LEAD, defined as an ABI of less than 0.9. All of the subjects in the SHEP study had isolated systolic hypertension and were randomized to receive antihypertensive therapy *(42,43)*. Using age and gender matched populations of normal individuals and patients with intermittent claudication, Jelnes and coworkers *(44)* tracked subject survival for more than 7 years. Mortality among patients with intermittent claudication was 44%, about twice that of controls *(44)*. In a large multicenter clinical trial performed in Italy, 2111 consecutive patients with LEAD were examined to identify predictors of cardiovascular events *(45)*. The data were analyzed as a prospective, observational, epidemiological study. Factors that increased the risk of cardiovascular events in patients with LEAD were evaluated in a multivariant analysis *(45)*. Hypertension *(45,46)*, a low ABI, and cigaret smoking independently increase the risk of cardiovascular events, as does a history of vascular surgery, that by itself can be considered as a marker of disease severity. Elevated white blood cell count and high

plasma fibrinogen levels are also independent predictors of cardiovascular events in patients with LEAD *(27,45)*.

WHAT DO PATIENTS WITH LOWER EXTREMITY ARTERIAL DISEASE ASSESSED WITH A LOW ANKLE–BRACHIAL INDEX DIE FROM?

Cardiovascular mortality can be predicted based on the low ABI *(47)*. Compared to those with normal ABI, patients with LEAD die from CAD or cerebrovascular disease *(48–50)*. Patients with LEAD have a fourfold risk of dying from CAD. Moreover, LEAD can be used as a prognosticator of mortality in coronary patients treated medically or with coronary artery bypass grafting (CABG) *(45,48,49)* or percutaneous coronary intervention (PCI) *(45,48,49,51)*. There is a graded correlation between survival and LEAD severity in patients with severe and symptomatic LEAD; only one in four patients survive 10 years *(41)*.

How is the diagnosis of CAD made in patients with LEAD without any cardiac symptoms at all?

As mentioned in Chapter 1, a detailed investigative history and meticulous physical examination should be performed on every patient. The resting electrocardiogram (ECG) should be examined carefully, and the ABI of each patient over 50 years of age should be assessed. After LEAD is established, carotid ultrasonography should be performed in order to detect carotid plaques, flow limiting or not and the intima/media thickness *(52)*.

Electron beam computed tomography (EBCT) *(53)* or ultrafast computed tomography (CT) *(54)* is a simple, quick, noninvasive, and inexpensive test compared to other cardiac tests and diagnostic methods. EBCT *(53)* is a newcomer to our diagnostic armamentarium that can indirectly assess coronary endothelial dysfunction, and, of course, coronary calcium deposits. EBCT *(53)* can detect calcium deposits in the coronary arterial wall. The coronary diagram based on EBCT can be used to motivate patients to modify their modifiable risk factors (Table 1). Coronary calcium deposits mean that their coronary problem has started. If the coronary calcium score is high, the lumen of the coronary arterial tree (luminogram) should be made known by performing coronary angiography. A very high calcium score is highly predictive of coronary events *(55)*. Coronary angiography (luminography) is not the *sine qua non* method to diagnose ischemic heart disease.

The most popular method to diagnose myocardial ischemia is a myocardial perfusion ccan utilizing single photon emission computed tomography (SPECT) *(56,57)*. Myocardial perfusion can be assessed (if

the patient can walk fast) using the standard or modified Bruce *(58)* treadmill exercise protocol or the asymptomatic cardiac ischemia pilot (ACIP) *(59)* protocol combined with the IV injection of one or two radionuclide substances like Thallium-201 (^{201}Tl) and/or Technitium 99m (^{99m}Tc) analogs. Tomographic images of the myocardium based on perfusion are obtained at rest and following exercise. With progressive exercise, the depth of the ST segment depression on the exercise ECG *(58–60)* may increase involving more ECG leads, and the patient may develop angina. Stress-induced ST elevation indicates impending acute coronary syndrome and should be considered a cardiac emergency *(61)*. Rarely, exercise-induced coronary spasm can cause ST elevation *(62)*. Ischemic ST segment displacement may be seen not only during the exercise phase. In about 10% of patients, the ischemic response may appear only in the recovery phase; the prevalence of ischemic ST segment change in the recovery phase only, is higher in asymptomatic patients compared with the symptomatic CAD ones *(63,64)*.

Dual isotope IV injection: both ^{201}Tl and a ^{99m}Tc analog are preferable to administering ^{201}Tl alone *(65)*; myocardial perfusion can be assessed more accurately, particularly in overweight individuals, but also left ventricular function: contractility, ejection fraction objectively, as the ratio of end-systolic radioactivity counts over the end-diastolic counts. Chamber size, post stress dilatation of the left ventricle, and many times right ventricular hypertrophy or infarct can be seen on SPECT images *(66)*. Soft tissue attenuation of the SPECT images, particularly as a result of large breasts, not necessarily gynecomastia, is a problem. Hence, breast markers are advisable. ^{201}Tl, a potassium analog, became available 30 years ago *(65)*. In the early 1990s, new ^{99m}Tc labeled compounds with better imaging characteristics and novel biological properties *(67)* were introduced for visualization of myocardial perfusion. Perfusion images are acquired with electrocardiographically synchronized gating. Thus, accurate information is obtained for both perfusion and function. A defect present on the stress images and not present or present to a lesser degree on the rest images, i.e., a reversible defect indicates ischemia *(68)*.

A persistent defect, that is unchanged and is present on both exercise and rest images indicates MI or scar tissue. Persistent defect or defects can also be caused by chronic severe ischemia (hibernating myocardium). In order to differentiate between severely ischemic, but still viable myocardium, from scar tissue, myocardial viability studies are indicated.

Unfortunately, a number of patients with LEAD and claudication cannot walk fast on the treadmill; they can even get claudication on the stationary bicycle. Hence, pharmacological vasodilatation with adenos-

ine or dipyridamole IV *(69–71)* or pharmacological stress with dobutamine provides useful alternative approaches.

There is an over 30-year experience with dipyridamole (persantine IV). Dipyridamole is infused over a 4-minute period (0.142 mg/kg/minute) *(72)* using an infusion pump or by slow hand injection. Approximately 4 minutes after the completion of the infusion there is maximal coronary vasodilatory effect and the isotope is injected IV immediately thereafter. Dipyridamole can dilate the normal or nearly normal coronary arterial segments, but cannot dilate to the same degree the diseased atherosclerotic coronary branches. In essence, dipyridamole creates a steal phenomenon. Blood flow follows the hemodynamic law of least resistance preferentially; there is significantly more coronary blood flow through the normal (nonatherosclerotic) dilatable segments compared to the atherosclerotic coronary segments. The decreased blood supply as detected by decreased thallium uptake (radiopenic myocardial segment) is called a perfusion defect or defects, depending on the number of left ventricular regions that are ischemic. In true ischemia, during the rest period the SPECT images show homogeneous uptake. A reversible defect or defects during stress indicates decreased uptake of the flow tracer, because there is relatively less blood flow in these segments compared to other areas. A reversible defect is diagnostic of myocardial ischemia *(73–75)*. If the defect on the myocardial images persists even after the rest period, then this defect is called persistent defect. Persistent defect usually indicates scar tissue. MI is the commonest cause of persistent defect. Less commonly, cardiomyopathy, dilative or ischemic presents as persistent defects. As mentioned previously, a persistent defect can also be caused by chronic severe ischemia, (hibernating myocardium). In order to differentiate between severely ischemic but still viable myocardium from scar tissue, viability studies are indicated, preferably with positron emission tomography (PET) *(76,77)*.

Intravenous infusion of dipyridamole blocks the cellular re-absorption of adenosine and, in turn, increases the concentration of adenosine, an endogenous vasodilator that can activiate specific receptors *(72)*. Coronary blood flow is autoregulated by adenosine to meet myocardial metabolic demands *(72)*. In patients without CAD, dipyridamole or adenosine infusion creates vasodilatation and increases coronary blood flow three to five times above baseline levels. In patients with coronary disease the resistance vessels distal to the stenotic area are already dilated, often maximally, to maintain normal coronary flow at rest. In these patients (coronary patients) dipyridamole IV or adenosine IV *(78)* does not (cannot) cause further vasodilatation in the atherosclerotic vascular

bed or beds. Elsewhere in the myocardium, supplied by normal coronary arteries, a substantial increase in myocardial blood flow occurs. Myocardial segments supplied by atherosclerotic arteries are relatively hypoperfused compared to regions supplied by normal arteries. Pharmacological vasodilatation by adenosine or dipyridamole does not precipitate myocardial ischemia *per se*. The patient should be instructed not to have xanthine derivatives or caffeine-containing beverages or chocolate the night before, and in general substances that can block adenosine receptors, in order to avoid false-negative images *(79)*.

Adenosine IV (maximum of 140/μg/kg/min) has been increasingly utilized as a pharmacological vasodilating agent in patients unable to walk fast as in LEAD *(78)*. Persantine IV works as a vasodilating agent via endogenous adenosine. Adenosine IV can cause chest discomfort, headache, dizziness, or bronchospasm. However, in many cases, the side effect profile of adenosine is similar to a small quantity of saline IV, i.e., no side effects. The great advantage of adenosine is that it is a very short acting agent, so the side effects dissipate spontaneously and quickly. The half-life of adenosine is only 30 seconds. Transient atrio-ventricular (A-V) block, first degree, second degree, or high degree of A-V block and profound sinus bradycardia are common, but transient. The expected pharmacologic effect of adenosine is transient bradycardia and transient hypotension. In severely ischemic coronary patients, mild tachycardia or even systolic blood pressure elevation has been observed. Probably the most significant side effect of adenosine is bronchospasm; if persistent and severe, the effect of adenosine can be reversed by aminophylline IV. Of course, if the patient suffers from bronchial asthma in addition to LEAD, then adenosine should not be used. In asthmatic patients and for those who take xanthine derivatives the choice of pharmacologic stressor is dobutamine.

Dobutamine is a pharmacologic stressor that can be used in patients with LEAD. Dobutamine acts by increasing the workload of the left ventricle in three ways: (1) by increasing the heart rate, (2) by increasing the systolic blood pressure, and (3) by increasing myocardial contractility. Hence, dobutamine increases myocardial oxygen demands. Coronary blood flow increases significantly to a degree comparable to that during physical exercise (twofold to threefold), but less than that with adenosine or dipyridamole. The increase in heart rate is lower than that with exercise. Dobutamine pharmacologic stress testing is not the first diagnostic option to assess patients with LEAD for CAD. Dobutamine should not be used in patients with atrial fibrillation, because it can increase the ventricular rate excessively. Any significant side effect of

dobutamine IV, either serious sinus tachycardia or other tachyrhythmias, or severe elevation of the blood pressure should be promptly treated with a short acting β-blocker.

DOBUTAMINE STRESS ECHOCARDIOGRAPHY

The resting transthoracic echocardiogram is compared very carefully, segment by segment with the echocardiogram during dobutamine infusion and during the recovery phase. The left ventricular contractility is analyzed in all segments and areas of hypokinesis, dyskinesis, or akinesis are studied and compared to the baseline echocardiogram.

As mentioned previously, in LEAD if the patient cannot exercise adequately myocardial perfusion imaging is done, utilizing one and preferably two radionuclide substances and a pharmacologic stressor. Commonly used pharmacological stressors are adenosine or dipyridamole IV, and if there is a contraindication to either of these two agents, such as in patients with history of bronchospasm or patients receiving xanthine containing products, then the choice should be dobutamine. In most LEAD patients who cannot walk fast the approach of choice is pharmacological stress testing with a vasodilating agent.

Stress echocardiography has high sensitivity and specificity to diagnose accurately myocardial ischemia if left ventricular hypertrophy is not present. The Treadmill protocol *(44)* is also utilized. In patients who cannot walk on the treadmill adequately or perform on a bicycle ergometer because of limiting claudication or other medical problems, pharmacological stress 2-dimensional echocardiogram is recommended utilizing dobutamine. Stress echocardiography is more sensitive and specific for detecting inducible ischemia than the exercise electrocardiogram (ECG) alone *(80,81)*, even in patients who can walk incrementally faster, something patients with LEAD cannot do. The resting echocardiogram is done first, analyzing carefully the wall motion of all left ventricular segments along with the endsystolic and end-diastolic volumes, dimensions, and hemodynamics. Then the infusion of the pharmological stressor, dobutamine begins. The patient, his or her symptoms, the BP response, the heart rate, rhythm, the regional wall motion, the ventricular volume changes, and the ST changes are observed carefully and diligently. The additional IV injection of a perfluoro carbon-based contrast agent or sonicated albumin (trade name optison) can give beautiful images of myocardial perfusion and impressive details. Dobutamine stress ECG with a contrast medium is an excellent method to study a patient with LEAD for ischemic heart disease with three preconditions: (1) there is no atrial fibrillation because dobutumine can increase the

ventricular rate excessively; (2) there is no left ventricular hypertrophy (LVH), so that wall motion analysis can be performed accurately; and (3) the resting BP is not significantly elevated. The transthoracic echocardiogram obtained at rest is compared with the one obtained during pharmacologic stress testing with IV dobutamine and in the post-stress period. Dobutamine or exercise echocardiogram combined with a contrast agent IV infusion is an excellent noninvasive diagnostic method to assess cardiac function. The contrast medium follows the coronary blood flow. An accurate diagnosis of myocardial ischemia, MI, or normally perfused myocardium can be made. Two-dimensional echocardiagraphy can also provide clinically useful information on cardiac chamber size, cardiac volumes, intracardiac pressures, and valve function. Information about the thoracic aorta can also be obtained.

In patients who have risk factors for LEAD and CAD in whom no LEAD and no demonstrable carotid disease or myocardial ischemia can be diagnosed, utilizing noninvasive techniques, a regular cardiovascular evaluation should be done once or twice a year, because of the high probability of developing cardiovascular disease as time goes on.

The accurate assessment of myocardial ischemia is useful, not only to establish the diagnosis of ischemic heart disease and prescribe antiischemic therapy: a β-blocker (even in the presence of LEAD), a statin to keep the LDL very low (statin therapy also reduces CRP levels), an antiplatelet agent such as 75 mg of clopidogrel every day or 325 mg per day of enteric coated aspirin. Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) have been introduced in our pharmacotherapeutic panoply of LEAD and CAD in order to improve endothelial function and left ventricular performance. In diabetic patients in particular, ARBs have been shown to slow the progression of diabetic microvasculopathy and protect them to some degree from diabetic nephropathy. Diabetic patients even in the absence of demonstrable LEAD and CAD have coronary endothelial dysfunction, and secondary prevention with a statin and an antiplatelet agent should be recommended.

Whenever the medical history, detailed physical examination, and the resting ECG were used, between 20 and 50% of patients with symptomatic LEAD were found to have evidence of CAD *(82)*. When myocardial perfusion studies or coronary angiography are applied, CAD was diagnosed in 60 to 90% of patients with LEAD. Indeed, many patients with LEAD also have CAD. The prevalence of cerebrovascular disease in patients with LEAD is also significant; again a detailed history and a meticulous physical examination alone resulted in prevalence values for

cerebrovascular disease ranging from 0.5 to 15% in patients with LEAD *(33)*. In order to detect cerebrovascular disease co-existing with LEAD, the clinician should use sensitive and specific noninvasive tests. The most widely used method is Doppler ultrasonography of the cranial arteries. Carotid ultrasound studies in patients with LEAD show an exponential increase of the risk for cardiovascular events when carotid stenosis exceeds 50% of the lumen, even after successful PCI *(51,83)*.

MANAGEMENT

The cornerstone of medical therapy in any type of cardiovascular disease, LEAD, carotid artery disease, and CHD is aggressive risk factor modification. The goal for systolic pressure is 120 mmHg in the arm, which shows the highest pressure. The goal for diastolic pressure should be less than 90 mmHg according to the most recent guidelines of the Joint National Committee (JNC7) on Hypertension *(84)*.

In patients older than 50 years of age, a systolic pressure of greater than 130 mmHg is more of a serious risk factor for atherosclerosis than a mildly elevated diastolic pressure is. In patients 50 years or younger both systolic and diastolic pressure elevations can be damaging.

The blood sugar ideally should be controlled with regular checks of the fasting blood glucose level and of hemoglobin A1C *(6)*. Hyperglycemia should be controlled by a diet low in carbohydrates and low in animal fat. Regular exercise such as brisk walking, use of treadmill or stationary bicycle for an average of 45 minutes a day or swimming should become routine for every cardiovascular patient. The pharmacotherapeutic armamentarium for diabetes mellitus keeps expanding. Pharmacologic agents, which can potentially improve endothelial function and tone are preferable.

EXTRA-LOW LDL CHOLESTEROL

Improvement of the lipid profile is beneficial to all vascular patients with or without demonstrable coronary disease. The goal for the LDL-C is well below 90 mg/dL or as low as possible, a natural occurrence in parts of China and Japan. The recommendation for an LDL well below 90 mg/dL stems from many clinical trials utilizing statins. The prevailing trend today based on recently completed large clinical trials is extra-low LDL-C; a median level of 78.9 mg/dL *(85)* and 62 mg/dL *(86)*. Patients with extra-low cholesterol levels had prospectively fewer cardiac deaths and fewer overall cardiovascular events. The optimally lowest level of LDL-C is unknown. Analysis of the ARBITER study *(87)* shows a greater likelihood of regression of atherosclerosis across a broad

range of LDL-C values with the greatest likelihood of regression at a LDL-C below 70 mg/dL. Regression of atherosclerosis demonstrated by intra vascular ultra sonography (IVUS) imaging in the coronary circulation or in LEAD is an established surrogate for clinical benefit—LDL-C reduction should go well below the current National Cholesterol Education Program (NCEP) (*88*) value of 100 mg/dL. Regression of carotid atherosclerosis demonstrated by carotid B-mode ultrasound is directly related to the absolute LDL-C level on statin therapy. The greatest regression was obtained with an LDL-C below 70 mg/dL *(89)*.

The axiom should be for all vasculopathic patients with regards to LDL-C: the lower the better.

The goal for HDL-C should be more than 40 mg/dL for men and more than 50 mg/dL for women *(8,90)*. Hypertriglyceridemia, which has been strongly associated with LEAD (*4*), should be aggressively treated with weight reduction, exercise, and a high protein, high fiber diet. High protein, high fiber diets, both short term and long term, reduce body weight significantly, reduce triglycerides, and hence improve endothelial function. Elevated triglycerides can induce endothelial cell dysfunction and serve as a marker for increased serum concentrations of lipoprotein remnant particles, which are highly atherogenic *(13,91–93)*.

Pharmacotherapeutically, a short-acting statin such as simvastatin 20 mg as a starting dose after dinner and fenofibrate 160 mg *(92,94)* with breakfast has been proven safe and effective for the reduction of triglyceride blood levels and LDL-C. The two pharmacologic agents should be given about 10–12 hours apart to avoid overlap of their half lives and potential liver toxicity. Fibrates should not be co-administered with long-acting statins; the possibility of hepatoxicity and rhavdomyolysis is real, particularly after a fall resulting into muscle trauma and/or unexpected renal dysfunction.

The Heart Protection Study (*95*) included 20,536 persons aged 40–80 years with a serum total cholesterol of 135 mg/dL or higher and prior MI (8510 persons), other CAD (4876 persons), or no CAD (7150 persons) *(95)*. Of the 7150 persons without CAD, 1820 had cerebrovascular disease, 2701 had LEAD, and 3982 had diabetes mellitus. Although treated hypertension was present in 8457 persons, only 237 persons were included on the basis of hypertension alone. Patients were randomized to simvastatin 40 mg daily or to placebo. Mean follow-up was 5 years.

Compared to placebo, simvastatin caused significant reduction in all-cause mortality by 13%, in any vascular death by 17%, in major coronary events by 27%, in any stroke by 25%, in coronary or noncoronary revascularization by 24%, and in any major vascular event by 24% *(95)*. In the 3500 persons with an initial serum LDL-C of <100 mg/dL,

reduction of the serum LDL-C level from 97 mg/dL to 65 mg/dL by simvastatin caused a similar reduction in risk as did treatment of patients with higher serum LDL-C levels *(95)*. Simvastatin significantly reduced all-cause mortality, vascular death, major coronary events, coronary or noncoronary revascularization, and any major vascular event regardless of initial levels of serum lipids, age, or gender *(95)*. On the basis of these data, the Heart Protection Study investigators recommended treating patients at high risk for vascular events with statins, regardless of the initial levels of serum lipids, age, or sex *(95)*.

In the reversal of atherosclerosis with aggressive lipid lowering (REVERSAL) study, intravascular ultrasound was used to measure progression of atherosclerosis in 502 patients with CAD randomized to pravastatin 40 mg daily or atorvastatin 80 mg daily *(85)*. The serum LDL-C level was reduced to 110 mg/dL in the pravastatin group and to 79 mg/dL in the atorvastatin group. At 18-month follow-up, compared with baseline values, patients treated with atorvastatin had no change in atheroma burden, whereas patients treated with prevastatin showed progression of coronary atherosclerosis *(85)*.

At 3-year follow-up of 1410 persons, mean age 81 years, with prior MI and a serum LDL-C level of 125 mg/dL or higher, reducing the serum LDL-Cl level by statins to <90 mg/dL was associated with a 20% incidence of new coronary events, whereas reducing the serum LDL-C level to 90–99 mg/dL was associated with a 48% incidence of new coronary events *(96)*. In this study, the incidence of new stroke was 7% if the serum LDL-C level was reduced to <90 mg/dL and 16% if the serum LDL-C was reduced to 90–99 mg/dL *(94)*.

In 4162 patients hospitalized for an acute coronary syndrome, the median serum LDL-C level was 95 mg/dL in patients randomized to pravastatin 40 mg daily vs 62 mg/dL in patients randomized to atorvastatin 80 mg daily *(86)*. At 2-year follow-up, the primary endpoint of death from any cause, MI, documented unstable angina pectoris requiring rehospitalization, coronary revascularization (performed at least 30 days after randomization), and stroke was 26.3% in the pravastatin group vs 22.4% in the atorvastatin group, a 16% reduction in favor of atorvastatin ($p = 0.005$) *(86)*.

The LDL-C should be kept well below 90 mg/dL, actually closer to 50 mg/dl, utilizing a low cholesterol, low animal fat diet, exercise, and a powerful statin *(97)*. Statins have pleiotropic effects: plaque stabilization, vasodilatation, anti-inflammatory, antithromotic, antiproliferative, and antioxidant *(98)*. Most statins are metabolized via the CYP 3A4 pathway. Hence, drugs which also use the CYP 3A4 metabolic pathway, like ketoconazole, nefazodone, cyclosporine, nifedipine, felodipine,

nisoldipine, macrolide antibiotics, and erythromycin in particular should not be used with most statins concomitantly. Sadly, many case reports have described the development of rhabdomyolysis when lovastatin was combined with erythromycin *(99)*. Erythromycin alone can cause QT prolongation *(100)*. If macrolide antibiotics are necessary, then the CYP 3A4 statin should be discontinued for the duration of the antibiotic therapy.

Intensive statin therapy bears benefits both short term and long term; it reduces the probability for cardiovascular events. Statin therapy also improves time to claudication.

In a recent study *(101)*, simvastatin 40 mg significantly increased treadmill exercise time to onset of claudication from baseline by 54 seconds (a 24% increase $p < 0.0005$) at 6 months after treatment and by 95 seconds (a 42% increase, $p < 0.0001$) at 1 year of treatment. At 6 months and 1 year after treatment with placebo, treadmill exercise time until onset of intermittent claudication was not significantly different from baseline exercise time *(101,102)*.

WHAT IS THE SIGNIFICANCE OF LOW HDL CHOLESTEROL?

Low serum HDL-C predisposes patients to the development of MI *(90)*, sudden death *(103)*, stroke *(104,105)*, carotid artery disease *(106)*, restenosis after angioplasty *(107)*, multivessel disease, and atheromatous plaque formation in the proximial left main coroanry artery *(108)*, and disability and loss of ability to execute activities of daily living in the elderly *(109)*. Low levels of HDL are highly prevalent and occur in approximately 35% of men and 15% of women *(24)*.

Conversely, high HDL-C is correlated with longevity and protection from atherosclerotic disease and cognitive impairment *(25)*. In the Framingham risk equation, an HDL >60 mg/dL is defined as a negative risk factor, and was given the numerical designation of –1. An early meta-analysis of data from the Framingham Study, the Multiple Risk Factor Intervention Trial, the Lipid Research Clinics Coronary Primary Prevention Trial, and the Lipid Research Clinics Prevalence Mortality Follow-up Study revealed that for every 1 mg/dL rise in serum HDL-C, risk of CAD in men and women decreased approximately 2% and 3%, respectively *(110)*. The most important antiatherogenic function of HDL-C is likely its ability to drive the reverse cholesterol transport (RCT) *(111)*. RCT consists of a series of reactions by which HDL is able to induce the net delivery of systemic cholesterol back to the liver for disposal as bile salts or to steroidogenic organs (e.g., adrenals, ovaries,

testes) for conversion to steroid hormones. As long as RCT is able to outpace the accumulation of cholesterol in the vascular wall, the progression of atherosclerotic disease is prevented. The net effect of RCT (increased bile salt formation) has been verified in clinical studies *(111)*. It is postulated that increased RCT provides the basis for the intravenous infusion of HDL or bioengineered HDL in order to induce regression of the atheromas in clinical trials *(112)*. HDL is able to reduce the oxidation of LDL via the activity of two of its constituent enzymes, paraoxonase *(113)* and platelet activating factor acetyllhydrolase *(114)*, thus rendering it less atherogenic. HDL inhibits endothelial cell apoptosis *(115)* and stimulates endothelial cell proliferation along denuded areas of the vessel wall *(116)*. HDL is able to reverse endothelial cell dysfunction by stimulating endothelial nitric oxide synthetase activity *(117)*. As HDL levels increase, the capacity for arterial vasodilation increases. High HDL decreases endothelial cell adhesion molecule (vascular cell adhesion molecule [VCAM]-1), and intercellular adhension molecule (ICAM)-1 expression *(118,119)*, thus the capacity for leukocyte infiltration and progression of inflammation within the vascular walls decreases *(117)*. HDL is antithrombotic by virtue of its ability to decrease platelet aggregability *(120)*, stimulate prostacyclin production *(121)*, and potentiate the activity of proteins C and S *(122)*.

As mentioned earlier, decreased HDL-C levels constitute a major risk factor for CAD and LEAD. A novel therapeutic approach to raise HDL is inhibition of cholesteryl ester transfer protein (CETP) *(123)*. Individuals with CETP deficiency as a result of molecular defects in the CETP gene, have markedly elevated plasma levels of HDL-C and apolipoprotein A-I *(124)*.

Torcetrapib, a potent inhibitor of CETP, markedly increases HDL-C levels and also decreases LDL-C levels, both when administered as monotherapy and when combined with a statin *(123)*.

Elevated triglycerides have also emerged as an independent risk factor for the development of coronary atherosclerotic disease *(125)* and LEAD in particular. The Prospective Munster Cardiovascular Study *(126)* showed that elevations in serum triglyceride levels increased risk for CAD independent of serum LDL-C and HDL-C. In the Copenhagen Male Study *(127)* there was a clear gradient of increasing risk for CAD as serum triglyceride levels rose. A meta-analysis of 17 trials supports the conclusions that hypertriglyceridemia is an independent risk factor for CAD, and the risk rises progressively as the severity of hypertriglyceridemia increases *(128)*. Elevated triglycerides can cause endothelial dysfunction *(129)* and serve as a marker for increased serum concentrations of lipoprotein remnant particles. Remnant particles are

incompletely catabolized very low density lipoprotein (VLDL) and chylomicra and are highly atherogenic *(129)*.

The combination of high triglycerides and low HDL-C significantly increases the risk for CAD and LEAD, and is a frequent finding in patients with insulin resistance *(130)*. Hyperinsulinemia induces a dysregulation of adipose tissue metabolism *(130)*. Adipocyte triglyceride catabolism increases, causing a flux of free fatty acids into the liver. The liver in turn increases VLDL secretion. Hyperinsulinemia is also associated with decreased lipoprotein lipase activity and increased hepatic lipase acitivty *(130)*. As lipoprotein lipase activity decreases, the hydrolysis of triglycerides in VLDL and chylomicra decreases. This results in hypertriglyceridemia and increased remnant particle formation. HDL-C levels drop in patients with insulin resistance *(130,131)*. The American Diabetes Association has defined optimal HDL-C and triglycerides in patients with type 2 diabetes mellitus at least 40 mg/dL and less than 150 mg/dL, respectively *(132)*. Based on multivariate analysis of risk factors from the United Kingdom Prospective Diabetes Study *(133)*, the two most important risk factors to modulate for decreasing CAD risk are the reduction of LDL-cholesterol and the elevation of HDL.

Fibrates increase serum HDL-C levels by stimulating the hepatic expression of apoprotein A-I and A-II (Apo A-I, Apo A-II) *(134,135)*. These apoproteins help to drive the formation of HDLs. Multiple studies have demonstrated a clear reduction in risk for CAD as serum levels of Apo A-I increase *(136,136)*. Fibrates may also increase rates of RCT by increasing the expression of ABCA1 in macrophages *(138)*. Fibrates stimulate triglyceride metabolism via a number of pathways.

Inflammation plays a key role in the etiology of atherosclerosis *(139)*. A plethora of interleukins, cytokines, intercellular matrix modifying enzymes, and cell types interact to promote foam cell, fatty streak, and atheromatous plaque formation *(140)*. PPAR-α has emerged as an important mediator of vascular inflammation and cellular redox status *(141)*. The nuclear factor κB (NF-κB) pathway regulates the expression adhession molecules, interleukin-6, cyclooxygenase-2, and endothelin-1 *(141)*. CRP is an important modulator of and marker for atherosclerotic disease *(142,143)*. PPAR-α agonism with fibrate therapy decreases serum CRP levels *(94,144)*.

Fibric acid derivatives have been used in a variety of clinical trials in an effort to demonstrate their efficacy in reducing both cardiovascular morbidity and mortality and the progression of atherosclerotic disease. These trials also demonstrate the importance of increasing serum HDL and reducing triglycerides when attempting to decrease risk for acute

cardiovascular and cerebrovascular events in both primary and secondary prevention trials. In the Veterans Affairs High-Density Lipoprotein Intervention Trial (VAHIT) *(145)*, men with CAD and mean LDL 111 mg/dL, mean HDL 31 mg/dL, and mean triglyceride 161 mg/dL were treated with either gemfibrozil (600 mg po bid) or placebo over a five-year follow-up period. The treatment group experienced a 6% elevation in HDL, no change in LDL, and a 31% decrease in triglycerides *(145)*. Among patients in the treatment group, gemfibrozil therapy resulted in a 22% ($p = 0.006$) reduction in the composite endpoint of all-cause mortality and nonfatal MI. Treatment with gemfibrozil reduced the risk of stroke and transient ischemic attacks by 31% ($p = 0.36$) and 59% ($p < 0.001$), respectively, and decreased the need for carotid endarterectomy by 65% ($p < 0.001$) *(145)*. A trend for reducing cerebrovascular event rates with gemfibrozil therapy was apparent within 6–12 months. The diabetic patients enrolled in VAHIT benefited the most from gemfibrozil therapy, with reductions of 32% ($p = 0.004$) in the combined endpoint, 41% ($p = 0.02$) in cardiac death, and 40% in stroke ($p = 0.046$) *(145)*. Among patients with hyperinsulinemia, the benefit of gemfibrozil therapy increased as the severity of insulin resistance rose *(145)*. Of considerable importance is the fact that VA-HIT was the first trial to show a reduction in cardiovascular and cerebrovascular events with an antilipedmic medication independent of changes in serum LDL. Most of the benefit of fibrate therapy in this trial was attributed to HDL elevation and the pleiotropic effects of gemfibrozil *(145)*.

Multiple angiographic trials with fibrates have demonstrated the ability of these drugs to reduce rates of atheromatous plaque progression. The Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT) *(146)* compared bezafibrate 200 mg po tid to placebo in 92 men who suffered a MI at age <45 years *(146)*. The study patients underwent coronary angiography at time of entry into the the trial and then after 2 or 5 years of therapy. The mean minimum luminal diameter decreased over the follow-up period by 0.06 mm and 0.17 mm in the bezafibrate and placebo groups, respectively. The change in rate of disease progression was statistically significant ($p < 0.049$) and was achieved with a 31% reduction in triglycerides, 9% elevation in HDL, and no change LDL *(146)*. In the Lopid Coronary Angiography Trial (LOCAT) *(147)*, 395 men who had undergone revascularization with CABG were randomized to either gemfibrozil (600 mg po bid) or placebo. Patients underwent coronary angiography at time of study entry and then after 32 months of therapy *(147)*. Gemfibrozil therapy significantly reduced rates of plaque progression in coronary vessels and the development of new

plaques in saphenous vein grafts. Gemfibrozil caused reduction in stroke rate in men with CAD and low HDL *(148)*. The benefit in LOCAT was attributed to a 9% elevation in HDL and a 33% reduction in remnant lipoprotein particle formation *(147)*.

Patients with hypertriglyceridemia may have low LDL levels. However, fibrate therapy stimulates the conversion of VLDL to LDL; triglyceride levels drop, but LDL can rise, sometimes substantially. In this situation, the addition of a short-acting statin such as simvastatin after dinner or a cholesterol absorption inhibitor such as ezetimibe (Zetia) *(149)*, may become necessary. In general, the combination of statins with gemfibrozil should be avoided. Gemfibrozil can inhibit the glucuronidation of statins, thereby reducing elemination of the statin thus raising the risk for hepatotoxicity and skeletal muscle toxicity, including rhabdomoylysis *(150–153)*. Fenofibrate is a much safer alternative because it does not adversely impact the metabolism of rosuvastatin *(154)* or pravastatin *(155)*. Moreover, unlike gemfibrozil, fenofibrate can provide additional capacity for further reducing serum LDL levels. Fenofibrate (Tricor) decreases the expression of a variety of inflammatory mediators (CRP, interleukin 1-β, soluble CD40, and soluble CD40-ligand) and substantially improves forearm flow-mediated dilatation of the brachial artery (a surrogate of coronary endothelial function) when combined with simvastatin to treat patients with mixed hyperlipidemia *(94)*.

In dislipilemic coronary patients with claudication, combination therapy with simvastatin 40 mg and ezetimibe *(149)* 10 mg (trade name of the combination preparation Vytorin) after dinner combined with fenofibrate (Tricor) 160 mg *(94)* with breakfast brings a significant improvement in walking time to onset of claudication, and time to absolute claudication (*see* Chapter 1) measured on the treadmill (personal observations). There is also a remarkable improvement of the patient's lipid profile with this combination therapy.

A combination therapy of rosuvastatin (Crestor) *(156)* and fenofibrate (Tricor) *(154)* has been gaining popularity among vascular physicians and cardiologists. Rosuvastatin can keep LDL very low and can also increase the plasma levels of HDL *(97,156)*. Fenofibrate *(94)* in the morning can bring a remarkable reduction of the serum triglycerides. Rosuvastatin and fenofibrate is a safe combination therapy with regards to liver toxicity and rhabdomyolysis *(154,156)*.

EFFECTS OF NIACIN

Niacin positively impacts lipoprotein metabolism through multiple mechanisms. Niacin appears to block HDL particle uptake and catabo-

lism by the liver cells without adversely affecting cholesteryl ester delivery to the liver *(157)*. Unlike statins and fibrates, niacin does not stimulate hepatic biosynthesis of HDL. Niacin has also been shown to reduce Lp(a) biosynthesis. Lp(a) is procoagulatory and is a highly atherogenic variant of LDL. Small dense LDL is believed to be more atherogenic than its large buoyant form because it has a higher susceptibility to oxidation, has a lower affinity for the LDL receptor on hepatocytes, and has increased access to the arterial wall subendothelial space by virtue of its smaller physical volume. Niacin has been shown to alter the distribution of LDL particle sizes by increasing the concentration of large buoyant LDL and decreasing the concentration of small, dense and highly small LDL particles *(158)*. Extended release niacin is safe *(159)* and beneficial to all components of the lipid profile *(160)*.

Niacin can induce significant skin flushing secondary to prostaglandin-mediated dermal vasodilation. Aspirin and other salicylates are potent inhibitors of cyclooxygenase and prostaglandin biosynthesis. Hence, salicylates can be used to prevent facial flushing, i.e., aspirin 325 mg concomitantly with long acting niacin. Niacin can cause gastric irritation, nausea, bloating, pruritus, and acanthosis nigricans *(161)*. Slo-Niacin was developed to slow the rate of absorption of niacin and decrease the risk of flushing. Both crystalline niacin and Slo-Niacin are associated with significant risk for hepatotoxicity, especially when used at doses that exceed 2 g/day *(161,162)*.

Niacin therapy can induce mild elevations in glucose levels. For this reason, many clinicians have been hesitant to use niacin therapy in patients with diabetes mellitus or impaired glucose tolerance (IGT) out of concern that it would significantly antagonize glycemic control *(161)*. The Assessment of Diabetes Control and Evaluation of the Efficacy of Niaspan (ADVENT) trial evaluated the effect of two doses of Niaspan vs placebo on glycemic control in 148 patients with LEAD and type 2 diabetes mellitus during 4 months of follow-up *(161)*. Glycosylated hemoglobin values at baseline and after 4 months of therapy were: placebo, 7.13% and 7.11%; Niaspan 1000 mg daily, 7.28% and 7.35% (p = 0.16 vs placebo); Niaspan 1500 mg daily, 7.2% and 7.5% (p = 0.48 vs placebo). In diabetic patients such small changes in overall glycemic intolerance control can be offset by titrating insulin or antiglycemic medication as indicated *(163)*. Among patients with carbohydrate intolerance (fasting blood sugar 110–125 mg/dL), high-dose niacin therapy can disturb glycemic control enough to meet criteria for diabetes mellitus (fasting blood sugar >126 mg/dL). Consequently, patients with established glucose intolerance should be monitored closely for increases

in serum glucose when placed on niacin therapy *(161,164)*. When evaluated over one year of follow-up, the Arterial Disease Multiple Intervention Trial (ADMIT) demonstrated that niacin at doses of up to 3 g/day given to 64 patients with diabetes mellitus had no effect on glycosylated hemoglobin levels (7.8% at baseline and after 48 weeks of therapy) *(163)*. Serum glucose levels were minimally increased in diabetics and nondiabetics by 8.7 and 6.3 mg/dL, respectively *(163,165)*.

Extended-release niacin (Niaspan: dosing range 375–2000 mg daily) used as monotherapy at a dose of 2000 mg daily decreases LDL by 17%, triglycerides by 35%, and Lp(a) by 24%, and increases HDL by 26% (166). These alterations in lipoprotein values are stable more than 2 years of follow-up (166). The rationale behind the bedtime administration of Niaspan is based on two important clinical issues. The first is that if flushing does occur the patient may simply sleep through it. The second has to do with the finding that during the nighttime fast the rate of adipose tissue lipolysis increases and serum triglycerides surge to their highest level at any point in a 24-hour cycle *(167)*. Niacin has been shown to inhibit this overnight surge in serum triglycerides *(167)*. This may have important additional consequences because postpradial hypertriglyceridemia is associated with significant endothelial dysfunction. Combination therapy of niacin and colestipol has beneficial effects on coronary atherosclerosis and coronary venous bypass grafts *(168,169)*.

In the Coronary Drug Project *(170)*, niacin therapy compared to placebo reduced the combined endpoint of nonfatal MI and CHD death by 14% ($p < 0.05$), nonfatal MI by 27% ($p < 0.005$), stroke and transient ischemic attack by 26% ($p < 0.05$), and the need for CABG by 67% ($p < 0.005$) during the 5-year follow-up period. After an additional 9 years of follow-up subsequent to completion of the study, all-cause mortality was 11% lower ($p < 0.001$) in the niacin-treated group compared to those patients given placebo *(171)*.

The HDL-C should exceed 50 mg/dL, preferably with exercise and vitamin B3, i.e., long-acting niacin *(166,167)* at a starting dose of 500 mg and gradual increments of the dose to 1 g or more, once a day after dinner and aspirin 325 g to ameliorate or abort facial vasodilatation as already described earlier. Aspirin decreases the cutaneous vasodilatation and hyperemia that may scare uninformed patients causing noncompliance to niacin therapy.

Long-acting niacin *(167)* in incremental doses at bedtime is very effective and safe in raising HDL-C. The starting dose can be 500 mg at bedtime for a week, 750 mg the second week, and 1 g or more thereafter. Long-acting niacin should be given after dinner. Improvement of the

lipid profile of vascular patients, HDL in particular, can also infer clinical benefits and vascular wall benefit, improvement of endothelial function, long term and short term. We should re-emphasize some of the key benefits of raising HDL:

- HDL inhibits endothelial cell apoptosis *(115)*.
- Stimulates endothelial cell proliferation along denuded areas of the vascular wall *(116)*.
- HDL reverses endothelial cell dysfunction by stimulating endothelial nitric oxide synthetase activity *(117)*.
- Long-acting niacin is safer than short-acting niacin with regard to liver toxicity *(162,166)*.
- Long-acting niacin should be given after dinner with enteric-coated aspirin, 325 mg, in order to prevent skin flushing caused by dermal vasodilatation. Aspirin and other salicylates are potent inhibitors of cyclooxygenase and prostaglandin biosynthesis *(161)*. Patient compliance is very important both short term and long term.
- The most common noncompliance side effect of niacin preparations is facial vasodilatation and flushing.
- Raising HDL can inhibit the progression of atherosclerosis by enhancing reverse cholesterol transport (RCT) *(110–112)*. RCT consists of a series of reactions by which HDL induces the net delivery of systemic cholesterol back to the liver for disposal as bile salts or to organs (adrenals, ovaries, testes) for conversion to steroid hormones *(111)*.

Low HDL-C levels constitute a major risk factor for CAD and LEAD. A novel therapeutic approach to raise HDL is inhibition of CETP *(123)*.

It is worth re-emphasizing the following observations: in dislipilemic patients with claudication, combination therapy with simvastatin 40 mg *(95)* and ezetimibe (zetia) 10 mg *(149)* after dinner combined with fenofibrate *(94)* (Tricor) 160 mg with breakfast brings a significant improvement in the walking time to onset of claudication and time to absolute claudication (*see* Chapter 1) measured on the treadmill (personal observations). There is also a remarkable improvement of the patient's lipid profile with this combination therapy.

A combination therapy of rosuvastatin *(97,156)* (Crestor) and fenofibrate (Tricor) *(94,154)* has been gaining popularity among vascular physicians and cardiologists. Rosuvastatin can keep LDL very low (a key goal) and also increase the plasma levels of HDL. Fenofibrate *(94)* in the morning can bring a remarkable reduction of the serum triglycerides *(94)*. Rosuvastatin and fenofibrate is a safe combination therapy with regards to liver toxicity and/or myositis *(154)*.

Obesity should be aggressively approached with dietary programs avoiding animal fat and sugars; regular exercise; and in morbidly obese

patients, the combination of the FDA approved phentermine taken every morning with a selective serotonin reuptake inhibitor (SSRI), preferably at nighttime, because in some patients antidepressants may cause somnilence. Overtly obese patients should be frequently counseled, and symptoms and stigmata of depression should be sought. Morbidly obese patients with CAD and LEAD can be referred for one of the newer gastric interventional procedures of bariatric surgery, provided of course that myocardial ischemia if present is adequately treated preoperatively.

High serum homocystein *(22,23,172)* is an important risk factor for both LEAD and CAD. Elevated levels of homocystein have been successfully dealt with green leafy salads and other foodstuffs rich in folic acid and also with pharmacologic preparations containing folic acid and vitamin B complex.

A pharmacologically safe strategy to deal with elevated Lp(a) is to keep the LDL-C very low with a statin and heart healthy, low animal fat diet. If LDL is already low and high Lp(a) stands out as an independent risk factor, then a long-acting niacin *(162,166)* preparation can be utilized. For dislipidemic vasculopathic patients with high serum triglycerides and high LDL-C, the combination of a low dose, short-acting statin like simvastatin *(95)* at bedtime and fenofibrate *(94,154)* with breakfast or alternatively long-acting niacin can be cautiously tried. Regular blood sample analyses of liver function and of the skeletal muscles both clinically and with CPK assessment is necessary in vasculopathic patients who have high LDL-C and low HDL-C, and are placed on combination therapy with extended-release niacin 1 g at bedtime with aspirin 325 mg to prevent vasodilatation and simvastatin 20 mg or lovastatin 20 mg. The lipid profile of the patient improves remarkably. There is minimal overlap of the half-lives of extended-release nicacin and simvastatin, if they are given 12 hours apart. For the forgetful patient with LEAD and CAD this author finds the combination therapy (one pill) approach of long-acting niacin with lovastatin (trade name Advicor) very gratifying, not only in improving the lipid profile but claudication as well. Sedentary patients should be aggressively motivated to participate in exercise programs, preferably as a form of group therapy for psychological and social support in tandem with antilipid therapy. Exercise therapy should be combined with pharmacotherapy and a diet rich in antioxidants like the Mediterranean diet. The Mediterranean diet improves endothelial function *(173)*.

By far, the most important therapeutic intervention in any patient with LEAD and CAD is smoking cessation. Smoking is the strongest risk factor for arterial disease progression, cardiovascular morbidity, and mortality from MI, thrombotic stroke, and cardiovascular death. Smok-

ing cessation should be approached by the clinician systematically with frequent calls to the patient and to his friends and family, encouraging him to start decreasing the number of cigarets smoked per day. Many smokers, men and women, can succeed to decrease the number of cigarets to five per day. Reduction below five cigarets per day in the unfortunate 10% of all the smokers who develop hardcore addiction to nicotine (an addiction similar to cocaine) is extremely difficult. Smoke cessation programs do exist and should be sought out by all physicians.

A successful pharmacotherapeutic approach is cutaneous nicotine patch combined with Zyban (welbutrin) as an aid to smoking cessation treatment *(174)*. This pharmacological combination should be continued for at least 6 months.

LEAD assessed clinically or more accurately by noninvasive testing is an adverse prognosticator in patients with CAD. LEAD increases the likelihood of cardiovascular mortality *(175–177)*. This increased mortality of CAD, when it coexists with LEAD, is true for any type of therapeutic modality applied for CAD: medical therapy, percutaneous coronary intervention (PCI), or coronary artery bypass grafting (CABG) *(37,44,48–50)*.

The risk of death among patients with LEAD is high; only one in four patients survive 10 years *(41)*. Co-existing CAD adversely influences the results of surgery for LEAD as the landmark study by Hertzer and coworkers *(176,178,179)* showed in 1000 patients, who underwent coronary angiography pre-operatively. Compared to normal subjects over a 10-year follow-up period mortality among patients with LEAD is about threefold for total mortality and about sixfold for cardiovascular and coronary disease mortality *(42)*. In this well-executed study *(42)*, the conclusion of a three-fold increase in all cause mortality and a sixfold increase in cardiovascular and coronary disease mortality was adjusted for age, gender, smoking, blood pressure (BP), LDL-C, HDL, triglyceride level, glycemia, and BMI.

LEAD is an independent risk factor for CAD and a prognosticator of cardiovascular mortality *(87–89,180)*. Cardiovascular death rates are higher in men than in women. In patients with LEAD, morbidity from coronary heart disease and stroke is increased: 2.5 times more likely to present with morbidity from all forms of cardiovascular disease compared to subjects who do not have LEAD *(44)*. There is a graded effect of the ABI on survival *(45)*. The 10-year survival estimates among those patients with an ABI of less than 0.4 was only 33%, whereas the group with ABI ranging between 0.4–0.85 had a survival rate of 51%. Three out of four patients with an ABI greater than 0.85 survive 10 years *(45)*.

Almost identical statistical associations were found by the frequently cited San Diego Study *(48)*.

A detailed medical history and physical examination combined with measurement of the ABI in patients over 50 years of age is a cost effective, sensitive, and noninvasive approach, even if the individual has no clinical evidence of cardiovascular disease. Because a low ABI predicts an increased risk for cardiovascular events including death *(87–89,180)*, the clinician should feel compelled to aggressively modify and treat all modifiable risk factors for cardiovascular disease and myocardial ischemia, no matter how silent the latter may be, if the particular patient has a low ABI. In patients with myocardial ischemia, silent or symptomatic, aggressive polypharmacy should be applied: statin therapy, beta blockade, coronary vasodilating agents like mononitrates and a long acting calcium channel blocker, antiplatelet agents, and newer agents to improve endothelial function *(85,94,97,98,131,154)*. Aggressive polypharmacy *(181)* should be combined with a low or no animal fat diet and exercise therapy. Heart healthy diet, exercise therapy, and pharmacotherapy (polypharmacy is not a bad word in treating patients with LEAD and CAD) should be continued for life, in addition to PCI or peripheral vascular interventions or surgery. It should be re-emphasized that LEAD has an adverse effect on coronary patients treated with either PCI or CABG. LEAD is a strong independent and adverse predictor of long-term mortality in patients with stable CAD *(87–89,180)*. Hence, aggressive medical therapy and global revascularization (Fig. 2) is warranted in this high-risk group of patients with LEAD and myocardial ischemia *(182,183)*.

An increasing number of cardiac surgeons prefer to do coronary bypass surgery in patients who also have LEAD off cardiac pump on the beating heart. Perioperative complications and embolic events are fewer and the length of stay following CABG shorter. The PCI of choice should be stenting with drug-eluting stents (DES) to minimize restenosis. CABG is recommended for diabetic patients with multivessel CAD.

After revascularized procedure, pharmacotheraphy and a heart healthy, low animal fat diet should be continued for life in patients with CAD and LEAD.

It should be noted that the prevalence of CAD in patients with LEAD is high; it depends on the diagnostic methodology used; if coronary arteriography is used *(179)* the prevalence of CAD in LEAD patients reaches 92%. However, as mentioned earlier in this chapter, screening for ischemic heart disease in patients with LEAD is done by myocardial perfusion studies: adenosine, depyridamole, and if there is a contraindi-

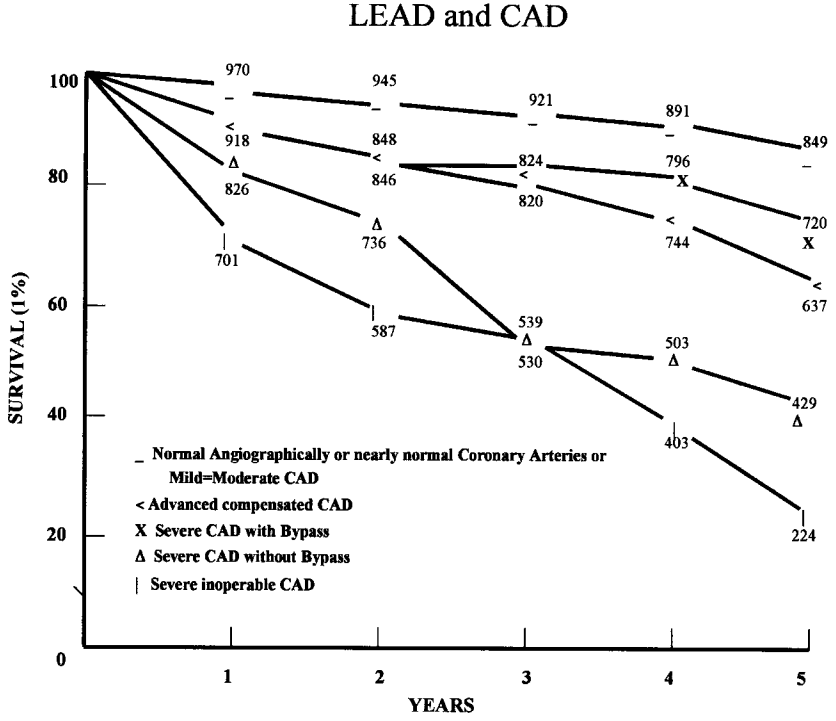

Fig. 2. The survival of patients with LEAD depends on the severity of the co-existing CAD. Adapted from refs. *178* and *179*.

cation to either one of these two agents, dobutamine, dual isotope single photon emission computed tomographic images or stress ECG, preferably with contrast.

Our patients with LEAD are LEADers: and we follow.

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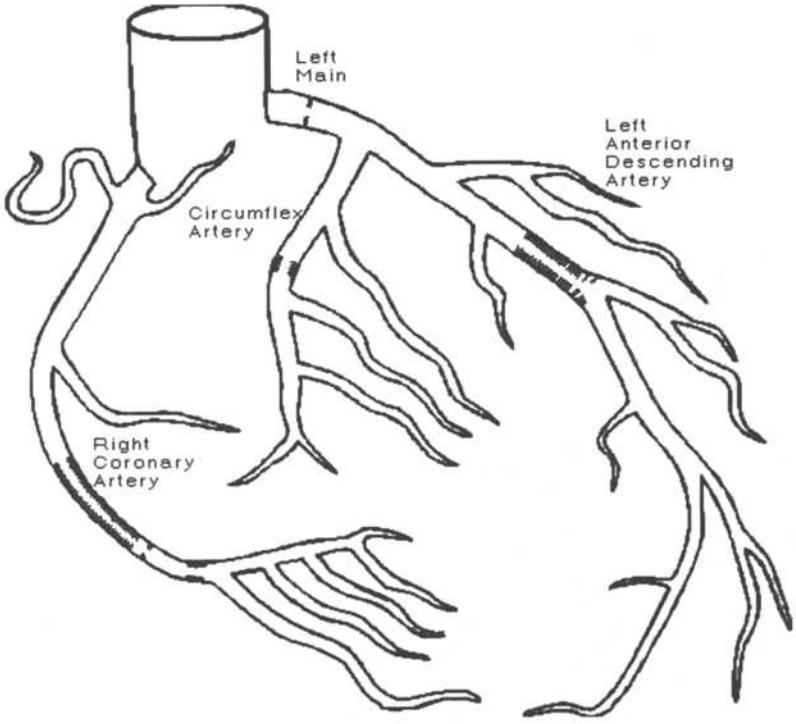

Fig. 3. Electron Beam Computed Tomography (EBCT): coronary diagram of a 68-year-old asymptomatic male with an ABI of 0.8 shows extensive calcium deposits totaling 1000, i.e., coronary calcium score 1000. Coronary angiography (luminography) was recommended.

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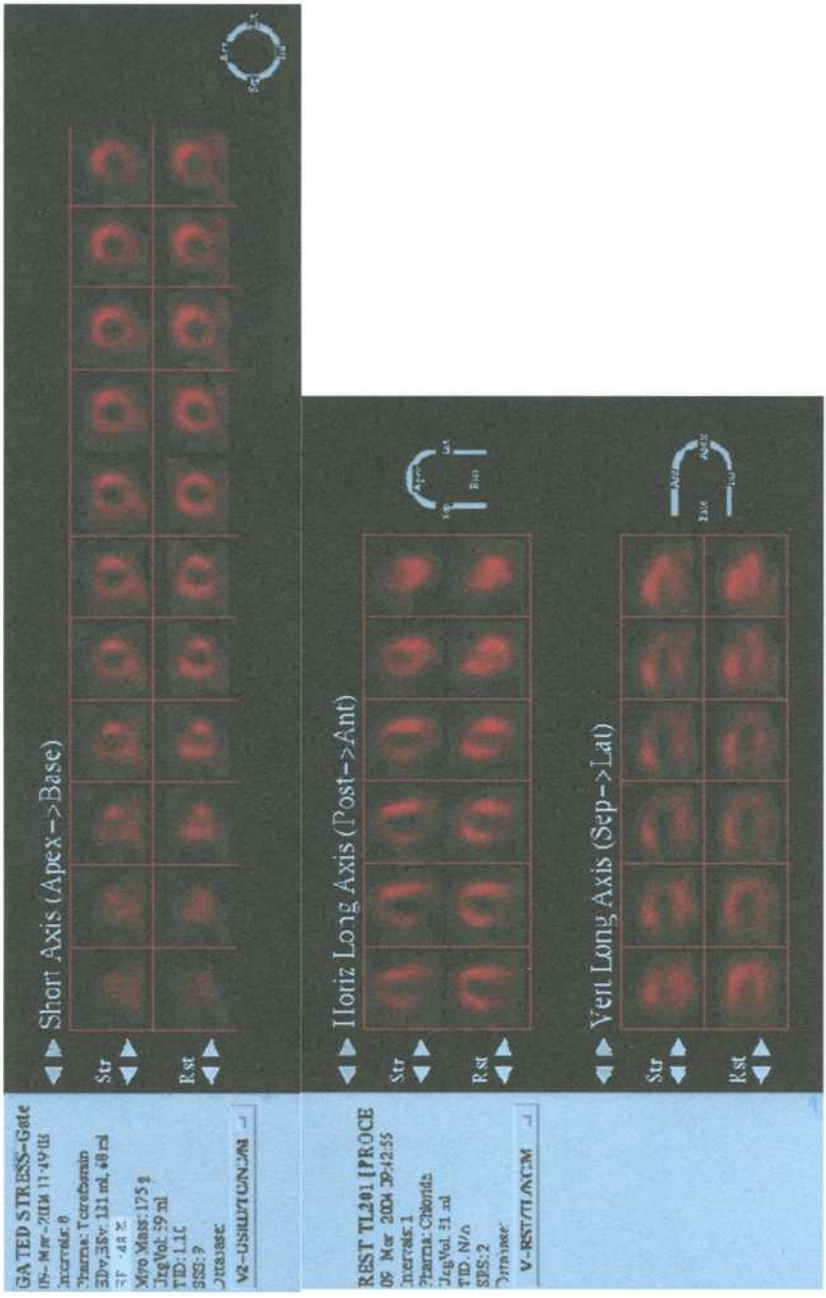

Fig. 4. Abnormal rest/stress myocardial perfusion study of a 69-year-old male with stable infrequent right and left calf claudication. Findings suggest adenosine-induced myocardial ischemia in the distribution of CAD and possibly right coronary artery.

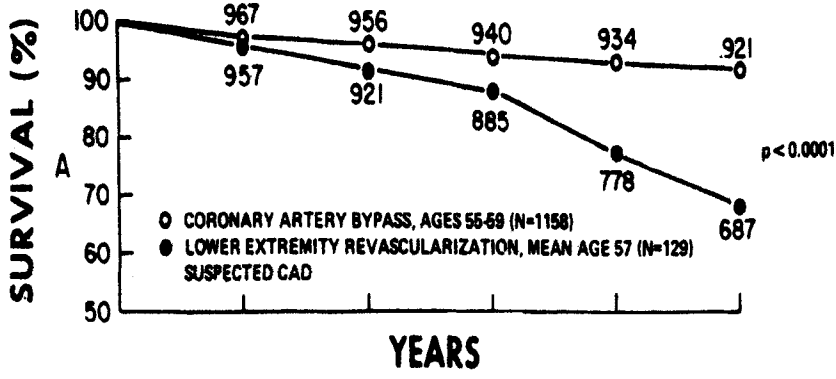

Fig. 5. Survival of patients with LEAD who underwent surgical revascularization procedures compared to patients who had CABG performed. (Adapted from ref. *180.*)

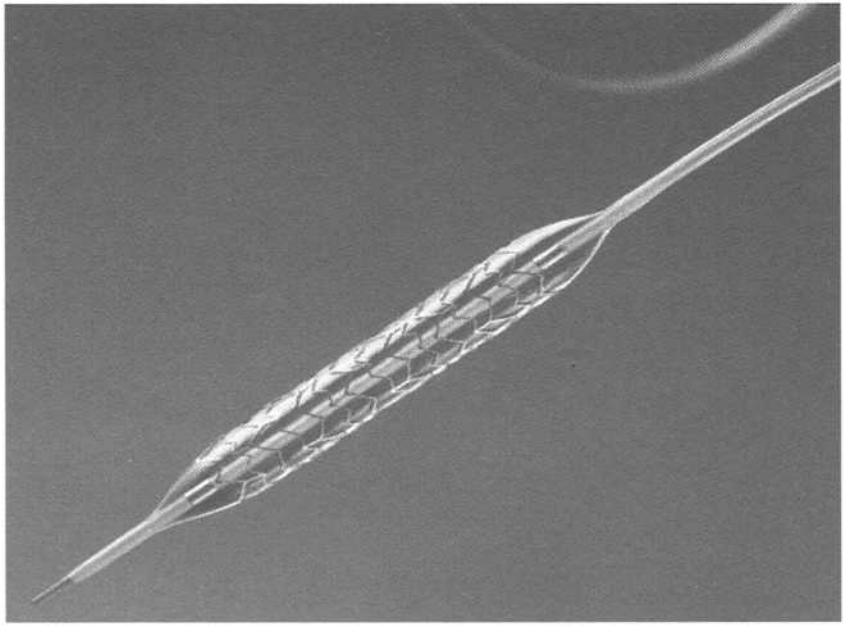

Fig. 6. The majority of percutaneous coronary interventions are performed with drug elating stents (DES) in order to minimize restenosis, a common phenomenon with balloon angioplasty and even bare metal stents.

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10 Medical Therapy of Claudication and Lower Extremity Arterial Disease

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CONTENTS

INTRODUCTION

The new millennium found lower extremity arterial disease (LEAD) and intermittent claudication in particular, pharmacotherapeutically orphan. Pharmacologically, intermittent claudication has been an orphan since it was first described many decades ago. Intermittent claudication, the cardinal symptom of LEAD occurs in 35 to 50% of patients with known LEAD documented by noninvasive diagnostic methods *(1)*. The development of effective pharmacotherapy for treating claudication has lagged behind that for treating angina pectoris and other manifestations of myocardial ischemia.

The mechanism of the symptoms of claudication has not been well understood and continues to be largely unknown. Claudication may be a manifestation of endothelial dysfunction of segments of the arterial tree of the lower extremities or a manifestation of skeletal muscle

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ischemia or both. In addition to lack of full knowledge of the pathophysiologic mechanisms that precipitate claudication, the methodology used in many clinical trials for the treatment of intermittent claudication was flawed by lack of placebo controls and blinded randomization. Additionally, the series of study patients has been small and the end-points inappropriate *(2)*. The lack of effective pharmacotherapy for decades has allowed LEAD to be considered a surgical disease. The primary care physician after taking a detailed history from the patient and performing a meticulous physical examination, looking for decreased capillary filling time and skin trophic changes, listening for bruits, assessing arterial pulse volumes, and testing for decreased vibratory sensation on the ankles, would then refer the patient for arteriography and subsequently to a vascular surgeon. LEAD is not an isolated, rare disease entity. LEAD affects a large portion of the general population with an age-adjusted prevalence of approximately 12% for men and women older than 40 years of age. The prevalence of intermittent claudication is between 3% and 7% of the general population. In the United States alone the number of patients with LEAD, symptomatic or asymptomatic, is estimated to be between eight and twelve million men and women. Until recently, the clinician would use one venue: history, physical examination → arteriography → vascular surgery. This approach for decades led to the establishment of vascular radiology as a subspecialty and vascular surgery as a major specialty.

For decades LEAD and its cardinal symptom claudication has been pharmacologically in standstill *(3,4)*. The Food and Drug Administration (FDA) has approved only two drugs in the United States, pentoxifylline and cilostazol, to treat claudication in patients with vascular claudication resulting from LEAD. In 1972, a new pharmacologic concept was introduced; a hypothesis really, which stated that if hemorheology is improved through the stenosed arterial segments by making the erythrocytes more deformable, more pliable and flexible, then claudication should improve. The drug pentoxifylline (Trental) was introduced to treat intermittent claudication based on this hypothesis *(5)*. Much later pentoxifylline was also found to have anti-inflammatory and antiprolepative effects *(6,7)*. In theory, pentoxifylline should improve stable angina pectoris because the same hemorrheologic hypothesis can be applied for the stenosed and diseased coronary arterial segments *(5)*. Pentoxifylline has not been used at any time as an antianginal agent, primarily because of lack of evidence that it is beneficial; the existing series studying pentoxifylline to treat stable angina pectoris are small and inconclusive.

The extensive utilization of pentoxifylline to treat arterial claudication was based on two prospective multicenter, but small clinical trials, which showed that pentoxifylline increases time to absolute claudication (when the patient reports the symptom is so severe, that he/she cannot walk anymore). The walking distance increased after the 24th week of treatment *(8–10)*. Time to absolute claudication is measured in minutes and should be distinguished from time to onset of claudication. Usually, as walking continues claudication gets worse and the patient has to stop to ease off the pressure, the pain, the cramp, the squeezing sensation, the burning, and the other symptoms of claudication. Time to claudication is the time in minutes (or even less) it takes for the onset of claudication. In these two series, time to absolute claudication improved by approximately 20% *(8–10)* after 24 weeks of treatment. It should be emphasized that pentoxifylline by its mere pharmocologic mode of action on the newly produced red blood cells, takes at least 120 days to manifest its benefits; new erythrocytes produced in the presence of pentoxifylline would be more pliable, flexible, elastic, and deformable. Thus, the hemorrheologic benefit may manifest itself after the life span of the older erythrocytes ends. The life span of the red blood cells is approximately 120 days. In two meta-analyses of randomized placebo-control trials, pentoxifylline was found to increase walking distance to onset of claudication by approximately 20 to 30 m and distance to absolute claudication by approximately 40 to 50 m *(11,13)*. A more recent meta-analysis showed that the quality of the reported data of all existing trials do not allow any reliable conclusion on the efficacy of pentoxifylline *(12)* in LEAD. Nonetheless, many patients with LEAD who have been treated with pentoxifylline 400 mg po tid are reluctant to discontinue taking it; some patients attribute the symptomatic improvement of their claudication to pentoxifylline, although detailed interviews by many cardiovascular physicians, including the author, show that in parallel with the initiation of pentoxifylline therapy many, patients with LEAD started engaging in a progressive community based exercise program for at least 30 minutes or more of walking every day, excluding the necessary for symptomatic relief pauses. Today, pentoxifylline is prescribed for certain oncology patients who may benefit from its anti-inflammatory and antimitotic effect *(6,7)*.

There is skepticism about the efficacy of pentoxifylline *(13–15)*. A meta-analysis concluded that a benefit for pentoxifylline was suggested, but that properly conducted large multicenter trials (would be) required to provide estimate of benefit *(11)*. This meta-analysis *(11)* estimated a mean improvement in maximal walking distance with pentoxifylline of

48 m (95% confidence interval 18–79 m), similar to the 64 m change with pentoxifylline in the study by Strandness and co-workers *(17)*. In the same study, pentoxifylline was no more effective than placebo.

CILOSTAZOL

In 1999, cilostazol was approved by the FDA specifically for the treatment of intermittent claudication. Cilostazol is a quinolinone derivative that inhibits phosphodiesterase III. The mechanism of action of cilostazol on the symptoms of claudication is not fully understood. Cilostazol and several of its metabolites decrease cyclic adenosine monophosphate (AMP) degradation by inhibiting the enzyme phosphodiesterase III; as a result cyclic AMP concentration increases in both platelets and the vascular wall, leading to decreased platelet aggregation and vascular dilatation *(18)*. Cilostazol can inhibit platelet aggregation introduced by a variety of stimuli including thrombin, ADP, collagen arachidonic acid, epinephrine, and shear stress *(19)*. The effect of cilostazol on circulating plasma lipids has been examined in human volunteers. After 12 weeks as compared to placebo, cilostazol at the dose of 100 mg po bid was associated with a reduction in the serum triglyeride level by about 15% and an increase in HDL-cholesterol by about 10% *(19)*. Cilostazol produces a nonhomogeneous dilatation of vascular beds, with greater dilatation in the femoral arterial beds; it has minimal effect on the vertebral beds, the carotids or superior mesenteric arteries. The renal arterial tree is not responsive to the effects of cilostazol. Cilostazol is not known to have any direct effect on the skeletal myocyte metabolism.

Several prospective randomized trials have reported that cilostazol improves walking distance in patients with intermittent claudication by 40 to 50%, compared to placebo after 12 to 24 weeks of treatment *(20,21)*. One of these placebo-controlled trials evaluated both pentoxifylline and cilostazol *(17)*. Pentoxifylline demonstrated no benefit in either onset of claudication or absolute claudication distance as compared to placebo. Cilostazol, however, significantly improved both distances compared to placebo *(17)*. A prevalent side effect of cilostazol is headache. Transient diarrhea, palpitations, and dizziness have also been reported. The FDA has issued a warning regarding the use of cilostazol in patients with congestive heart failure, because of the increased possibility of sudden cardiac death observed with other forms of diesterase type III inhibitors. Thus, it has become routine practice to assess cardiac function clinically and echocardiographically prior to initiating therapy with cilostazol for claudication, and periodically thereafter. As a result of the modest vasodilatation, heart rate may increase by a mean of 5.1 and 7.4 beats per

minute in patients treated with cilostazol 50 and 100 mg po bid, respectively. In 264 patients evaluated with ambulatory electrocardiogram (ECG) recordings, more cilostazol-treated patients demonstrated an increase in ventricular ectopic activity and nonsustained ventricular tachycardia events (runs of six or less consecutive ventricular ectopic beats), than did placebo-treated patients; these increases of ventricular ectopy were not dose related *(23)*.

A comparison of cilostazol with pentoxifylline for the treatment of intermittent claudication demonstrated a great improvement in walking distance with cilostazol *(17)*. After 24 weeks of treatment, the increase in the absolute claudication distance was 107 m (54%) with cilostazol compared to 64 m (30% increase) seen with pentoxifylline or the placebo effect of 65 m (30%). The beneficial effect of cilostazol on walking distance in claudication patients rapidly dissipates when the drug is withdrawn *(24)*. There was a decline in walking distance with crossover to placebo within 6 weeks; this observation provides support that the initial improvement with cilostazol was caused by the medication's action. Further evidence of clinical benefit with cilostazol is the subjective improvement in walking performance. There was a significant improvement in the physical component scale score of the Medical Outcome Scale Health Survey (SF-36) with cilostazol relative to placebo *(18,19)*. Using the Walking Impairment questionnaire, the cardiovascular physician can see an improvement in the walking speed, walking distance, and in specific measurements of walking difficulties with cilostazol therapy *(20,21)*. Patients with LEAD describe claudication symptoms to have improved at the end of treatment with cilostazol as compared to placebo. A global therapeutic assessment found that significantly more patients rated their outcome as *better* or *much better* with cilostazol compared to placebo *(21,25)*.

Two trials demonstrated an approximate 9% increase in the ankle–brachial index (ABI) with cilostazol *(20,26)*. Although the overall significance of this finding is uncertain, some have suggested that this provides evidence of its vasodilating properties *(20,26)*. A trial involving 516 patients with moderately severe intermittent claudication found that 50 mg of cilostazol given twice daily also improved walking distance with a possible dose response *(21)*. The standard dose for cilostazol is 100 mg po bid. Important pharmacokinetic issues of cilostazol include rapid oral absorption, extensive binding to plasma proteins, 95% hepatic metabolism by cytochrome P450 isoenzymes (especially CYP3A4 or CYP2C19) with formation of active metabolites, and elimination primarily by the renal route (74%) *(28)*. Pharmacokinetic studies have suggested that dose adjustment is not required in renal insufficiency or mild

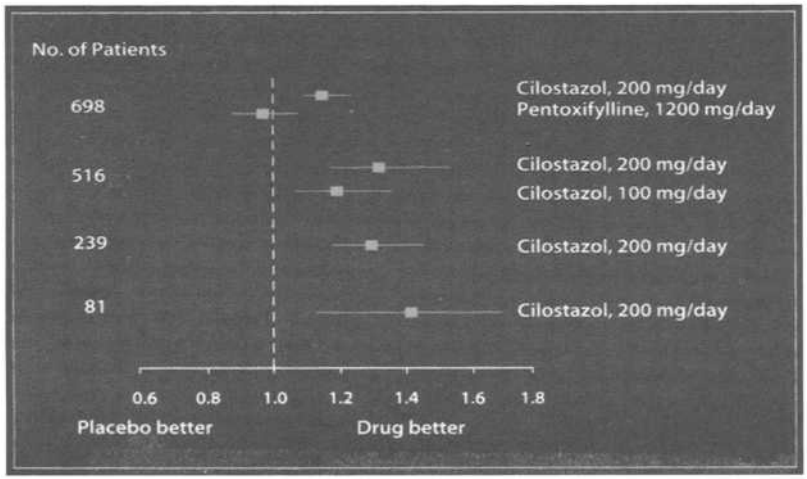


Fig. 1. Geometric mean ratios of the maximal treadmill walking distance (on the horizontal axes) and 95% confidence intervals for cilostazol as compared with placebo. Results of four randomized trials. From ref. *22*, with permission.

hepatic impairment, but caution should be exercised in patients with moderate or severe hepatic impairment. Dose adjustments are necessary during co-administration of inhibitors of CYP3A4 or CYP2C19 such as ketoconazole, erythromycin, diltiazem, and omeprozale *(28,30)*.

Side effects of cilostazol have been reported in about 25 to 30% of patients including gastrointestinal cramping, loose bowel movements or diarrhea, headache, dizziness, and palpitations *(22)*. The side effects reported are transient and some appeared to be dose dependent *(21)*. The most common side effect is headache, which is mild and responds well to over-the-counter analgesics. The rate of withdrawal among patients who use cilostazol was similar to those receiving placebo or pentoxifylline *(24)* (Fig. 1).

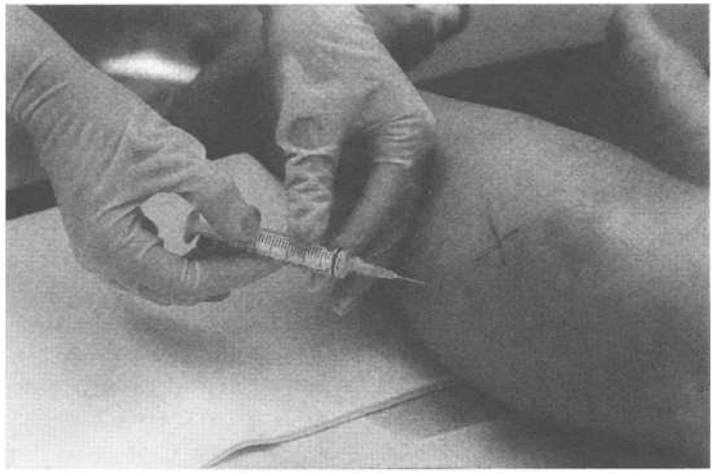
The insert of cilostazol and the *Physicians Desk Reference* contain a *black box* warning that it is contraindicated in patients with congestive heart failure of any severity. As mentioned earlier, this is a caution resulting from the findings with other phosphodiesterase III inhibitors in a subpopulation of patients with congestive heart failure. Oral milrinone that has a significant positive inotropic action, had a detrimental effect on mortality in patients with New York Heart Association class III-IV heart failure *(29)*. Cilostazol has not been adequately studied in patients with congestive heart failure. The long-term effects of phosphodiesterase III inhibitors are not known. It is prudent to evaluate every patient prior to initiating cilostazol therapy both clinically and with echocardiography

looking for congestive heart failure. During therapy with cilostazol periodic evaluations of the heart rhythm and of the cardiac function should be performed, and also an assessment of possible myocardial ischemia silent or symptomatic in view of the fact that on cilostazol the patient with LEAD may be able to walk for a longer distance and become potentially tachyacardic. In the United States, clinical trials conducted with more than 2000 patients found no serious adverse effects like myocardial infarction (MI) or mortality attributed to cilostazol for up to 6 months of therapy with it *(22)*. Other phosphodiesterase inhibitors, like NM-702, have shown benefit in increasing walking distance in patients with claudication as a result of LEAD *(31)*.

The effects of propionyl-L-carnitine and L-arginine in claudication owing to LEAD have been tried in small series, but no conclusions have been reported as yet.

Angiogenetic growth factors in the treatment of LEAD and intermittent claudication are very promising *(32–42)*. There is ongoing clinical research with angiogenic growth factors, specifically with basic fibroblast growth factor (bFGF) *(43)* and vascular endothelial growth factor (VEGF) *(44)*. Angiogenesis is the formation of new blood vessels by sprouting from pre-existing vessels. Arteriogenesis is the process of progressive enlargement and remodeling of existing collateral vessels *(42)*. The cellular and molecular mechanisms in angiogenesis are complex and involve a series of steps including cell proliferation, migration, cell–cell and cell–matrix interactions, extracellular matrix turnover, and eventual formation of a primitive capillary tube that then evolves through a process of myocyte and pericyte recruitment into a blood vessel *(42)*.

Angiogenesis is promoted by intramuscular (IM) injections (Fig. 2) in the ipsilateral gluteal, femoral, tibial areas; there appears to be chemotactic affinity between regional ischemia of the limb or limbs and growth factors, which promote angiogenesis. One study *(44)* suggests that intramuscular gene transfer therapy with phVEGF 165 promotes healing of ischemic ulcers in patients with critical lower extremity limb ischemia. In a more recent study *(45)*, intrafemoral arterial administration of recombinant fibroblast growth factor increased treadmill exercise time significantly compared to placebo. The TRAFFIC study, a randomized trial, is the prototype for other trials of therapeutic angiogenesis *(43)*. Probably the most promising therapeutic angiogenesis is the IM injection of commercially available human, HLA compatible, platelet concentrate into the ischemic limb. Platelets induce collateral vessel formation by supplying VEGF *(45)*. The patient's own platelet concentrate can be injected into the ischemic muscle in order to promote angiogenesis. The IM injection of the patient's own thrombocytes into his or



Intramuscular gene therapy in chronic critical limb ischemia.

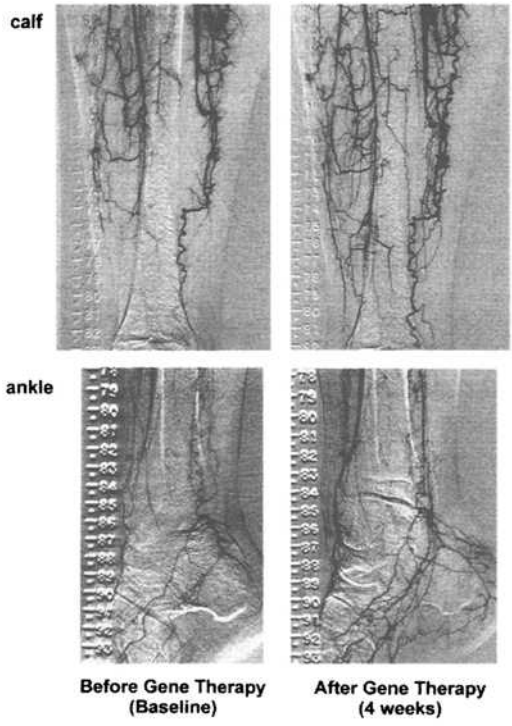


Fig. 2. Serial angiographies after intramuscular gene therapy using phVEGF$_{165}$ showing small-sized newly visible collateral networks at the ankle level. (Coffman JD, Eberhardt RT. Contemporary cardiology. In: Peripheral Arterial Disease. Humana Totowa, NJ, 2003; p. 197.)

her ischemic muscles is becoming a very exciting experience in therapeutic angiogenesis.

AGGRESSIVE RISK FACTOR MODIFICATION

The risk factors associated with atherosclerosis should be aggressively treated in patients with LEAD. *First and foremost is tobacco smoke cessation.* Tobacco use is known to damage vascular endothelial function; it promotes intravascular coagulation and enhances the rate of atherosclerosis progression *(46–50,53,54)*. Cigaret smoking is associated with increased failure rate for percutaneous and surgical revascularization procedures in patients with LEAD. Cigaret smoking in patients with LEAD carries an increased rate of amputations *(47–49)*. Smoking cessation can slow disease progression, reduce the risk of MI and of cardiovascular death *(46,49,50)*. Smoking cessation strategy should include smoking cessation programs and ongoing education from the patient's physician, physician's assistant, family, and friends.

It has been a useful strategy to invite the smoker on a significant date, like his or her birthday, wedding anniversary, or other professional or social event, to announce in front of his family, friends, and business associates, "I am stopping all forms of smoking now. I am quitting *now*!" In preparation for that important date, the smoker's health date, the smoker and their significant other should eliminate all cigaret lighters and other smoking paraphernalia from the home, workplace, and car. The smoker should avoid visiting bars, restaurants, and other areas where smoking is even partially allowed. The spouse of the smoker should suppress smoke odor at home by spraying curtains, furniture, etc. with a pleasant natural aroma .

Nicotine replacement therapy in the form of transdermal nicotine preparations combined with sustained-release bupropion *(51)* has been successfully tried in some hard core nicotine addicts, after the above social and practical measurements have been taken. The combined therapy of transdermal nicotine and bupropion should last for at least 6 months, and in many cases much longer if the crave to smoke still exists. Sustained-release bupropion therapy alone resulted in a 23.1% tobacco use cessation at one year in a placebo-controlled trial *(51)*. Combination therapy of bupropion and high dose transdermal nicotine has been successful for smoking cessation, but the physician should be certain that his or her patient is not prone to coronary spasm. In premenopausal women, nicotine has been identified as the most important risk factor for coronary spasm *(52)*.

Patients with *diabetes mellitus* have a high relative risk for LEAD, three- to fourfold actually *(53,54)*. Co-existing diabetic neuropathy

increases the risk for dermal ulceration; the symptoms may mimic ischemic rest pain. *Euglycemic control of blood glucose* utilizing both fasting blood sugar samples and hemoglobin A1C assessment should be a key target for every diabetic patient with LEAD. Tight control of blood sugar level prevents small vessel complications of diabetes, but similar benefits on the microcirculation have not been proven at least with today's clinical diagnostic methodologies *(56)*. Large trials of intensive glucose control have shown a reduction in cardiovascular events, including MI, but have not demonstrated a significant reduction in the prevalence of LEAD or amputation. The Diabetes Control and Complication trials compared intensive and conventional insulin therapy in 1441 type 1 diabetics *(56)*. Intensive therapy caused a trend toward reduction in cardiovascular events ($p = 0.08$), but had no effect on the risk for the development of LEAD *(56,57)*. The United Kingdom Prospective Diabetes study *(58)* evaluated 3867 patients with type 2 diabetes comparing intensive therapy with insulin or sulfonylureas to dietary therapy. Intensive therapy was associated with a trend toward reduction in MI ($p = 0.05$), but had no impact on the risk of death or amputation caused by critical lower limb ischemia *(58)*. Nevertheless, the established benefits of intensive therapy on macrocirculatory diabetic complications include retinopathy, nephropathy, and neuropathy with a trend for reduction in cardiovascular events. Clearly, optimization of serum glucose in patients with LEAD is warranted *(59)*. The goal should be to keep all patients with LEAD euglycemic.

Systemic hypertension, is strongly associated with LEAD *(60)*. The risk of MI and cardiovascular death is increased significantly in patients with LEAD and hypertension *(60)*. Untreated or undertreated hypertension can lead to serious cardiovascular complications. In addition to the strong statistical association between systemic hypertension and cardiovascular events there is pathophysiological evidence as well, i.e., an association between the activation of the renin-aldosterone-angiotensin system and increased risk of adverse cardiovascular events in patients with hypertension *(61)*. The Heart Outcomes Prevention Evaluation (HOPE) trial *(62)* evaluated the effect of an angiotensin-converting enzyme inhibitor (ACEI), ramipril, on the reduction of adverse cardiovascular events in patients with arteriosclerosis *(62)*. In this study *(62)*, more than 4000 (44%) patients had LEAD, defined as symptoms of intermittent claudication, history of peripheral revascularization procedure or an ABI below 0.9. In the same study *(62)*, 17.7% of individuals in the placebo group died or suffered nonfatal MI or stroke compared to 14.1% of the ramipril treated group; this difference accounts for a 22% reduction of the composite (MI–stroke–death) event risk. There was no

significant difference in the protective effect of ramipril between subjects with LEAD and those without it. The HOPE study *(62)* emphasizes the importance of including patients with LEAD in studies of secondary prevention of adverse cardiovascular events. Angiotensin-converting enzyme (ACE) inhibition can reduce risk of ischemic events in the LEAD patient population. In diabetic patients with hypertension and LEAD, ACE inhibitors may be beneficial in decreasing the rate of progression of renal dysfunction and overall nephropathy. In patients who cannot tolerate ACE inhibitors well, either as a result of cough mitigated by the bradykinin effect or other side effects, angiotensin receptor blockers (ARBs) have been increasingly utilized: valsartan, losartan, etc., either alone or more preferably combined with a low-dose hydrochlorathiazide or chlorthalidone. Traditionally, the goal and target for blood pressure (BP) control have been set by the Joint National Committee (JNC) on Hypertension. This committee consists of experts on hypertension invited by the National Heart Blood and Lung Institute to confer and come up with recommendations in a consensus forming paper based on existing data. The latest *(55)* JNC 7 provides a new guideline for hypertension prevention and therapy. The JNC 7 Committee gives us conclusions and important guidelines: (1) in persons older than 50 years, systolic BP of more than 140 mmHg is a much more important cardiovascular disease (CVD) risk factor than diastolic BP; (2) The risk of CVD, beginning at 115/75 mmHg, doubles with each increment of 20/10 mmHg. Individuals who are normotensive at 55 years of age have a 90% lifetime risk for developing hypertension; (3) Individuals with a systolic BP of 120 to 139 mmHg or a diastolic BP of 80 to 89 mmHg should be considered as prehypertensive and require health-promoting lifestyle modifications to prevent CVD; (4) Thiazide-type diuretics should be used in drug treatment for most patients with uncomplicated hypertension, either alone or combined with drugs from other classes. Certain high-risk conditions are compelling indications for the initial use of other antihypertensive drug classes (ACE inhibitors, ARBs, β-blockers, calcium channel blockers); (5) Most patients with hypertension will require two or more antihypertensive medications to achieve goal BP (<140/90 mmHg, or <130/80 mmHg for patients with diabetes or chronic kidney disease); (6) If BP is more than 20/10 mmHg above goal BP, consideration should be given to initiate therapy with two agents, one of which usually should be a thiazide-type diuretic; and (7) The most effective therapy prescribed by the most careful clinician will control hypertension only if patients are motivated. Motivation improves when patients have positive experiences with, and trust in their clinician *(55)*.

EXTRA-LOW LDL CHOLESTEROL

Improvement of the lipid profile is beneficial to all vascular patients with or without demonstrable coronary disease. The goal for the LDL-cholesterol (LDL-C) is to be well below 90 mg/dL or as low as possible, a natural occurrence in parts of China and Japan. The recommendation for an LDL well below 90 mg/dLis based on many large clinical trials utilizing statins. The prevailing trend today based on recently completed large clinical trials is extra-low LDL cholesterol; a median level of 78.9 mg/dL *(71)* and 62mg/dL *(72)*. Patients with extra-low cholesterol levels had prospectively fewer cardiac deaths and overall cardiovascular events. The optimal low level of LDL-C is unknown. Analysis of the ARBITER study *(73)* shows a greater likelihood of regression of atherosclerosis across a broad range of LDL cholesterol values with the greatest likelihood of regression at a LDL-C below 70 mg/dL Regression of atherosclerosis demonstrated by intravascular ultrasonography (IVUS) imaging in the coronary circulation or in LEAD is an established surrogate for clinical benefit—LDL-C reduction should go well below the current National Cholesterol Education Program (NCEP) *(76)* value of 100 mg/dL. Regression of carotid atherosclerosis demonstrated by carotid B-mode ultrasound is directly related to the absolute LDL-C level on statin therapy. The greatest regression was obtained with an LDL-C below 70 mg/dL *(74)*.

The axiom should be for all vasculopathic patients with regards to LDL-C: the lower the better.

The goal for high-density lipoprotein cholesterol HDL should be over 40 mg/dL for men and over 50 mg/dL for women. Hypertriglyceridemia, which has been strongly associated with LEAD, should be aggressively treated with weight reduction, exercise, and a high protein–high fiber diet. High protein, high fiber diets, both short-term and long-term, reduce weight significantly and can improve endothelial function by avoiding sugar and animal fat.

Pharmacotherapeutically, a short-acting statin like simvastatin 20 mg as a starting dose after dinner and fenofibrate 160 mg *(77)* with breakfast has been proven safe and effective for the reduction of triglyceride blood levels and LDL-C. The two pharmacologic agents should be given about 10–12 hours apart to avoid overlap of their half lives and potential liver toxicity. Fibrates should not be co-administered with long-acting statins; the possibility of hepatoxicity and rhabdomyolysis is real, particularly after a fall resulting into muscle trauma and/or unexpected renal dysfunction.

Long-acting niacin *(78–80)* in incremental doses at bedtime is very effective and safe in raising HDL-cholesterol. The starting dose can be 500 mg at bedtime the first week, 750 mg the second week, and 1 g or more thereafter. Long-acting niacin should be given after dinner; improving the lipid profile of vascular patients, can also infer clinical benefits and vascular wall benefit, improvement of endothelial function, long-term and short-term. Long-acting niacin *(78–80)* is safer than short-acting niacin with regard to liver toxicity and should be given after dinner with 325 mg of enteric-coated aspirin, in order to prevent the facial vasodilatation and flashing caused by niacin; smaller doses of aspirin do not prevent flashing. Patient compliance is very important both short-term and long-term. The most common noncompliance side effect of niacin preparations is facial vasodilatation and flashing. Long-acting niacin 500 mg or more combined with a low-dose statin, i.e, 20 mg lovastatin trade name (Advicor) can increase time to claudication and improve lipid profile (author's observations). Raising HDL can inhibit the progression *(75)* of atherosclerosis.

In a recent study *(63)*, 40 mg of simvastatin significantly increased treadmill exercise time to onset of claudication from baseline by 54 seconds (a 24% increase, $p < 0.0001$) at 6 months after treatment, and by 95 seconds (a 42% increase, $p < 0.0001$) at 1 year of treatment. At 6 months and 1 year after treatment with placebo, treadmill exercise time until onset of intermittent claudication was not significantly different from baseline exercise time *(63,69)*.

In dislipilemic patients with claudication combination therapy with simvastatin 40 mg and ezetimibe (zetia) *(70)* 10 mg after dinner combined with fenofibrate *(77)* (Tricor) 160 mg with breakfast, brings a significant improvement in walking time to onset of claudication and time to absolute claudication measured on the treadmill (not yet published data). There is also a remarkable improvement of the patient's lipid profile with this combination therapy.

A combination therapy of rosuvastatin *(81)* (Crestor) and fenofibrate (Tricor) *(77)* has been gaining popularity fast among vascular physicians and cardiologists. Rosuvastatin can keep LDL very low and also increase the plasma levels of HDL. Fenofibrate *(77)* in the morning can bring a dramatic reduction of the serum triglycerides. Rosuvastation and fenofibrate is a safe combination therapy with regards to liver toxicity and myositis. Nevertheless, it is prudent to periodically evaluate the patient clinicaly and with liver enzymes and creatine phosphokinases.

Exercise therapy and a heart healthy diet should be preconditions for any long-term pharmacotherapy. A beneficial diet for every vascular

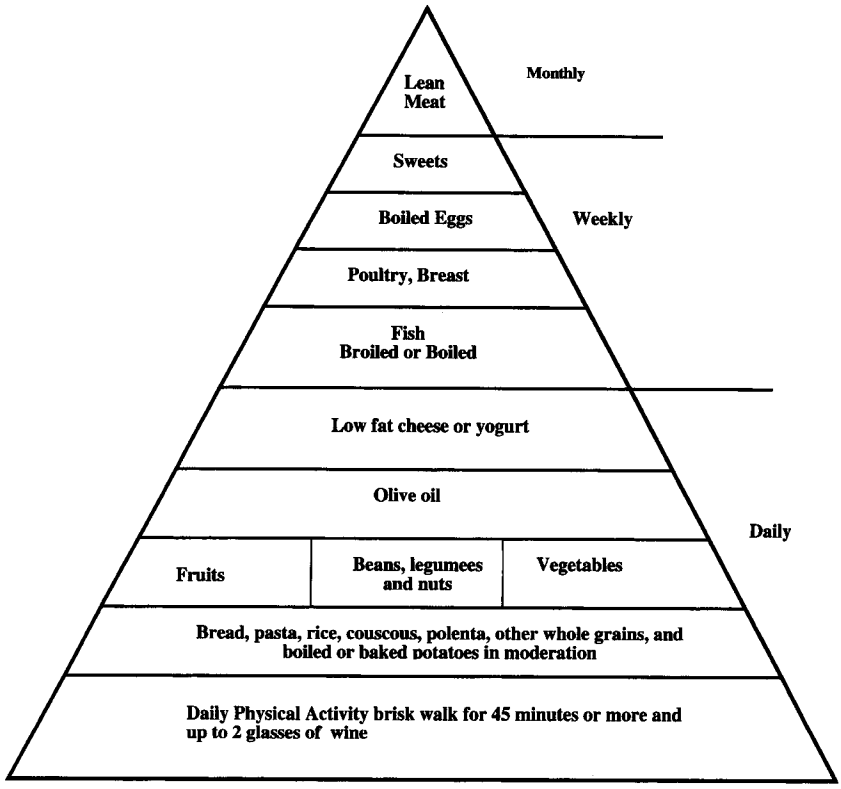


Fig 3. Food Pyramid reflecting the traditional healthy Mediterranean diet. Adapted from Oldways Preservation and Exchange Trust.

patient is the Mediterranean diet, a time honored diet rich in antioxidants (*see* Fig. 3).

ANTIPLATELET THERAPY

Patients with arterial disease caused by atherosclerosis, carotid, coronary, aortic, or LEAD are prone to thrombosis.

In the Peripheral Arterial Disease Detection, Awareness, Risk and Treatment: New Resources for Survival (PARTNERS) study *(64)*, 6979 patients age 70 years or older or age 50–69 years with diabetes mellitus or history of smoking were evaluated by history and ABI. LEAD cases were classified as such, if the ABI was <0.9 or if there was a history of limb revascularization. A main outcome measure evaluated the treatment of LEAD patients compared with other forms of arterial disease.

Although patients with LEAD had similar risk profiles compared to patients with coronary heart disease, antiplatelet medications were recommended less often in patients with newly diagnosed LEAD (35%) and known LEAD (54%) only, compared to 71% of patients with cardiovascular disease only ($p < 0.001$) *(64)*.

Indeed, antiplatelet therapy is underutilized in patients with LEAD. In the Minnesota Regional Peripheral Artery Disease Screening study, 40% of patients with LEAD were receiving no antiplatelet therapy at all *(65)*. A number of landmark studies have concluded that antiplatelet therapy reduces the risk of nonfatal MI, stroke, and cardiovascular death in patients with cardiac and vascular disease. A collaborative meta-analysis of randomized antiplatelet therapy for the prevention of cardiovascular death, MI, and stroke in high-risk patients *(66)* included more than 135,000 high-risk patients; and the main conclusion was that antiplatelet therapy reduced the risk of adverse cardiovascular events from 13.2% in the control group to 10.7% in the treatment group, a reduction of the odds ratio by 25%. Antiplatelet medications should be recommended for secondary disease prevention in patients with known cardiovascular disease. In a substudy of the same meta-analysis *(66)*, patients with symptomatic LEAD receiving antiplatelet therapy showed a statistically significant reduction (23%) in severe cardiovascular events compared to controls.

The Physicians' Health Study *(67)* evaluated the effects of aspirin (325 mg/day), compared to placebo in more than 22,000 male physicians over an average treatment period of 5 years. Those in the aspirin group had a 50% reduction of surgical limb revascularization procedures compared to the placebo group; this difference is remarkable. However, the incidence of intermittent claudication was not statistically different in the two groups (aspirin or placebo).

The CAPRIE trial *(68)* clopidogrel vs aspirin in patients at risk of ischemic events compared the efficacy of clopidogrel (plavix) to aspirin in more than 19,000 patients with atherosclerosis manifested as ischemic stroke, MI, or LEAD defined by an ABI of 0.85 or less, history of revascularization or amputation secondary to ischemia. In the CAPRIE trial, there were more than 6400 patients with LEAD. Clopidogrel was associated with an overall relative risk reduction of 8.7% for adverse cardiovascular events. Patients with LEAD received the greatest benefit, with a relative risk reduction of 23.8% compared to aspirin alone *(68)*.

Patients with LEAD may present with symptoms of intermittent claudication (for a detailed diagnosis and differential diagnosis, *see* Chap-

ter 1). Intermittent claudication can adversely impact the quality of life and impose severe limitations on overall daily activities. Severe arterial stenoses may threaten the viability of the lower extremity, one or both, as a result of critical ischemia necessitating emergent revascularization or even amputation.

Fortunately, the emerging theropeutic angiogenesis and regression therapy of the atherosclerotic plaque burden should turn down the trend for amputation caused by ischemia.

The author's prediction is that in approximately 5 years an amputation will be considered a defeat for the attending physician of the vascular patient.

Medical therapy of claudication and LEAD need a multifaceted approach; aggressive risk factor modification; smoking cessation; body weight reduction; euglysemic control of diabetes optimization of the lipid profile with medications, a heart healthy diet; regression therapy; antiplatelet therapy; angiogenesis; and exercise therapy, community based or supervised at a medical facility. Exercise therapy among its many benefits, reduces high sensitivity C-reactive protein levels as well.

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11 Exercise Therapy for Lower Extremity Arterial Disease

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CONTENTS

INTRODUCTION

Atherosclerosis is a progressive, generalized disease affecting the entire arterial system. The development of atherosclerotic lesions in the large arteries of the lower extremities eventually leads to significant stenosis. Consequently, blood flow distal to the lesions is significantly impaired. The condition is referred to as lower extremity arterial disease (LEAD).

In the early stages of the disease patients are asymptomatic when performing low-intensity daily activities. As the severity of the disease progresses, arterial blood flow distal to the lesions is progressively impaired. Symptoms occur when blood flow requirements to meet the metabolic demand of the lower extremity musculature are not met. The earliest and most frequent presenting symptom is intermittent claudication (aching or cramping) primarily of the calf and thigh muscles during daily activities requiring walking *(1,2)*. The ischemic pain discourages patients from walking or participating in any form of exercise. Conse-

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quently, muscular and cardiovascular capacities deteriorate, fostering an even more sedentary lifestyle, and further deterioration of muscular and cardiorespiratory functions. Limitations in the walking ability of the patients become more evident as the disease progresses and the patient's capacity to perform occupational, leisure, or social activities is severely limited *(3,4)*. It is estimated that the maximal walking distance on a flat surface at 4 km per hour is less than 1000 meters for about 84% of LEAD patients without pain at rest *(3)*. Eventually, patients develop pain at rest.

There is no established pharmacotherapy that is proven efficacious in improving the functional capacity of LEAD patients or resulting in any significant remission in the course of the disease. Only limited pharmacological agents that alter blood rheology *(5)* and others that improve ischemic skeletal muscle metabolism *(6)* are available, with limited success in improving physical performance for LEAD patients.

LEAD is classified on the scale of I–IV. The classification is based on symptoms.

Class I	Blood flow reduction does not produce visible symptoms and patients are asymptomatic
Class II	Intermittent claudication is precipitated by moderate exertion i.e., walking fast, uphill, or distances longer than 200 m
Class III	Claudication occur when walking a short distance of approximately 50–100 m
Class IV	Ischemic pain occurs at rest

RATIONALE FOR EXERCISE AND THERAPY FOR LOWER EXTREMITY ARTERIAL DISEASE PATIENTS

Approximately 2500 years ago the ancient Greek physician Hippocrates wrote:

> *Speaking generally, all parts of the body which have a function if used in moderation and exercised in labors in which they are accustomed, become thereby healthy and well developed and age slowly, but if unused and left idle, they become liable to disease, defective in growth, and age quickly.*

These observations were largely forgotten for centuries and the relationship between a physically active lifestyle and health was not recognized until the early 1950s. Since then, a number of large epidemiologic and prospective studies provided consistent and robust evidence that a sedentary lifestyle is deleterious to health. Conversely, physical activity reduces the risk of premature cardiovascular disease and all-cause mortality *(7–9)* even in patients with established risk factors *(10,11)*. There

is also strong and consistent evidence documenting the protective and therapeutic effects of increased physical activity on the traditional cardiovascular disease risk factors including diabetes, hypertension, and dyslipidemia *(12–14)*. In light of these data, national health organizations including the American Heart Association, American College of Sports Medicine, and the Centers for Disease Control and Prevention strongly recommend that increased physical activity alone or as an adjunct to pharmacologic therapy is implemented for the prevention and management of cardiovascular disease and the aforementioned risk factors *(15–17)*.

LEAD patients often suffer from comorbidities, including hypertension, diabetes mellitus, dyslipidemia, and coronary artery disease. In addition, the sedentary lifestyle fostered by claudication accelerates the deterioration of cardiorespiratory and muscular functions, and leads to a decline in the overall health and quality of life for the LEAD patient *(4)*. Thus, treatment for intermittent claudication should include lifestyle changes for positive modification of the traditional cardiovascular disease risk factors.

Exercise training should be an integral part of such therapeutic approach. An exercise training program of low to moderate intensity can attenuate the deleterious effects of the aforementioned comorbidities associated with LEAD, and provide an attractive and conservative alternative therapy for these patients. For example, walking, the preferred form of exercise for LEAD patients, has a relatively low risk–benefit ratio, it is relevant to daily living, inexpensive, easily implemented to large populations, contributes to the overall health, can be used alone or as an adjunct to pharmacotherapy, and does not interfere with the surgical possibility that may be deemed necessary in the future. In addition, the well-recognized local vasodilatory effects of exercise and the preservation of lean body tissue can only be of benefit to these patients.

EXERCISE THERAPY FINDINGS

The obvious need to improve blood flow to the affected lower extremities in LEAD patients, and the well known local vasodilatory effects of physical work prompted early investigators to assess the possible therapeutic effects of structured exercise programs for these patients. Scientific assessment in patients with mild and moderate claudication began in the 1960s *(18–21)*. In a pioneering study, Hillestad *(18)* assessed calf muscle blood flow at different workloads. He described the different effects speed and distance had on time to claudication. He also suggested that not only blood flow limitations, but additional fac-

tors are involved in determining the walking performance. Interestingly, the effect of excess weight on walking performance was also assessed, a factor largely ignored by more contemporary investigators. The concepts and findings of this study provided the background and direction for studies that followed.

Exercise rehabilitation is now recommended and implemented as the first line of therapy for LEAD patients in stage I or II alone, in conjunction with medical therapy, and after reconstructive arterial surgery in patients with significant hymodynamic improvements *(22)*. Primarily, two exercise training protocols have been used extensively. One requires the patient to walk to the onset of pain (claudication), rest until the pain subsides, and repeat this intermittent walking several times. The other protocol is similar with the exception that the patients walk until near-maximal or maximal claudication is reached.

Without exception, exercise training studies involving LEAD patients have yielded substantial and clinically significant improvements in walking distance to the onset of pain or maximal pain *(18–40)*. A Meta-analysis of 21 studies revealed that, following exercise rehabilitation, the average walking distance to onset of claudication increased by 179% and the distance to maximal claudication by 122% *(23)*. Significant increases in peak oxygen uptake along with improvements in maximal and pain-free walking time have also been reported *(30)*.

There is also evidence of a synergistic effect when exercise is combined with pharmacologic therapy or reconstructive arterial surgery than either therapy alone. After six months of either antiplatelet therapy, exercise therapy, or a combination of antiplatelet therapy plus exercise, walking distance improved in all groups. However, the greatest improvements were observed in the combined therapy group. Pain-free and maximal walking distances increased by 120% and 105% in the combined therapy group, 90% and 86% in the exercise alone group, and only 35% and 38% in the antiplatelet only group *(37)*.

Exercise training following reconstructive arterial surgery also merits special attention. In one study, patients were randomized to reconstructive surgery, exercise alone, or reconstructive surgery plus exercise. Performance was assessed at baseline and 48 weeks following intervention. The symptom-free and maximal walking distance in the exercise alone group increased by 179% and 151%, respectively. In the surgery only group, the increase was 376% in the symptom-free distance and 173% in the maximal walking distance. In the group that combined surgery with exercise, symptom-free and maximal walking distance increased by 698% and 263%, respectively *(35)*.

The findings of the two studies presented (arterial reconstructive surgery and the antiplatelet therapy) support that a well-implemented exercise program can play an integral role in the therapy of the LEAD patient as an adjunct to either therapy.

Exercise Mode

The mode of exercise for most studies is walking combined with some other forms of leg exercise such as running, cycling, stair climbing, dancing, jumping, and other dynamic and static leg exercises *(23)*. Several studies have also used walking alone and two studies combined and compared walking with resistance (strength) training *(31,38)*. Walking appears to be superior to other forms of exercise training, especially when the exercise protocol requires that patients perform intermittent bouts of walking to near-maximal or maximal pain *(24)*. This is not surprising considering that exercise-induced adaptations are specific to the imposed demand (SAID Principle) and the method of assessing performance (treadmill walking) is similar to the training mode (walking). In other words, in patients whose exercise training consists of walking, performance will be greater and assessments more accurate if they are assessed by a walking test than any other method (i.e., bike test). Greater improvements with walking to near-maximal or maximal pain also suggests that the intensity of the stimulus is directly related to the degree of physiological adaptations.

It is interesting that the efficacy of resistance training as a therapeutic modality for the LEAD patient has been considered in only two studies *(31,38)*. Although both studies reported that resistance training was inferior to walking and no additional benefits to the patient were added when the walking program was supplemented with resistance training, the possible therapeutic effects of resistance exercise for the LEAD patient should not be dismissed.

It is probable that endurance of the leg musculature becomes the limiting factor in LEAD patients after aerobic capacity has improved via a walking program. This is supported by recent findings suggesting that aerobic capacity in the elderly may be compromised by muscle weakness and decline in muscle mass and strength *(41–43)*, characteristics also exhibited by LEAD patients *(44)*.

The failure of the two studies to yield favorable findings may be explained, at least in part, by the resistance training protocol used in these studies. It is important to note that both studies implemented high resistance to reach maximal fatigue of the lower extremity muscles within five to six repetitions. Such training is more conducive to muscular

strength rather than endurance gains. Yet, most daily activities for healthy individuals, and perhaps more so for the LEAD patients, require muscular endurance rather than muscular strength. Therefore, if resistance training is considered as an adjunct to a walking exercise program (aerobic component) for the LEAD patient, it should include resistance training with high repetitions *(15–20)* and low weight. Such an exercise program is more conducive to muscular endurance rather than strength, and reflects more closely the demands of daily living activities. The findings of a recent study supports that such a program can enhance the ability of LEAD patients to perform daily activities. Healthy adults between the ages of 60 and 83 participated in a 6-month resistance training exercise program consisting of 8 (low) and 13 (high) repetitions. Gains in aerobic capacity and treadmill time to exhaustion were higher in the group that trained with high repetitions *(45)*.

Evidence also supports that in certain conditions glycolytic pathways may play an integral role in the performance of the LEAD patients. Undoubtedly, improved oxidative metabolism will be of benefit for a certain period of exercise duration and intensity. However, at increased workloads (i.e., faster or uphill walk) the demand on the glycolytic pathways also increases. A compromised capacity of type IIa (glycolytic) and the intermediate IIb (oxidative-glycolytic) muscle fibers will lead to greater lactate accumulation at the local level and subsequent claudication pain, forcing the patient to stop *(36)*. The type II fibers respond better to forceful muscle actions that largely depend on anaerobic energy metabolism *(46)*. Therefore, it is likely that challenging the muscle groups of the lower extremities via high-repetition resistance training will improve the glycolytic capacity of the type IIa and IIb fibers, enhance muscular endurance, and ultimately improve the ability of LEAD patients to perform daily tasks.

Intensity

The intensity of the exercise programs is not well described in any of the studies. It is assumed that the exercise intensity depends on the onset of claudication pain *(38)*. It is estimated that only 16% of patients who do not experience pain at rest can walk a distance of 1000 m or more on a flat surface at 4 km/hour or 2.5 miles/hour *(3)*. The average metabolic equivalent (MET) level calculated from the studies included in the meta-analysis was about 3.8 *(23)*. Collectively, this information supports an exercise intensity of approximately 2–4 miles/hour walking speed on a flat surface *(47)*.

Duration and Frequency

In recent studies, the exercise duration was 30 to 60 minutes per session and some reported an exercise duration of 30 minutes or less. Improvements in the onset of claudication pain and maximal claudication pain walking distances are significantly greater in patients exercising 30 or more minutes per session when compared to those exercising less. There is also evidence to support that exercising three or more sessions per week yields greater improvements in claudication distance when compared with fewer than three times per week *(23)*. Thus, it appears that most improvements in physical performance for LEAD patients occur when exercising for at least 90 minutes per week. However, the interaction between duration and frequency cannot be discerned from the existing literature.

Length of the Training Program

Several studies used exercise programs of at least 6 months in duration and approximately as many studies trained the patients for less than 6 months. Improvements in walking distance were noted even after four weeks of training. However, longer training periods are clearly more successful. Greater improvements in walking distances were associated stronger with the length of training lasting 6 months or longer *(23)*.

Limitations in Exercise Studies

The findings of exercise studies make a strong and convincing argument that exercise training is an efficacious therapy for the LEAD patient. Although the consistently favorable findings reported thus far favor an optimistic view, it is necessary to recognize that serious design flaws exist. In several studies, the number of patients studied is relatively small *(19,20,25,27,32,37)* and, therefore, the statistical power of the studies is compromised. In addition, a control group has not been used in a number of studies *(3,20,21,24,40)*. There is also considerable variability in the distance walked among the exercise studies. For example, the increase in distance to onset of pain ranged from 72 to 746% and to maximal pain 61 to 739% *(23)*. Part of the wide variation may be explained by differences in criteria used to terminate the test *(18)*, and the use of handrails for support during the test, with greater variability when the patients use handrails to support their walking *(20,48)*. In addition, differences in the components of exercise programs used *(22)* and graded vs constant-load protocols with the former yielding less variability for both initial and maximal claudication distance *(18,49,50)*.

Finally, despite the aforementioned limitations of previous studies, the overwhelming improvements in performance following exercise training strongly suggest that exercise training is an attractive, efficacious therapy for LEAD patients. Previous study limitations do no more than underscore the need for more well-controlled exercise studies in this area.

POTENTIAL MECHANISMS

Various mechanisms have been proposed to explain the improvement in walking distance following exercise training. The compromised blood flow to the lower extremities in LEAD patients and the well-known vasodilatory effects of exercise deserve attention.

Peak arterial blood flow to the symptomatic limb during exercise is 25 to 40% of that observed in the asymptomatic limb *(18)*. This suggests that reduction in exercise performance and peak oxygen uptake in LEAD patients may be explained by the decrease in arterial blood flow *(51)*. The significant increase in maximal arterial blood flow to the calf muscle following reconstructive arterial surgery and its association to improvements in walking distance lends further support to the blood flow theory *(35)*. However, findings on leg blood flow changes following exercise training are inconsistent. Some investigators reported a significant relationship between blood flow changes and walking performance *(20,21)*. The average maximal blood flow increase to the calf following exercise training is 19% and the ankle–brachial index or the ratio of systolic blood pressure measured in the ankle and arm increase is 7% *(34–37)*. Others, however, found either no increase in peak blood flow or no significant relationship between blood flow and improvements in exercise performance *(3,25,26,30,36,37)*.

Despite the inconsistencies in exercise-induced blood flow increases, even small changes in blood flow are likely to play a favorable part in performance. However, it is not likely that the relatively small increase of 19% in blood flow can account entirely for the large increases in walking performance reported, suggesting that improvements in other physiological systems may be involved. This is supported by the significantly greater improvement in performance achieved when physical training was added to patients following reconstructive arterial surgery *(35)*.

In healthy subjects, aerobic exercise training leads to an increase in oxidative enzyme activity and an increase in the capacity of the involved musculature to perform aerobic work *(52)*. In addition, the number and size of mitochondria and capillary density increase resulting in a more

efficient use of oxygen *(53,54)*. Greater utilization of oxygen as a result of higher concentration of oxidative enzymes *(25,32,36)* and decreased reliance on anaerobic metabolism *(40)* have also been proposed as adaptations resulting from exercise training in LEAD patients. Mitochondrial and glycolytic enzymes were assessed at baseline and 6 months following therapy in LEAD patients randomized to either exercise training alone, reconstructive arterial surgery, or surgery supplemented with exercise. Relatively high mitochondrial enzyme activity was observed at baseline in all patients suggesting a compensatory response for the compromised blood flow. The enzymatic activity was positively correlated with the maximal walking performance. Following therapy, the metabolic adaptations were reversed in the surgery-only group, but remained high in the surgery plus exercise group. In the exercise only group, however, muscle enzyme activities of cytocrome-c-oxidase, citrate synthase, and 3-OH-acyl-CoA-dehydrogenase were increased beyond baseline. The increased enzymatic activity was correlated with improvements in symptom-free walking performance. Thus, improved local muscle metabolism with increased oxidative capacity may explain why aerobic exercise training improves walking performance in LEAD patients. This suggests a causal relationship *(36)*.

Improved hemorrheological properties *(28)* and improvement in walking efficiency *(55)* are consistent with exercise training and have been suggested as possible contributing factors in the improved walking performance of the LEAD patients.

In summary, the mechanisms responsible for the exercise-induced improvements in walking distance remain illusive. The decline in physical performance observed in LEAD patients is likely the collective outcome of deterioration on several physiological systems. Likewise, the exercise-induced improvements in the functional capacity of these patients is the result of favorable changes in several of these systems.

DESIGNING AN EXERCISE PROGRAM

The exercise program for the LEAD patient must be tailored to meet the needs and abilities of the individual patient. Existing comorbidities in certain patients should be considered. Ultimately, the purpose of the exercise program should be to minimize the risk–benefit ratio.

Exercise Mode

The preferred mode of exercise should be walking. However, activities that involve the large muscles of the legs (cycling, stair climbing, tennis, dancing, etc.) can be implemented in the exercise program. Such

approach can improve participation and reduce the attrition rate. In addition, supplementing walking with some resistance training exercises for the lower extremity muscles should be considered. Such a program should emphasize relatively low resistance and high repetitions(15–25 repetitions) performed one to two times per week.

Frequency

The frequency of an exercise program should be at least two times per week for the first two weeks and progressively increase to three to five times per week. Exercise sessions should be performed in nonconsecutive days when possible. It is also essential to recognize that more frequent exercise sessions may increase the incidence of musculoskeletal injuries and reduce compliance.

Duration

The cumulative duration of exercise of 30–60 minutes per session is preferred. Longer exercise durations offer added benefits. However, benefits diminish substantially beyond 60 minutes, while the risk of musculoskeletal injuries increases, compliance decreases.

For those unable to sustain longer exercise periods, intermittent bouts can also be implemented at different times throughout the day. For those with low cardiorespiratory fitness, the duration of exercise can begin at any duration level with rest between bouts. The duration can increase progressively over a period of weeks until the desired goal is achieved. The rate of progression should be specific for each patient.

Intensity

Intensity is difficult to define. Improvements in performance have been noted when patients exercise at intensities that elicit the manifestation of claudication. However, greater improvements have been reported when patients exercise at intensities eliciting maximum or near-maximum pain. However, the interaction between intensity and duration has not been assessed. The average MET level of the studies included in the meta-analysis is 3.8 *(23)*. Although the pain threshold is likely to vary among patients, it is recommended that patients push to the intensity that elicits maximal or near maximum claudication pain.

Rate of Progression

Individual goals and baseline cardiorespiratory fitness and other patient characteristics should be considered when determining the rate of progression. Initial changes in progression should be relatively slow.

Gradual increases should be emphasized in the duration and frequency of exercise rather than intensity. An increase in the weekly duration of no more than 10% is relatively safe for most patients.

Risks Associated With Exercise

The risk of cardiovascular events during physical activity in patients with underlying cardiovascular disease and especially those who are habitually sedentary is significant *(56)*. The risk of a cardiovascular event in LEAD patients has not been assessed. However, it is prudent to assume that these patients are at a relatively high risk. Thus, the initial phase of exercise training should be supervised by trained personnel.

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12 Endovascular Strategies for Management of Claudication and Lower Extremity Arterial Disease

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INTRODUCTION

In 1964, Dotter and Judkins introduced therapeutic percutaneous angioplasty of atherosclerotic peripheral plaques, a technique that used multiple coaxial catheters of various sizes to dilate the stenosis. In the mid-1970s, Andreas Gruentzig, at the University of Zurich, Switzerland, developed a double-lumen balloon catheter—used to dilate lesions in the iliac and femoral arteries safely and with a high rate of procedural success. The modern era of cardiovascular interventions had begun. Miniaturization of Gruentzig's balloon catheters system led to the first percutaneous transluminal coronary angioplasty in 1977 in Zurich, per-

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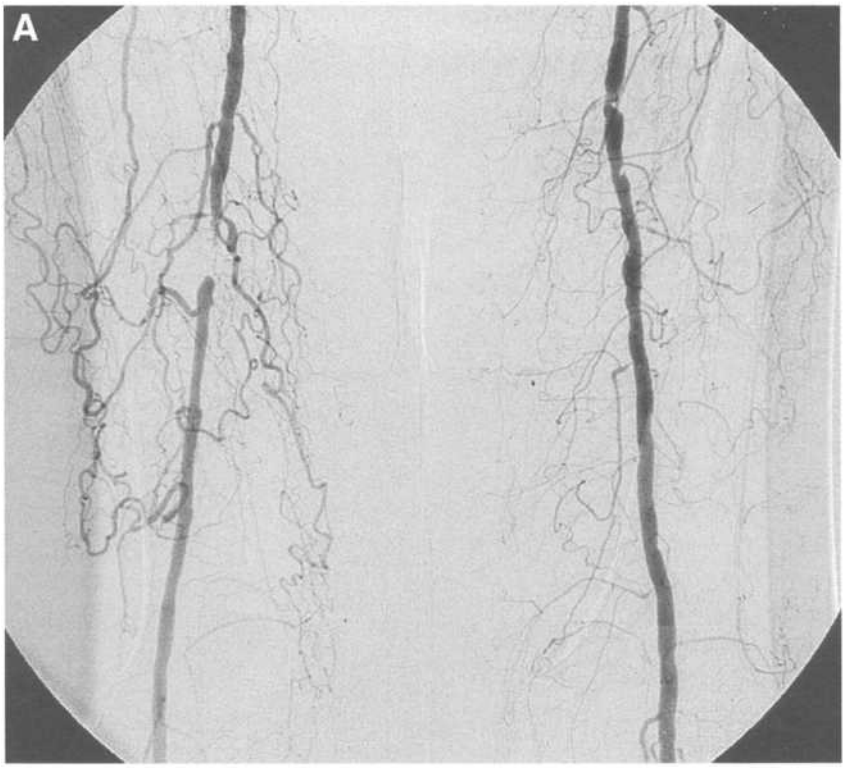

Fig. 1. (A): Pre-intervention angiography. There is significant atherosclerosis of both superficial femoral arteries (SFA) and a discrete 100% stenosis in the disal right SFA. This complete occlusion was felt to be the culprit for the disabling claudication in this 80-year-old woman.

formed by Gruentzig. Since the initial percutaneous interventions, there has been explosive growth in the field of interventional transcatheter therapeutics. This expansion has been fueled by the introduction of an array of new devices, adjuvant pharmacologic therapies, and novel imaging and physiologic measurement catheters designed to overcome some of the limitations of balloon angioplasty. Devices and techniques for lower extremity arterial disease (LEAD) are balloon angioplasty, rotational atherectomy, stents, rheolytic thrombectomy, and distal embolic protection devices. Table 1 lists their indications, potential advantages, limitations, and complications. *See* Fig. 1 for angiography examples.

Whereas, technological breakthroughs of the expandable balloon dramatically improved the initial percutaneous interventional technique of serial dilatation with larger and larger catheters, many attempts have

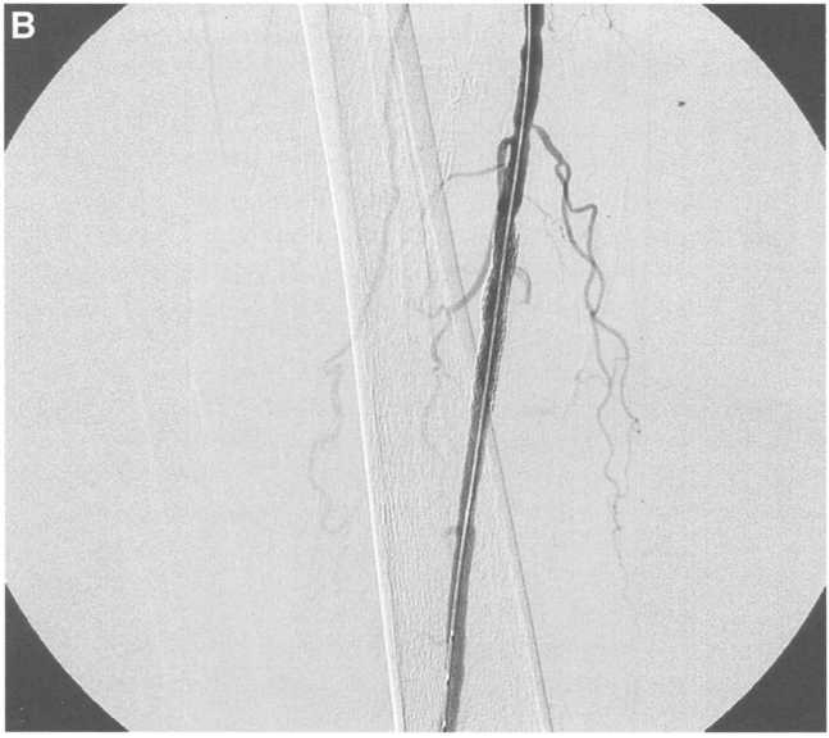

Fig. 1. (*continued*) **(B):** Intervention angiography. A balloon angioplasty was performed on the 100% lesion in the right SFA with inadequate results. The lesion was then stented with an excellent angiographic appearance and 10% residual stenosis. The stent, an Exceed 7 x 40 mm self-expanding device, was placed across the lesion.

been made to improve upon or replace this technique. These have included attempts to better control the depth of vessel injury by cutting the plaque with atherectomy catheters, as well as attempts to selectively vaporize the plaque with laser catheters. More recent attempts have been aimed at performing balloon dilation, but controlling the response to injury either by instilling an agent transmurally through the balloon at the site of dilatation in order to modify the body's response to injury or the use of local irradiation to perform a similar function. It has gotten to the point where an acronym *POBA* has been coined to refer to the unmodified technique of *plain old balloon angioplasty*. Many of the innovations touted to be alternatives to POBA have been relegated to either obscurity or minor complimentary techniques to improve upon the good results obtained with balloon dilatation. In fact, the major advancements affecting the success rates of endovascular therapy include hydro-

Table 1
Transcatheter Therapies of LEAD

Device/Technique	*Indications*	*Advantages*	*Limitations, Disadvantages*	*Complications*
Balloon angioplasty	Any noncalcified vascular stenosis	Simple, easy to use	Ostial locations, calcified lesions	Dissection, acute or threatened closure
Rotational atherectomy	Resistent, nonyielding lesions	Has a niche in certain lesions; ulcerated eccentric or resistant	Limited availability for large diameter vessels	Dissection, distal embolization, no reflow, perforation, increased restenosis
Stents	Most medium and large vessels with eccentric or compliant lesions	Treat dissection and threatened closure, with more predictable outcome in some targets	Limited experience with infrapopliteal arteries	Stent thrombosis, in-stent restenosis may be minimized by drug-eluting stent). Studies needed to be completed for appropriate dosage of larger vessels
Rheolytic thrombectomy	Thrombotic lesions, degenerated vein grafts, peripheral thrombosis or embolus, dialysis access thrombosis	Reduces distal embolization	Can be used primarily in large vessels	Dissection, hemolysis
Cutting balloon	In-stent restenosis, ostial lesions	May be efficacious in resistant plaques	Tortuous arteries	More acute complications (e.g., dissections, perforations)

Brachytherapy	In-stent restenosis	Effective in preventing recurrences	Bulky devices, used primarily in medium and large coronary arteries only; increased radiation exposure, have to coordinate with radiation physicist	Dissection; late thrombosis if new stent is deployed. Long-term effects are unknown
Distal embolic protection devices	Vein graft and carotid interventions, possible use in other peripheral interventions (e.g., renal arteries)	Reduce periprocedural infarction and potentially stroke	Unable to use in large conduits	Ischemia with balloon-occlusion devices
Intravascular ultrasonography (IVUS)	Obtain more anatomical information, evaluate lesion morphology or adequacy of intervention (e.g., stent expansion)	Simple to use	Time-consuming; does not provide physiologic information. Expensive	Need for anticoagulation, dissection

Adapted from Khalil AA, Catheter-based treatment. Postgraduate Medicine 2004;115:16.

philic guide wires and lower profile balloon catheters, which enable the balloon to be more successfully placed at the target lesions.

Therefore, this chapter will focus upon the use of POBA and other intravascular therapeutic interventions above and below the level of the inguinal ligament. Adjunctive techniques to POBA that allow for dilatation of resistant calcified lesions such as atherectomy; or allow for improved balloon dilatation of compliant lesions or treatment of complications of angioplasty such as intravascular metallic stents, and finally therapy to improve the results of balloon dilatation for occlusions with thrombolytic therapy will be discussed.

PERCUTANEOUS TRANSLUMINAL ANGIOPLASTY FOR CLAUDICATION

Angioplasty for claudication can be separated into two anatomic areas for purposes of applicable techniques and expected results. The suprainguinal region describes stenoses in the aortoiliac vessels, whereas the infrainguinal area of pathology includes the femoral and popliteal arteries as well as vessels below the knee.

Aortoiliac angioplasty (with or without stenting) is competitive to aortic bifurcation bypass grafting, a well-established, durable, and safe operation without lengthy recovery period. The major determinants for separating patients between surgical disease and percutaneous transluminal angioplasty (PTA), aside from surgical candidacy, are expected short- and long-term patency results. A joint committee consisting of several councils of the American Heart Association prepared "Guidelines for Peripheral Percutaneous Transluminal Angioplasty of the Abdominal Aorta and Lower Extremity Vessels" which attempts to separate lesions more amenable to angioplasty from those more suitable for a surgical approach. Their conclusions demonstrated that noncalcified, more focal nonostial stenoses are most favorably treated by balloon dilatation. This would also presume the absence of other indications for surgical approach, such as associated aneurysmal disease.

Focal stenotic disease of the abdominal aorta is generally seen in young female cigaret smokers with thigh, hip, and buttock claudication predominant over calf claudication. When present in males it commonly is associated with impotence (Leriche syndrome often associated with aortoiliac disease). Although initially treated as long as three decades ago with a *kissing balloon*technique from a bilateral transfemoral approach, the development of larger diameter, higher-pressure balloons has made a single transfemoral approach possible with satisfactory patency rates *(1)*.

More extensive data is available for lesions involving the iliac arteries with or without concomitant disease of the distal abdominal aorta. Whereas comparison between different studies is very difficult because of patient selection and exclusion criteria, the technical success rate for iliac stenoses in most series is greater than 90% with the more favorable lesions approaching 100%. Tegtmeyer et al. *(3)* obtained a 79% overall patency, and 85% patency of those procedures initially successful when 200 patients with aortoiliac disease were followed for an average of 29 months mean follow-up. The complication rate was just over 10% *(2)*. Whereas there is a wide body of literature concerning outcome of iliac angioplasty, comparison of success rates in the same patients can vary by as much as 30% when different criteria for evaluating the success of the intervention are utilized *(3)*. The long-term data do not reflect more recent procedures that improve the initial technical and clinical success rate, such as thrombolytic therapy for cases of occlusion and/or intravascular stents for occlusions or suboptimal results after initial angioplasty.

The percutaneous approach to infrainguinal arterial disease is much like that of the aortoiliac region, but with diminished longevity like its surgical counterpart.Similar to the surgical tenant of trying to stay above the knee in therapy for intermittent claudication, the percutaneous approach is primarily guided towards short stenoses of the superficial femoral and popliteal arteries above the knee with an infrageniculate approach primarily reserved for patients with debilitating claudication and/or symptoms at rest.

Although studies vary greatly *(3)*, particularly in whether or not they exclude technical failures from their long term follow-up, most series that select patients with good run off (which should include most patients who suffer from intermittent claudication) have 5-year patencies of approximately 50 to 60% with somewhat worse results for occlusions instead of stenoses, and long length of diseased segment *(4,5)*. The American Heart Association Committee again classified lesions that are most favorable for angioplasty to be single nonostial stenoses less than 3 cm in length that are not heavily calcified and above the knee. Lesions approaching 10 cm in length (including occlusions) with runoff disease of increasing severity are more appropriate for surgical therapy *(6)*. Similar findings have been suggested in cost-effectiveness analyses *(7)*.

Whereas the initial success rates of femoral popliteal angioplasty is currently in the 90% range for stenoses and 80% range for occlusions, much of the recent improvement is again a result of the presence of hydrophilic guide wires, digital subtraction angiography with road mapping, the increased availability of antispasmodic pharmacological

agents, and lower profile catheters and balloon catheters *(8)*. Although perhaps beneficial from a marketing standpoint, the use of lasers in an attempt to improve long-term results over POBA has been unsuccessful *(9,10)*.

ATHERECTOMY AND COMPLICATIONS OF ANGIOPLASTY

Although it was initially hoped that directional atherectomy would demonstrate a lower degree of restenosis as has been suggested in some coronary interventional data, long-term follow-up results of femoral or popliteal directional atherectomy has demonstrated disappointing results compared to POBA *(11,12)*. So that in general, directional atherectomy is reserved for therapy of lesions resistant to balloon dilatation at safe inflation pressures, for treatment of either eccentric lesions or complications of angioplasty such as dissection.

Complications of angioplasty are generally related to the catheter size and location of the puncture site, severity of the patient's disease, and associated comorbid conditions such as hypertension or a need for anticoagulation or thrombolysis. The major complications are all in the order of 2 to 5% and include puncture site hematomas and/or subsequent pseudoaneurysms, intimal dissection (which may, depending upon its severity, be a normal sequela of balloon dilatation rather that a complication), arterial rupture, and distal embolization *(13,14,15)*.

THE USE OF INTRAVASCULAR STENTS

The first bare metal stent approved for peripheral arterial use in the United States by the US Food and Drug Administration is the Palmaz Balloon-Expandable Intraluminal Stent (Johnson & Johnson International Systems, Warren, NJ). It was initially approved for use in the iliac artery after suboptimal results following POBA. The use of this stent has been effective in reducing residual gradient across lesions following iliac angioplasty that may have been caused by either recoil of the vascular wall or flow limiting intimal dissections. Long-term studies indicate that iliac artery stenting provides better long-term patency than angioplasty alone in prospective randomized trials of iliac artery primary stent placement vs POBA *(16)*.

Flexible, self-expanding metallic stents have been used in scientific studies for suboptimal iliac artery results with balloon dilatation and in treating complete occlusions. These stents have the advantage of being more flexible for use in either tortuous arteries or from the contralateral

femoral approach but, may have somewhat less exact placement positioning characteristics compared to balloon expansion delivery systems.

Metallic stenting has significantly less restenosis rate compared to balloon angioplasty for lower extremity arteries. Leading examples of impressive, long-term patency are the stents deployed in the iliac artery. Whereas early PTA failures in infrafemoral sites can often be salvaged by the use of a bare metallic stent, the long-term results have been plagued by intimal hyperplasia and stent occlusion. Although no large prospective, methodologically impeccable randomized study is available, it appeared until recently that short-term patencies are similar to those of PTA alone.

The introduction of drug-eluting stents, Sirolimus-Eluting, may solve the problem of in-stent restenosis in LEAD *(17)*; a gratifying experience similar to that with drug (rapamycine or taxol) eluting stents for coronary lesions.

Stents *(17)* that allow elution of antiproliferative medications have been developed and are proven to reduce instent restenosis to nearly negligible rates (4 to 9% in some trials). The first drug-eluting stent is approved for the US market: the Cypher stent has a polymer coating that elutes sirolimus (Rapamune) during a 2-week period with few or no systemic effects. More stents with different drug coatings will soon be available for high grade stenosis of the arteries of the lower extremities.

Drug-eluting stents are a very promising advance. They could be the answer for lesions at increased risk of restenosis as predicted by smaller vessels, longer lesions, and the presence of diabetes mellitus. Like any new technology, however, this advance raises many unanswered questions. Drug-eluting stents have not been adequately and long term evaluated in LEAD or vein graft lesions.

Biodegradeable stents are the products of another technological advancement. A potential advantage of biodegradeable stents is that they leave a larger potential surgical target.

THROMBOLYSIS

Selective arterial thrombolysis is usually utilized in the setting of acute limb jeopardy. However, acute or subacute onset of claudication can be treated with thrombolytic therapy. In addition, chronic occlusions, particularly in the iliac artery, can sometimes be better recanalized with the adjunctive use of thrombolytic therapy allowing the placement of a balloon catheter and subsequent intravascular stent.

For claudication resulting from either thrombotic or embolic occlusions, urokinase has been extensively utilized over the past two-and-a-

half decades. The choice of urokinase over other thrombolytic agents, stems from studies asserting its higher efficacy to complication rates when used in the peripheral arterial system. In general, thrombotic occlusions respond much better than emboli to regional thrombolysis. The therapy is rarely effective if a guidewire cannot transverse the occlusion, and the results are most efficacious when a multiside holed catheter can be placed to infuse the agent directly into the thrombus. If successful, thrombolytic therapy can demonstrate a short stenosis that usually underlies an occlusion for thrombotic disease or completely lyse an embolus, if it is fresh enough to respond. In the case of chronic occlusions (especially in the iliac region) an infusion of urokinase may allow recanalization of a channel through which further intervention can be performed. Whereas thrombolytic therapy may not always allow for successful treatment of the underlying disease by percutaneous means, it can often reduce the extent of surgical intervention and will rarely compromise such procedures.

There are three absolute contraindications to the initiation of thrombolytic therapy: (1) active uncontrollable hemorrhage, (2) likely persistent proximal source of embolization, or (3) neurovascular compromise in the affected extremity, where it is likely that a reperfusion injury such as myonecrosis may develop. Relative contraindications include recent intra-abdominal or intrathoracic surgery, uncontrollable hypertension, a recent intracerebral event that is likely to be hemorrhagic, and possibly the recent use of a porous intra-arterial graft material. Thrombolytic complications are proportional to the duration of therapy and usually consist of hemorrhage, often related to concomitant need for anticoagulation.

CONCLUSION

Indeed, there have been advances in techniques allowing for more bare metal stents and drug-eluting stents and occlusions to be crossed by the angioplasty balloon, thereby increasing the initial success rate. Few techniques have improved upon the long-term durability of POBA. The long-term success rate for angioplasty in the iliac region remains in the upper 80% to lower 90%, 60 to 70% patency in the femoral popliteal region, and approximately 50% below the knee. Most of the interventions, including these to help support angioplasty, do not make a subsequent surgical repair more difficult. Hopefully, with advancements already made in controlling intimal hyperplasia by deploying drug-eluting stents, long-term results of angioplasty will continue to improve in a dramatic way. Any revascularization procedure, mechanical or surgi-

cal, should be followed by medical therapy for life to control risk factors for atherosclerosis and potentially reverse its process.

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13 Severe Lower Extremity Arterial Disease

Limb Salvage and Revascularization

Chad E. Jacobs, MD
and Walter J. McCarthy, MD

CONTENTS

INTRODUCTION

Innovations in surgery, radiology, and medical management during the past 30 years have allowed patients who once faced certain amputation as a result of leg ischemia to be offered a variety of alternatives. Patients with limb-threatening ischemia differ from those with intermittent claudication in regard to both natural history and treatment requirements. Management of patients with claudication involves risk factor modification, exercise regimens, and pharmacologic intervention. This

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group rarely requires amputation. Patients with critical limb ischemia are most often treated surgically in order to preserve limb functionality. Five year rates of limb salvage for this patient population have been shown to be 81% for femoropopliteal bypass and 47% for infrapopliteal bypass *(1)*. However, despite continued additions to our treatment armamentarium, it has been shown that amputation rates have remained the same over the past decade *(2)*. The mortality from major amputations is generally reported to be 3 to 10% *(3)*, but in selected populations, such as those over 80 years old, mortality may be as high as 82% *(4)*. In addition, the rehabilitation outcome after amputation for nonreconstructible arterial disease is usually overestimated. One recent study *(5)* reported that only 26% of patients were able to ambulate outdoors with a prosthesis 2 years after major amputation. Another study predicted that the number of amputations performed in the geriatric population will double by the year 2030 *(6)*. The implications for future health resource allocation are somewhat daunting once these statistics are reviewed.

Our goal as clinicians in the field of peripheral arterial disease should be to apply the multiple technological advances that we have toward limb salvage and the prevention of amputation. This chapter will discuss the surgical, percutaneous, and medical adjuncts available in this regard.

SURGICAL INTERVENTION

The surgical treatment of lower-extremity ischemia must be based on the severity of the patient's symptoms and the overall medical condition of the individual. Atherosclerosis is a systemic process, and these patients are predisposed to stroke, myocardial infarction, and hypertension caused by the involvement of other arterial segments. The extent of such involvement requires full evaluation prior to lower extremity surgery, and some patients may require carotid endarterectomy or coronary artery bypass grafting or stenting before infrainguinal reconstruction is undertaken.

Indications for surgical revascularization include disabling claudication and limb-threatening ischemia. Patients with moderate symptomatic ischemia present with claudication and are candidates for a reconstructive operation only if this disability severely limits their lifestyle or occupational activity. Most patients who claudicate do not require an operation to prevent limb loss, and several large studies have shown that such patients have only a small risk (approximately 5%) of requiring a major amputation within five years of onset of symptoms *(7,8)*. Therefore, most patients who present with intermittent claudication are managed nonsurgically. Such treatment includes formal exer-

cise programs, smoking cessation, risk factor management, and pharmacological interventions as discussed elsewhere in this text.

Interventions for limb salvage are required if a patient develops rest pain, nonhealing ulcers, or gangrene of the toes or foot. After appropriate treatment, rest pain disappears, ulcers heal with standard attention, and gangrenous tissue can be amputated with prompt healing. This allows substitution of a minor toe or forefoot amputation for an otherwise inevitable below- or above-knee amputation. All effort is made to preserve as much extremity as possible so that the potential for rehabilitation is optimal.

OPERATIVE OPTIONS

Selecting the appropriate procedure comes after careful patient evaluation including medical history, physical examination, noninvasive testing, and arteriography. Because there is often arterial disease in several areas, it is essential to determine the arterial segment with the most hemodynamically significant stenosis. The occlusion may be proximally at the aortoiliac level, thus affecting inflow to the lower extremity. It is a fundamental principle that inflow should be restored prior to correcting outflow lesions to the foot. This prevents a newly placed bypass graft from thombosing as a result of inadequate blood flow through the graft.

If in fact the aortoiliac segment is diseased, an aortofemoral bypass graft may alleviate the patient's symptoms. Patients who cannot tolerate an abdominal operation may undergo axillofemoral reconstruction, supplying the lower extremities with blood flow from an axillary artery through a graft tunneled in the subcutaneous tissue. However, the distinct disadvantage of axillofemoral reconstruction is the much shorter patency rate when compared with the traditional aortobifemoral bypass graft *(9)*. If a patient has adequate blood flow to the contralateral leg, a femoral-femoral bypass graft can be used to provide sufficient inflow. Percutaneous angioplasty and stent placement are also common techniques used in the management of iliac artery occlusive disease, and will be discussed in greater detail later.

Once it has been ascertained that inflow to the level of the femoral arteries is adequate, infrainguinal reconstruction can be undertaken. The two traditional approaches for correcting arterial occlusion are either to open the artery and remove the obstruction by endarterectomy, or to fashion a bypass around the obstruction. Longstanding experience has established direct bypass of obstruction as the dominant and superior technique. However, endarterectomy is still used as an adjunct to bypass grafting, and even sometimes alone, such as in the case of a profundoplasty to repair a stenotic profunda femoris artery.

Determining the most appropriate bypass procedure involves identifying the exact level of occlusion, the best proximal site for inflow, and the best distal site for outflow. The proximal anastamosis may be sewn to the common femoral artery, the profunda femoris artery, the superficial femoral artery, the popliteal artery, or even the limb of an aortobifemoral or femorofemoral bypass graft. The distal end of the graft is anastamosed to the most cephalad portion of the leg that allows unobstructed blood flow to the foot. The most common point of arterial occlusion in the lower extremity is at the inferior portion of the superficial femoral artery as it courses through the medial adductor muscles just above the knee, a location known as Hunter's canal. A femoral-popliteal bypass graft is therefore one of the most frequently performed arterial reconstructions. If the above-knee popliteal segment is diseased, the graft can simply be anastamosed to the below-knee segment of the popliteal artery.

In the event that there are no tibial branches remaining in continuity with the popliteal artery, the graft may be anastamosed directly to any one of the three tibial arteries. Because of technique, body habitus, and image quality the tibial vessels may be difficult to adequately visualize on preoperative angiogram. In such cases, an intraoperative arteriogram performed immediately prior to bypass can be quite helpful in determining adequacy of distal target vessels. The tibial vessel with largest diameter, least amount of occlusive disease, and best runoff to the foot should be selected for the distal anastamosis. After completion of the bypass procedure, a completion arteriogram should be performed in the operating room to inspect the reconstruction. If technical flaws are discovered, they can be repaired while the patient is still anesthetized and the graft and anastamosis are still exposed.

CONDUIT OPTIONS

Autogenous Vein

The long-term success of bypass grafts performed below the inguinal ligament depends largely on the type of conduit utilized. Despite general agreement that autogenous saphenous vein provides the best long-term success for distal lower extremity bypass, there is some controversy regarding the optimal technique for constructing such bypasses. The two most widely used techniques include reversal of the saphenous vein and the *in situ* technique. Each technique has inherent advantages. The reversed technique must be used if a patient has inadequate or previously harvested saphenous vein in the ipsilateral extremity. Furthermore, use

of a reversed segment does not require valve lysis as is necessary for *in situ* use. One of the disadvantages of the reversed technique is the size mismatch that is necessitated when the distal, smaller end of the vein is anastamosed to the proximal, larger artery. Similarly, the larger proximal vein must be anastamosed to the smaller, distal artery. Despite these apparent shortcomings, the reversed saphenous vein technique is one of the most commonly used and most successful bypasses performed. Reported patency rates for infrainguinal reversed saphenous bypass grafts are between 75 and 80% at 5 years with a limb salvage rate of 90% *(10–12)*.

The *in situ* technique, first described by May et al. *(13)*, eliminates the concern of anastamotic size mismatch. Use of the *in situ* technique has the theoretical advantage of preserved endothelial function resulting from an intact vaso vasora as well as improved graft hemodynamics, but to date this has not been supported by conclusive data from laboratory studies. Important principles for *in situ* use include valve lysis and ligation of side branches to prevent arteriovenous fistulae. Valve lysis is usually performed after creation of the proximal anastamosis, so that arterial pressure distends the vein to the level of the most proximal valve. A valvulotome is then introduced into the vein through either a side branch or the distal most end and is passed through the valves to cause their lysis. Valves are disrupted in a systemic fashion so that pulsatile uninterrupted blood flow is established through the entire vein graft. Whereas the majority of the *in situ* bypass graft remains undissected in the subcutaneous tissue, the distal end must be dissected and mobilized in order to perform the distal anastamosis. Once this has been completed, side branches must be ligated to prevent arterial blood from entering the venous circulation. Side branch points may be identified by Doppler ultrasound, by small incisions and direct visualization, or by angioscopy, in which case the branches may be not only identified but coil embolized as well. Patency rates for the *in situ* saphenous vein technique have been reported as 65 to 85% with a limb salvage rate of 82% *(12,14–16)*.

Unfortunately, autologous saphenous vein may not always be of sufficient length or diameter for the contemplated procedure, or it may have been harvested for use in another procedure. Alternative autologous vein may be obtained from several locations, including the lesser saphenous vein, the superficial femoral vein, and upper extremity veins. A common approach is to utilize arm vein grafts as the next alternative when saphenous vein cannot be used. In preparation for using arm veins as a conduit, preoperative ultrasound vein mapping can be useful in identifying the location, patency, and diameter of veins. Some surgeons use angioscopy

as well to identify webs, bands, or other sequelae of pre-existing injury to arm veins as a result of venipuncture or cannulation. However, the results of arterial reconstruction with arm vein grafts are not as encouraging as those with saphenous vein grafts. In a recent large study *(17)*, evaluating arm vein as a conduit for arterial bypass, the 3-year primary patency rate was 52%, the 3-year secondary patency rate was 60%, and limb salvage was 80%.

Synthetic Conduits

Synthetic conduits are another option for limb salvage in patients requiring arterial reconstruction as a result of severe atherosclerotic occlusive disease. Despite many clinical studies, the role, indications, and optimal use of synthetic conduits in lower-extremity bypass continue to be a controversial topic. The synthetic graft most widely used today is expanded polytetrafluoroethylene (ePTFE). Initially used industrially as a wire insulator, ePTFE was first used in humans as a vascular conduit in 1976 *(18)*. Polyethylene terephthalate (Dacron) is another synthetic fabric that can be woven or knitted into a bypass conduit. Dacron grafts are sometimes used for lower extremity arterial reconstruction today, and are used frequently by many surgeons for aortic reconstruction. Demonstrating a renewed interest in the use of Dacron for lower extremity bypass, a recent study compared ePTFE vs Dacron for above-knee femoropopliteal bypass, and showed no statistically significant difference between the two synthetic graft choices in regard to primary or secondary patency *(19)*. Regarding ePTFE, several studies in the past have shown that in comparison to autologous saphenous vein, when used for above-knee femoropopliteal bypass, 4- and 7-year patency rates are not statistically different between the two conduits *(20,21)*. However, a more recent randomized trial demonstrated that for above-knee bypasses, autogenous vein had a significantly better 5-year patency rate than ePTFE (76% vs 52%) *(22)*. In addition, when used for reconstructions below the knee, ePTFE has been shown to be considerably inferior *(20,23)*.

Because ePTFE grafts have less than optimal results compared to native vein, several techniques have been employed when autologous vein is in short supply and prosthetic graft material must be used for limb salvage. The use of a vein cuff or patch at the distal anastamosis improves the patency of ePTFE grafts in below-knee vessels *(24,25)*. This may be caused by a reduction in compliance mismatch, as well as alteration in the shear stress flow patterns at the distal anastamosis *(26,27)*. A recent large study *(28)* found that such composite grafts had a 5-year primary patency of 58%, 5-year secondary patency of 75%, and limb salvage rate

of 80%. These results are clearly superior to infrageniculate bypass using ePTFE alone.

Another alternative in certain situations is the use of a composite-sequential bypass. This technique may be applied if a patient has occlusive disease requiring bypass both above and below the knee (i.e., superficial femoral and trifurcation disease), but has a patent isolated popliteal segment. Under these circumstances, a prosthetic bypass can be performed down to the popliteal segment, and an autogenous vein graft is then placed between either the prosthetic or the native popliteal artery and the distal infrageniculate outflow vessel. This technique was designed to avoid the poor patency rates that accompany an infrageniculate bypass using ePTFE. Published data with this reconstructive technique reveal a 1-year patency and limb salvage rate of 80% and 88%, and a 4-year patency and limb salvage rate of 40% and 70% *(29,30)*.

Allografts

In 1975, Dardik *(31)* introduced the human umbilical vein for use as a bypass graft, and since that time surgeons have acquired a relatively large experience with this conduit. The umbilical vein is fixed in gluteraldehyde, which enhances crosslinking of collagen at the molecular level and theoretically results in a stronger graft. The gluteraldehyde also destroys the endothelium and minimizes the immunogenicity of the graft. Once the human umbilical vein is fixed, it is not viable and is essentially a musculocutaneous tube. One disadvantage of human umbilical vein when compared to ePTFE is the greater difficulty of handling, tunneling, and anastamosing the graft. The graft tissue is fragile, friable, and does not tolerate traction. If not handled appropriately in the course of rinsing off the tanning agent and tunneling during the procedure, the umbilical vein is prone to develop mural dissection. Suturing the graft may be difficult owing to the thickness of the graft and the necessity of incorporating not only the vein but the polyester wrap as well. After early animal studies demonstrated a significant incidence of human umbilical vein graft dilatation *(32)*, manufacturers added a Dacron mesh around the graft to prevent dilatation and aneurysm formation.

In terms of graft patency, a large prospective randomized Veterans Affairs Cooperative study *(33)* compared human umbilical vein with ePTFE and autogenous saphenous vein for lower extremity bypass. For patients in whom a bypass was performed for limb salvage (critical ischemia), 5-year cumulative assisted primary patency rates for human umbilical vein were 53%, which was better than the ePTFE rate of 37%

but not as good as the saphenous vein patency rate of 68%. However, the rate of perioperative (30 day) graft thrombosis was significantly higher in the umbilical vein group (12.2%) compared to the ePTFE group (2.2%). Interestingly, the rate of limb salvage was significantly higher for patients who received umbilical vein graft (90.4%) vs ePTFE (87.5%).

Despite the addition of the Dacron mesh wrap, the nemesis of the human umbilical vein graft continues to be dilatation and aneurysm formation. Aneurysm formation in the human umbilical vein graft is most likely a consequence of biodegradation, and most studies have found that clinically apparent aneurysm formation occurs an average of five years after graft implantation. These studies have shown the incidence of aneurysm formation to be between 33% and 57% *(34–36)*, but reoperation is only required in 0.04 to 3.3% of cases *(33,37)*. Aneurysmal rupture has been an extremely infrequent event *(33)*. Nonetheless, the combination of technical difficulty when dealing with the graft material, relatively high rates of early graft thrombosis, and increased aneursymal degeneration has made the human umbilical vein graft a complicated graft with which to work.

Fresh nonfixed arterial conduit is a form of allograft that can be used as a transplant in the absence of adequate autogenous vein. An intact endothelial lining is important for improved long-term patency in distal bypass grafts *(38–40)* and this can only be provided with fresh grafts implanted immediately after harvest. Tissue preservatives such as gluteraldehyde and dialdehyde starch may predispose to graft thrombosis or biodegradation leading to aneurysm formation. The concept of providing a fresh arterial graft with intact endothelium was tested in a study using arterial allografts harvested from brain-dead donors. The data demonstrated that with low dose immunosuppression, a limb salvage rate of 75% at 1 year could be achieved *(41)*.

With the advent of cryopreservation techniques, it is now possible to preserve the arterial endothelium and smooth muscle cells and their properties of contractility and vessel wall relaxation *(42,43)*. A large retrospective multicenter study examined a population in which either fresh or cryopreserved arterial allograft was used for limb salvage infrainguinal reconstruction. The data from this report demonstrated suboptimal 5-year primary and secondary patency rates of 16% and 26%, but a respectable 74% 5-year rate of limb salvage *(44)*. While it does have drawbacks, the use of arterial allograft, either fresh or cryopreserved, represents another available conduit with which to allow reconstruction for limb salvage.

COMBINED REVASCULARIZATION AND FREE TISSUE TRANSFER

Sometimes a patient may have suitable size and length of autogenous vein for a lower extremity bypass, but the appropriate distal target vessel is in close proximity to nonviable tissue or exposed tendon or bone. Extensive tissue loss, proximal foot osteomyelitis, or joint involvement sometimes leaves patients with little recourse other than amputation. These patients often have wounds that would not heal, even with normalized arterial circulation and partial foot amputation. A viable alternative in such cases would be revascularization with free tissue transfer. A number of tissue coverage flaps, including omentum, rectus abdominis, latissimus dorsi, temporalis, gracilis, scapular, and radial forearm flaps can be surgically mobilized and a vascular pedicle preserved. The distal bypass is then performed, the free flap is surgically revascularized from the graft, and adequate coverage and healthy tissue are thus provided for wound healing. The transferred tissue flap not only provides wound coverage, but also increases the flow through the newly constructed graft and may increase its patency *(45)*. A recent study *(46)* using this technique of combined arterial reconstruction and free tissue transfer demonstrated an 86% limb salvage rate in this desperate patient population. Clearly, this technique represents an extension of our ability to achieve reliable limb salvage in patents with severe leg ischemia.

SYMPATHECTOMY

In 1913, Leriche and Jaboulay *(47)* first described and tested the concept of sympathetic denervation as therapy for ischemia secondary to arterial occlusive disease. Despite less than optimal initial results, others soon applied the technique with better long-term results, and by the mid-1920s, sympathectomy had emerged as the only alternative to amputation for severe arterial occlusive disease. By the early 1960s, advances in pain control and arterial reconstruction combined with considerable debate over the true value of sympathectomy led to a substantial decline in its use. Today, sympathectomy remains a well-described technique with a diminishing number of applications.

The sympathetic nervous system helps regulate heat loss by providing resting tone to cutaneous arteriovenous anastamoses. These anastamoses are large diameter (50 μm) arteriovenous shunts that bypass nutrient capillary beds and typically do not supply subcutaneous tissue or muscle. An increase in sympathetic outflow results in less blood flow through these cutaneous anastamoses, as well as piloerection and sweat-

ing. Sympathectomy, therefore, results in unopposed parasympathetic stimulation and results in a net increase in blood flow to an ischemic limb. Cronenwett, using radiolabeled microspheres in a canine model, demonstrated that after sympathectomy there was an eightfold increase in total extremity blood flow, which was directly a result of increased flow through the cutaneous arteriovenous (AV) anastamoses *(48)*. Only a modest change in muscle perfusion, either at rest or upon exertion, was identified *(49)*. Vasodilatation and increased net limb perfusion are seen immediately after sympathectomy, but begin to decrease at one week and are diminished almost to baseline levels by 6 months *(50)*. This may be related to either nerve fiber regeneration or development of hypersensitivity to circulating catecholamines.

Despite experimental evidence that muscular perfusion is not significantly enhanced with sympathectomy, there is clinical evidence that certain patient populations may benefit from sympathectomy. Up to 80% of patients with an ankle–brachial index of greater than 0.30, no known neuropathy, and limited tissue loss may obtain relief of their symptoms with sympathectomy *(51)*. Yao and Bergan *(52)* demonstrated the presence of a threshold in regard to arterial inflow by showing that in 90% of limbs with an ankle–brachial index of less than 0.30, no response was obtained with sympathectomy. An ankle–brachial index of 0.30 may therefore represent a marginal level of perfusion that may suffice to achieve limb salvage if slightly improved by sympathectomy *(53)*.

Unfortunately, there have been no randomized controlled studies to date that evaluate the efficacy of sympathectomy in treating advanced arterial occlusive disease. There have been several uncontrolled studies *(51,54–57)* that demonstrate relief of rest pain and healing of ischemic ulcers in 28 to 73% of patients and acute limb salvage rates of 60 to 94%. The wide range of these results and lack of a randomized and controlled setting brings into question the effect of placebo as well concomitant medical therapies and conditions.

In treating limb-threatening ischemia, sympathectomy has a limited role because arterial construction is more reliable and yields a far greater chance of limb salvage. Patients who are candidates for sympathectomy are truly nonreconstructible and have at least some inflow (as manifested by an ankle–brachial index greater than 0.30), no evidence of neuropathy, and limited tissue loss. Response to preoperative chemical sympathetic blockade may predict success for operative sympathectomy. In carefully selected patients, sympathectomy may provide the small increase in limb perfusion needed to tip the scales in the direction of ulcer healing and resolution of rest pain.

Percutaneous Intervention

Percutaneous intervention is now an accepted technique for management of patients with severe lower extremity arterial occlusive disease. The two most common forms of percutaneous treatment involve arterial dilatation via balloon angioplasty and intravascular stent placement. Arterial dilatation is achieved by passing a catheter-mounted balloon over an angiographic guidewire through the offending lesion. Arterial access is typically obtained at a site remote from the lesion so as to allow maximum maneuverability. Once the lesion is crossed with a balloon that has been sized at or slightly greater than the size of the native vessel, the balloon is inflated to six to eight atmospheres of pressure and kept inflated for one to two minutes. Effacement of any waist or indentation in the balloon indicates successful angioplasty of the lesion. Balloon inflation in an area of stenosis causes plaque fracture and stretches the media and adventitia of the vessel, thereby enlarging its diameter. The balloon is then deflated and an arteriogram is performed to assess for residual stenosis, dissection, spasm, or recoil. Repeat balloon dilatation, stent placement, or administration of spasmolytic agents can then be performed if any of these conditions exist. In addition, pre- and post-intervention intra-arterial pressure measurements across a lesion can be obtained to determine not only the degree of significance of a stenosis, but to assess the adequacy of treatment as well.

In 1987, Johnston and colleagues *(58)* published a sentinel report that described 5-year results of a prospective study of percutaneous angioplasty. During an 8-year span, 984 angioplasties were performed in vessels ranging from the aorta to the tibial vessels, with 13.1% of these interventions being performed for limb salvage (rest pain, ulceration, or gangrene). Successful angioplasty was defined as one level of clinical grade improvement as well as either an increase in the ankle–brachial index of 0.10, improvement in Doppler waveform or pulsatility index, or doubling of treadmill exercise distance. The overall initial success rate was 88.6% with a 5-year success rate of 48.2%. However, for patients in whom the indication was limb salvage the initial success rate was 71% with a 5-year success rate of 34%. Further differences were seen depending on location of the angioplasty. In all patients, angioplasty in the common iliac artery had a 5-year success rate of 60% whereas angioplasty in the femoropopliteal segment had a 5-year success rate of 40%. Predictors of success in this study were found to be indication (claudication vs limb salvage), angioplasty site (common iliac vs other), severity of lesion (stenosis vs occlusion), and status of runoff (good vs poor). From this data, the authors predicted that for limb salvage, a

patient with common iliac stenosis and good runoff has a 50% chance of 5-year success after angioplasty. A similar patient with femoropopliteal stenosis would have a 38% chance of 5-year success.

Iliac Angioplasty and Stenting

Because of the early data demonstrating a significant difference in percutaneous results for iliac versus femoropopliteal angioplasty, they are considered to be distinct entities with different management algorithms. Iliac angioplasty has a reported 1-year success rate of 84 to 90%, and a 5-year success rate of 50 to 72% *(59–61)*.

Introduction of metallic stents has allowed for intravascular placement of devices that augment our percutaneous capabilities. Stents serve as an intravascular scaffold and oppose the elastic recoil of the media and adventitia that often occurs after angioplasty. They can also radially compress a fractured plaque that is often the result of forceful dilatation of a stiff or calcified lesion, and in doing so a stent seals off any potential or real dissection planes. Sealing off the arterial media from exposure to circulating blood may limit production of factors that can lead to intimal hyperplasia, thus theoretically improving long-term patency. Arterial stent placement is most commonly indicated after suboptimal angioplasty, and some specific indications include flow-limiting dissection, residual pressure gradient (>15 mmHg), residual stenosis >30%, and failure to establish initial patency. Some controversy surrounds the concept of primary stent placement in the iliac arteries, as will be discussed below.

In 1992, Palmaz and colleagues *(62)* reported multicenter trial data with use of the Palmaz stent in iliac arteries. Stents in this trial were placed in 496 patients, 156 of which were for limb salvage (32%). The mean pre-stent arterial pressure gradient was 39.1 mmHg, which was reduced to a mean of 1.3 mmHg after stent placement. Immediate success rates were 99.2%, and 43-month success rates were 68.6%. Note that this data includes both claudicants and patients with severe limb-threatening ischemia. Other studies using combined populations have shown similar data, demonstrating a 2-year success rate of 71% using Wallstents *(63)* and 5-year success rates of 79% using Strecker stents *(64)*.

Given the variable ranges of success among different reports examining iliac angioplasty and stent placement, Bosch and Hunink *(65)* in 1997 performed a meta-analysis comparing these two treatment modalities. When data from six angioplasty studies and eight stent studies were combined, analysis suggested superiority of stenting for iliac lesions. In patients with critical ischemia, 4-year primary patency rates for

Table 1
Four-Year Primary Patency Rates for Limb Salvage Patients *(65)*

	Iliac stenosis	Iliac occlusion
Angioplasty	53%	44%
Stent	67%	53%

angioplasty were 53% for iliac stenoses and 44% for iliac occlusions that were treated. With stent placement, the same patient population had 4-year primary patency rates of 67% for iliac stenoses and 53% for iliac occlusions (Table 1). There was no statistically significant difference in complication or mortality rates with either approach.

Results such as these have ignited a controversy over whether the percutaneous management of iliac lesions should involve primary stent placement, i.e., stent placement regardless of angioplasty outcome. The only prospective randomized data to date comparing primary stent placement to angioplasty has been reported in abstract form by Richter and colleagues *(66,67)*. The updated abstract in 1993 described 123 patients who received stents and 124 patients who underwent angioplasty alone. Cumulative 5-year angiographic patency rates in the stent group were 93.6% compared to 64.6% in the angioplasty group. Early success, late success, and patency rates were all significantly better in the stent group. Although these early results seem to favor primary stent placement for iliac occlusive disease, published data in a prospective randomized fashion is lacking and further studies are needed before the question can be definitively answered. It is clear, however, that percutaneous management of iliac artery occlusive disease, be it via angioplasty alone, angioplasty and stenting, or primary stenting, is another important tool to assist us in salvaging limbs in patients with limb-threatening ischemia.

Infrainguinal Angioplasty and Stenting

In 1992, Johnston and colleagues (68) published re-analyzed data regarding angioplasty of the femoral and popliteal arteries. Of a total of 254 patients, 50 (19.7%) underwent femoropopliteal angioplasty with the intent of limb salvage for critical limb ischemia. Whereas the overall 5-year success rate was 38.1%, the 5-year success rate in the limb salvage population was closer to 30%. This echoed the authors' previous findings that angioplasties performed for claudication generally fared better long-term than those done for limb salvage. Other variables found to be predictive of long-term success included severity of lesion (stenosis vs occlusion), quality of runoff, and initial ankle–brachial index of

greater than 0.57. This is in general agreement with the findings of other authors, with the addition of poorer long-term results with longer lesions, especially those greater than 10 cm. Interestingly, although the 5-year success rate for below-knee angioplasty was lower than that of above-knee angioplasty, this difference did not reach statistical significance. Other studies of infrageniculate angioplasty have reported fairly dismal results, with 3-year success rates of only 20% *(69)*.

Because of the overall discouraging results from studies evaluating short- and long-term infrainguinal angioplasty alone, stents have been employed to attempt to increase success rates in this area. As discussed previously, intravascular stents serve to oppose post-angioplasty elastic recoil as well as to provide the radial scaffolding that compresses fractured plaque and seals dissection planes. A number of studies have evaluated intravascular stent placement for superficial femoral atherosclerosis, and the results are quite variable (63,70–75). These studies, as most others discussed, evaluate a mixed population in which indications include both claudication and limb salvage. The percentage of procedures performed for limb salvage in the above referenced studies ranged from 6 to 50%. One-year primary patency rates ranged from 22 to 81% with an average of 58%, and secondary patency rates at one year ranged from 43 to 96% with an average of 73%.

Gray and colleagues *(75)* evaluated 55 patients who underwent superficial femoral artery stenting after suboptimal angioplasty. This indication for intervention in this patient group was 50% claudication and 50% limb salvage. Stents were placed after suboptimal angioplasty as defined by flow-limiting dissection, residual pressure gradient of >15 mm Hg, residual stenosis of >30%, or failure to establish initial patency. The mean lesion length in this study was 16.5 cm, which is longer than the lesions treated in the previously referenced articles (3.7–13.5 cm). The authors found a mean increase in ankle-brachial index of 0.23, a 1-year primary patency rate of 22%, and a 1-year secondary patency rate of 46%. In the patients treated because of critical limb-threatening ischemia, amputation was eventually performed in 5 of 29 patients, yielding a limb salvage rate of 83%. Lesion length, occlusion versus stenosis, number and type of stent, presence of diabetes mellitus, preprocedural ankle–brachial index, and smoking status were all found to be not statistically significant factors in predicting success for infrainguinal stent placement.

Reviewing the available data, several guidelines can be recommended regarding infrainguinal angioplasty and stent placement for limb salvage in patients with lower extremity arterial occlusive disease. Initial

success with angioplasty is more likely if a stenosis rather than an occlusion is being treated, whereas late success is more dependent on adequate runoff. Length of lesion appears to inversely correlate with successful percutaneous intervention, either angioplasty or stent placement. In contrast to the iliac system, there is no data to support the notion of primary stenting in the infrainguinal vessels. Rather, stents in this setting should be used as an adjunct to suboptimal angioplasty results or recurrent stenoses after angioplasty. Whereas surgical revascularization remains the treatment of choice, for patients without adequate conduit or for those who are a prohibitive surgical risk, angioplasty and stenting are techniques that can help provide limb salvage.

SUBINTIMAL ANGIOPLASTY

Subintimal angioplasty is a technique that has shown some promise in treating patients with limb-threatening ischemia that have complete infrainguinal vessel occlusion and long-segment disease, two distinct factors that have been shown to correlate with poor results when treated with standard angioplasty. In 1990, Bolia and colleagues *(76)* first reported the technique of subintimal angioplasty. Unintentional dissection of the arterial wall occurs not uncommonly and is usually without sequelae, but until recently was considered an indication to abandon further attempts at angioplasty. Bolia observed that a successful outcome could be achieved if the accidentally created subintimal false lumen was pursued, with re-entry in to the true lumen beyond the distal extent of the occlusion. Thus, the concept of deliberate subintimal angioplasty was born.

Several studies have evaluated subintimal angioplasty of the femoropopliteal segment and demonstrated primary technical success rates of 74 to 85%, and hemodynamic patency rates of 59 to 75% at 1 year and 58% at 3 years *(77–79)* McCarthy and colleagues (79) recently reviewed their experience of 69 subintimal angioplasties, 43 (62%) of which were performed for critical limb ischemia. The procedure was successfully completed in 31 (72%) of these limb-salvage patients, and of those 31 patients, there was a 68% rate of limb salvage and a 79% rate of hemodynamic patency at 6 months.

Others have evaluated the efficacy of subintimal angioplasty in the infrageniculate vessels *(80–82)*. They have shown that this technique can be successfully performed in 78 to 86% of attempted patients, with a 1-year patency ranging from 53 to 56% and limb salvage rates ranging from 81 to 94%. Importantly, technical failure does not preclude con-

ventional revascularization surgery, and complications of this technique may often be corrected with standard endovascular procedures.

Subintimal angioplasty is a technically difficult procedure with a long learning curve. Results are inferior to surgical bypass, and this technique is not widely performed in the United States. Nonetheless, it does represent yet another option for limb salvage in the management of select patients with severe lower extremity occlusive disease.

Nonoperative Nonpharmacological Intervention

High-Pressure Intermittent Compression

The concept of manual compression therapy for arterial insufficiency is an old one, being mentioned as far back as the first volume of *Lancet* in 1835 *(83)*. One of the proposed mechanisms by which compression therapy works is via augmenting the arteriovenous pressure gradient in the lower extremity. The difference between arterial and venous pressure is the driving force behind perfusion. In the legs of normal patients, the large gradient between arterial and venous pressures drives blood out of the arteries, through the muscle beds, and into the venous circulation. In patients with arterial occlusive disease, decreased arterial pressures translate into a lower arteriovenous gradient that results in decreased perfusion. However, in the presence of competent venous valves, if the leg is briefly compressed with an external pressure that exceeds venous pressure, the veins will empty and the venous pressure is reduced by approximately 15 mmHg *(84)*. Until the veins refill, this decreased venous pressure partially restores the arteriovenous gradient and helps favor increased forward flow. The veins refill after about 14 seconds, at which point another compression is needed to continue the increased perfusion *(85)*.

Another proposed mechanism is the release of vasoactive substances by the shear stress that results from mechanical compression of vessel walls. Animal studies have demonstrated increased endothelial-derived relaxing factor (EDRF) production in response to compression of arterial segments *(86)*. Others have shown that short periods of shear-stress application result in an increase in mRNA production for nitric oxide (NO) synthase *(87)*. It has also been shown that the vasodilatation response is attenuated if NO production is halted by the administration of drugs that inhibit NO synthase *(88)*. Other studies show that limb compression leads to systemic vasodilatation in a manner that is dependent on shear stress *(89)*. To produce adequate shear stress on the vessel wall, compression must be prompt rather than gradual.

In 2001, van Bemmelen and colleagues *(90)* published their long-term data using a high-pressure intermittent compression device for limb

salvage in patients who were unsuitable for surgical revascularization. The study included 14 limbs, 13 of which had forefoot tissue loss prior to treatment. These patients were considered not to be appropriate surgical candidates based on lack of target vessels, lack of conduit, previous failed bypass, or comorbid medical condition. After 3 months of treatment, the authors reported an increase in the pulse-volume recording amplitude in 70% of limbs, all of which were salvaged at maximal follow-up of 2.5 years. For the four limbs that required amputation, hidden hour counters within the compression device revealed a much lower rate of compliance in adhering to the prescribed home protocol. Patients were advised to use the device for 4 hours per day; the limb salvage group averaged 2.38 hours per day whereas the amputation group averaged 1.14 hours per day. A more recent study from Canada *(91)* reported 3-month limb salvage rates of 58% (or 86.4% if the renal failure patients were excluded) with the same device in a similar patient population. These studies appear to indicate that high-pressure compression therapy may be a useful addition to our treatment options for patients with limb-threatening ischemia who are unsuitable candidates for surgical revascularization.

GENE THERAPY

Gene therapy, defined as therapeutic manipulation of a gene product by either over- or underexpression, represents a novel treatment strategy for patients with critical limb-threatening lower extremity ischemia. One of the main hurdles of gene therapy has been optimizing gene delivery systems so that transfection of the desired gene product is maximal. When free DNA comes into contact with cell membranes, only a minimal amount is transported into the cell, and the little that is transported is rapidly degraded by cytoplasmic nucleases *(92)*. Therefore, multiple vectors have been developed to facilitate DNA entry into cells. The ideal vector delivers the gene efficiently to the target tissue, allows effective gene expression for a desired time frame, has no systemic toxicity, and remains to be discovered. Most commonly used in current therapeutic trials are viral vectors, which include the retrovirus, lentivirus, adenovirus, and adeno-associated virus. Nonviral vectors include plasmids and liposomes.

Intimal Hyperplasia

The two focal areas of interest in gene therapy as it pertains to peripheral vascular disease are intimal hyperplasia and angiogenesis. Intimal hyperplasia is felt to be a result of flow perturbations, endothelial injury, and cellular proliferation. Accordingly, gene therapies for the preven-

tion of intimal hyperplasia attempt to modify the local vascular injury response and prevent cellular proliferation. A promising therapy to prevent intimal hyperplasia involves augmentation of nitric oxide production by delivery of nitric oxide synthase. Nitric oxide is a diffusable molecule that plays an important role in the vascular system via influence on endothelial maintenance, development of atherosclerosis, hypertension, vasospasm, and ischemia-reperfusion *(93)*. It has been shown to inhibit platelet aggregation, leukocyte chemotaxis, and smooth muscle cell proliferation in addition to promoting endothelial regeneration *(92)*. Because of these protective properties, experiments have been devised to examine the effect of increased nitric oxide production at sites of vascular injury. Several animal studies have reported a decrease in intimal hyperplasia after delivery and transduction of nitric oxide synthase to balloon-injured arterial segments compared to controls *(94–96)*. These results are highly encouraging and suggest the great utility that nitric oxide synthase may have in preventing intimal hyperplasia. Studies are currently underway which should elucidate the effects of nitric oxide on intimal hyperplasia in the human cardiac and vascular systems.

Angiogenesis

Therapeutic angiogenesis is defined as treating ischemia by increasing the number of blood vessels within the ischemic tissue. Studies from tumor growth and cardiovascular development have identified vascular endothelial growth factors (VEGF) and fibroblast growth factors (FGF) that are expressed in response to hypoxia and cytokine release *(97,98)*. Animal studies using VEGF and FGF have demonstrated increased collateral blood vessel development in response to the presence of limb ischemia *(99,100)*. Baumgartner and colleagues *(101)* reported their data on ten limbs with critical ischemia that were treated with intramuscular injections of plasmid DNA encoding for VEGF. At an average of 6 months of follow-up, they documented an increase in ankle–brachial index from 0.33 to 0.48, newly visible collateral vessels in seven limbs, and limb salvage in eight limbs (80%). Whereas such results are encouraging, similar trials have failed to show such definite improvement. Furthermore, there are some potential drawbacks to angiogenic therapy, such as hemangioma formation, formation of nonfunctional leaky vessels, and acceleration of incidental tumor growth *(92,102,103)*. Nonetheless, angiogenesis is a frontier that is being extensively studied and likely will have future use for limb salvage therapy in carefully selected patients.

CONCLUSION

Multiple treatment modalities exist to assist us in providing limb salvage for patients with critical lower extremity ischemia. Distal bypass with autogenous vein has clearly been shown to be the superior option, with the longest patency rates and highest rates of limb salvage. In the absence of adequate autologous vein, other conduits such as ePTFE, Dacron, and human umbilical vein may be used alone or in combination with some autologous vein (composite grafts). Percutaneous intervention, either angioplasty alone or in conjunction with stent placement, is a viable option for certain lesion types or for patients whose comorbidities preclude surgical revascularization. Therapeutic manipulation of gene expression holds great promise for future treatment in the patient with limb-threatening lower extremity ischemia.

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