Peripheral artery disease (PAD) is initiated by athero-
genic mechanisms common to all major vascular terri-
tories but encompasses several distinctly important clinical
sequelae. The global burden of PAD is large and rising,
with substantial morbidity and mortality found in high-
, middle-, and low-income countries. The atherosclerotic
occlusive disease underlying PAD is associated with an
exercise limitation in the majority of patients, whereas the
classic symptoms of intermittent claudication, ischemic
rest pain, and ischemic ulceration are less common mani-
festations. Patients with PAD are also at increased risk of
myocardial infarction, ischemic stroke, heart failure, re-
ovascular hypertension, and vascular death reflecting the
systemic atherosclerotic burden. The epidemiology of

Pathogenesis of the Limb Manifestations and Exercise
Limitations in Peripheral Artery Disease

William R. Hiatt, Ehrin J. Armstrong, Christopher J. Larson, Eric P. Brass

Abstract: Patients with peripheral artery disease have a marked reduction in exercise performance and daily
ambulatory activity irrespective of their limb symptoms of classic or atypical claudication. This review will evaluate
the multiple pathophysiologic mechanisms underlying the exercise impairment in peripheral artery disease based
on an evaluation of the current literature and research performed by the authors. Peripheral artery disease results
in atherosclerotic obstructions in the major conduit arteries supplying the lower extremities. This arterial disease
process impairs the supply of oxygen and metabolic substrates needed to match the metabolic demand generated
by active skeletal muscle during walking exercise. However, the hemodynamic impairment associated with the
occlusive disease process does not fully account for the reduced exercise impairment, indicating that additional
pathophysiologic mechanisms contribute to the limb manifestations. These mechanisms include a cascade of
pathophysiological responses during exercise-induced ischemia and reperfusion at rest that are associated with
endothelial dysfunction, oxidant stress, inflammation, and muscle metabolic abnormalities that provide opportunities
for targeted therapeutic interventions to address the complex pathophysiology of the exercise impairment in
peripheral artery disease. (Circ Res. 2015;116:1527-1539. DOI: 10.1161/CIRCRESAHA.116.303566.)

Key Words: exercise ■ metabolism ■ peripheral artery disease ■ peripheral vascular diseases ■ physiopathology ■ therapeutics
Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ABI</td>
<td>ankle-brachial index</td>
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<tr>
<td>ERR</td>
<td>estrogen related receptor</td>
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<td>ETA</td>
<td>endothelin receptor A</td>
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<td>endothelin receptor B</td>
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<td>FFR</td>
<td>fractional flow reserve</td>
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<td>PAD</td>
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<td>PPAR</td>
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PAD is linked to that of the other common atherosclerotic processes, such as coronary and cerebral artery diseases, and affects 15% of the adult population aged >45 years.7 Predominant risk factors for PAD include advancing age, smoking, and diabetes mellitus; hypertension and lipid disorders contribute less risk when compared with their association with ischemic stroke and myocardial infarction.2,8 Patients with PAD have cardiovascular outcomes similar to patients with coronary artery disease or ischemic stroke, with the risk of myocardial infarction, stroke, or vascular death >5% per year.9-15

The diagnosis of PAD can be ascertained by simple hemodynamic measurements, in particular, the ankle-brachial index (ABI), a test performed by measuring the systolic blood pressure in each arm and the dorsalis pedis and posterior tibial arteries of each ankle. The highest of the dorsalis pedis or posterior tibial artery pressures is the numerator of the ABI specific to each leg, whereas the highest arm pressure on either side is the denominator. A ratio of <0.90 in either leg is considered hemodynamic evidence of PAD.16 When imaged, PAD can be characterized as one or more arterial stenoses or occlusions involving the distal aorta, iliac, femoral, popliteal, or tibial arteries.17

The underlying mechanisms responsible for the functional limitations in PAD are hemodynamic in origin, which affects the supply of oxygen and substrates to metabolically active tissue: skeletal muscle in the case of intermittent claudication and skin and subcutaneous tissues in the case of critical leg ischemia. However, additional pathophysiologic mechanisms contribute to the limb manifestations, where a reduction in blood flow alone does not fully account for the exercise limitation. Recent research into the pathophysiology of this exercise limitation has identified several important sequelae resultant from the chronic hemodynamic deficit. These changes affecting the vasculature and skeletal muscle distal to the primary hemodynamic lesions likely contribute to the mechanisms of exercise limitation in PAD. These advances are reviewed here with an additional focus on identifying possible future therapeutic strategies.

Symptomatic Manifestations and Exercise Limitations in PAD

In the coronary circulation, plaque rupture leading to acute ischemic events is a common clinical presentation; in PAD, progressive atherosclerosis leading to chronic stenosis and occlusion, often in series, in the arteries supplying the lower extremities dominates the symptomatic manifestations.6

Limb Symptoms

Patients with PAD may present with a range of limb symptoms. Most patients are either asymptomatic or have atypical limb symptoms during exercise, whereas only a third have typical symptoms of claudication.18 The clinical characteristics of PAD have been characterized in a longitudinal cohort study that defined typical and atypical limb symptoms. Typical claudication is defined by exercise-induced calf discomfort that is relieved by rest.19 Atypical limb symptoms can be characterized as exertional calf symptoms that do not begin at rest but are otherwise not consistent with classical claudication, such as muscle symptoms that do not include the calves, and pain at rest and pain with exertion.18 These symptoms should not be confused with other causes of atypical limb pain, including statin-induced myalgias, spinal stenosis, and other orthopedic conditions. The pathogenesis can usually be differentiated with a careful history and physical examination, although some patients may have PAD concomitant orthopedic disease.

Atypical exertional leg symptoms are more common than classic claudication and are also associated with decreased overall functional exercise capacity.20 Patients with hemodynamic evidence of PAD in the peripheral circulation (eg, low ABI) consistently demonstrate a marked reduction in peak exercise performance and daily ambulatory activity.21-23 Thus, the pathophysiology, which results in reduced exercise performance in PAD, affects a broad spectrum of patients irrespective of their limb symptoms. In this context, although patients with typical intermittent claudication have been the best studied and the term is in common usage, the mechanisms underlying the exercise limitation are likely common to all patients regardless of the nature of their limb symptoms. Therefore, using the term claudication to describe all symptomatic patients with PAD is inappropriate, and in this review, the functional deficit associated with PAD will be referred to as an exercise limitation and includes otherwise asymptomatic patients who also have exercise impairment.

Severity of the Exercise Impairment

The severity of the functional limitation in PAD has been well characterized. On a population basis, patients with PAD have an >50% reduction in peak exercise performance when compared with an age-matched healthy cohort.21 This exercise limitation is associated with marked impairments in daily physical activity as assessed by questionnaire and daily energy expenditure.24,25 Patients with PAD also have an accelerated functional decline over time that is related to the underlying hemodynamic severity of the disease in the leg.26 The primary treatment goal for all patients with PAD is cardiovascular risk reduction, whereas on the basis of the above considerations, an additional goal is to improve exercise performance, daily functional activity, and quality of life.

Arterial Hemodynamics in PAD

The reduction in blood flow to the lower extremity as a consequence of the arterial occlusive disease process is a contributor to the exercise impairment in PAD. Ischemia with exercise followed by reperfusion is also an important initiator of the subsequent pathophysiology in skeletal muscle.
Resting Hemodynamics

Volumetric blood flow in the major conduit arteries delivering oxygen to the lower extremities is determined by the driving pressure, geometry of the vessel and associated stenosis, and blood viscosity. Poiseuille first described these fundamental relationships by showing that flow was inversely proportional to the length of a tube, directly proportional to the pressure gradient, and directly proportional to the fourth power of the tube diameter. Under normal conditions, flow is laminar and there is minimal pressure drop from the heart to the distal arterial circulation. However, with an arterial stenosis, there is a drop in pressure and flow across the stenosis. This pressure drop is accentuated by a loss of kinetic energy because of turbulent flow induced by the stenosis (Figure 1). In the lower extremity, a series of arterial stenoses, for example, a stenosis or occlusion in the external iliac, superficial femoral arteries, and popliteal arteries, will be hemodynamically additive with the overall flow limitation of the sum of the individual components. These hemodynamic manifestations of PAD can be assessed with the ABI, which reflects the total hemodynamic burden of stenoses and occlusions from the central circulation to the ankle. Except for patients with noncompressible tibial vessels where the ABI does not reflect the intra-arterial pressure, patients with PAD will typically have an ABI <0.90 at rest. However, these patients have no limb symptoms at rest in the absence of more severe hemodynamic compromise causing critical leg ischemia. Under conditions of rest, skeletal muscle oxygen demand is low and limb oxygen delivery is adequate despite the drop in systolic pressure from the arm to the ankle. Resting calf muscle blood flow in patients with claudication is similar to that in an age-matched control population, indicating that under the conditions of low muscle metabolic demand at rest, flow can be maintained by a corresponding decrease in peripheral resistance to compensate for the drop in arterial pressure across the stenosis.

Exercise Hemodynamics

In normal subjects during exercise, the increase in limb oxygen uptake is driven by an increase in metabolic demand. In healthy adults, the system is dynamic in the rest-to-exercise transition, with blood flow increasing 10-fold at low levels of exercise and 40-fold at high levels. At steady-state exercise, this leads to a close coupling of ventilatory oxygen update in the lungs, delivery of oxygen by the heart and arterial circulation to active skeletal muscle, and muscle mitochondrial oxygen uptake at the tissue level. In patients with PAD, there is a markedly blunted flow response in the rest-to-exercise transition that plateaus at flows well below that obtained in healthy subjects. This flow limitation is the result of the fixed stenoses becoming flow limiting (a concept termed critical arterial stenosis). Furthermore, the vasodilator response in the peripheral circulation is also abnormal and is unable to fully compensate for the fixed resistance lesions.

A related approach to understanding the change in arterial hemodynamics with exercise is the fractional flow reserve (FFR), which is the ratio of the flow distal to a stenosis divided by the pressure proximal to the stenosis under conditions of maximal vasodilation. For example, an FFR of 0.80 indicates that the stenotic vessel can only deliver 80% of the flow of a normal vessel when the distal arteriolar bed is maximally vasodilated. The vasodilator response in PAD can also be impaired by endothelial dysfunction, as discussed below. In humans with coronary artery disease, these relationships have

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Figure 1. Hemodynamic alterations in peripheral artery disease. Top, A healthy arterial bed with normal large vessel and microcirculatory flow characteristics. Bottom, A peripheral artery with atherosclerotic occlusive disease. ABI indicates ankle-brachial index. Reprinted from Hiatt and Brass.
been confirmed to have physiological significance for quantifying coronary ischemia. In the coronary circulation, clinical studies have shown that an FFR of <0.80 is physiologically significant and that this FFR value identifies a clinically significant cutoff point where symptomatic patients benefit from coronary revascularization. In patients with claudication, initial studies have shown that FFR measurements correlate with the lesion peak systolic velocity, a noninvasive measurement of lesion severity. Further studies will be necessary to correlate the clinical use of FFR measurements to potentially guide revascularization or other treatment strategies in PAD.

A complementary approach to determining the physiological significance of flow limitations in patients with PAD is the assessment of tissue oxygenation. Blood-oxygen-level-dependent MRI imaging techniques can measure the extent of tissue deoxygenation during postocclusive reactive hyperemia. Near-infrared spectroscopy has also been used to make dynamic assessments of tissue oxygenation in patients with PAD. For example, the phosphodiesterase-5 inhibitor sildenafil improved muscle oxygenation as assessed by near-infrared spectroscopy without improving walking performance in a small study of patients with PAD. These approaches have the potential to differentiate the effect of oxygen delivery versus oxygen use on muscle dysfunction.

**Endothelial and Microcirculatory Dysfunction**

As described above, optimizing muscle blood flow during exercise is dependent on vasodilation in the limb to minimize the overall resistance to flow delivery to active muscle. This vasodilation is an active process with several important vasoactive mediators, including nitric oxide. Patients with PAD have significant abnormalities in endothelium-dependent vasodilation. Oxidant stress leads to generation of superoxide anion and other mediators of endothelial dysfunction. Exercise-induced release of endothelin-1, a potent vasoconstrictor, can also antagonize exercise-induced skeletal muscle vasodilation. Through these mechanisms, impaired endothelial function as assessed by flow-mediated dilation has been correlated with the clinical severity of PAD. Furthermore, in studies where leg blood flow was measured by using a thermodilution catheter, there was a correlation (r=0.71) between maximal leg blood flow and performance on a bicycle ergometer.

Interestingly, endothelial dysfunction was recently associated with walking impairment independent of the ABI, suggesting that endothelial dysfunction may contribute to the exercise impairment in PAD. Other studies have suggested a correlation between ABI and endothelial dysfunction as assessed by flow-mediated dilation. Exercise training, which improves functional status in patients with PAD, also improves flow-mediated dilation. A recent study of patients undergoing a 12-week exercise training program demonstrated an improvement in flow-mediated dilation compared with baseline, suggesting that improvements in endothelial function may be one mechanism mediating reduced symptoms of claudication after exercise training. In addition, in an animal model of chronic atherosclerosis that simulated some features of PAD, a pharmacological intervention that improved exercise-induced blood flow also improved the animals’ functional status. The impaired vasodilation in patients with PAD is likely a sequela of the obstruction, and improvement in physiological vasodilation with exercise offers a potential therapeutic target.

Microcirculatory dysfunction and local skeletal muscle flow can also be assessed by novel noninvasive imaging techniques, including arterial spin-labeling MRI and contrast-enhanced ultrasound. In a pilot study, arterial spin-labeling MRI demonstrated that microvascular flow was relatively preserved among patients with mild PAD (ABI>0.50), but more severe PAD (ABI<0.50) was associated with a significant decrement in microvascular blood flow. This technique can also be used to image calf muscle flow at peak exercise and may be a useful tool to assess changes in calf blood flow after therapeutic interventions. In another small study, perfusion imaging of the skeletal muscle microcirculation was performed by contrast-enhanced ultrasound at rest and with exercise. In a multivariable model, the treadmill time to the onset of claudication was correlated with microcirculatory exercise flow and the difference between resting and exercise microcirculatory flow. Contrast-enhanced MRI measures of peak exercise blood flow have also been shown to correlate with exercise performance and 6-minute walk times.

**Rheological Factors**

Alterations in blood hemorheology can also impair arterial flow. Changes in the blood concentration of fibrinogen and von Willebrand factor can increase blood viscosity, particularly at the microcirculatory level, which according to Poussette law could contribute to reduced arterial flow—but less so than a change of equal magnitude in the percent stenosis. Activated leukocytes may also promote microvascular plugging and thrombosis, thereby further increasing the resistance to arterial flow. In a large cohort of patients from the Edinburgh Artery Study, blood viscosity and fibrinogen were independently associated with hemodynamic disease severity of PAD, and fibrin degradation products were also associated with progression of PAD. These findings suggest that increased blood viscosity may be a component of the functional limitation or simply secondary to the systemic atherosclerotic disease process.

**Existing Medical Therapy for the Exercise Limitation in PAD**

Current medical therapy for the exercise limitation in PAD is limited. Reasonable evidence exists for the use of phosphodiesterase type 3 inhibition, particularly cilostazol. Several meta-analyses have demonstrated a consistent clinical benefit of this medication despite a lack of detailed understanding of its mechanism of disease benefit. Specifically, it is not clear why either the inhibition of platelet aggregation or the stimulation of vasodilation—both mediated by phosphodiesterase type 3 inhibition—would translate into functional benefit in patients with PAD. As noted above, a nonspecific vasodilator, such as cilostazol, has the potential for steal of flow by healthier, more responsive vessels. Also, no convincing explanation exists for why the clinical benefits of phosphodiesterase type 3 inhibition continue to increase during 24 weeks of treatment when the direct pharmacological effects—vasodilation and inhibition of platelet aggregation—are immediate.
time-dependent improvement suggests a complex physiological response to treatment. Although modest improvements in ABI have been observed with phosphodiesterase inhibition, it is unlikely that this effect alone accounts for the mechanism of benefit. The efficacy signals from 2 additional phosphodiesterase type 3 inhibitors support a disease benefit for the class, albeit one that is not completely understood.

Older medications, such as pentoxifylline and naftidrofuryl, are approved for use in patients with PAD within and outside of the United States, respectively. The overall data supporting any functional benefit for treatment of patients with PAD with pentoxifylline are weak. The limited benefit of naftidrofuryl in patients with PAD also cannot be linked to any well-defined mechanism of action.

Exercise training has been a mainstay of treatment for PAD, with a well-established benefit during a typical 12-week training program. Exercise training may affect several pathways associated with clinical benefit, including improved skeletal muscle metabolism, endothelial function, and the biomechanics of gait. Given the established efficacy of exercise training, it would be logical to use pharmacotherapy as an adjunct to structured exercise rehabilitation in patients with PAD. Candidates for pharmacotherapy might include drugs with mechanisms that augment or complement the beneficial effects of training. In part to due to lack of good candidates and the complexity of studying drug therapy against a background of exercise training, there is a paucity of data addressing this hypothesis. A randomized trial of exercise training added to optimal medical therapy, including cilostazol, demonstrated a clear additive benefit of training, but the design did not address if cilostazol added benefit compared with exercise training without the drug. In addition, a study combining treatment with propionyl-l-carnitine and exercise training failed to show significantly better improvement in walking performance when compared with exercise training alone.

Endovascular revascularization is a commonly used intervention to improve skeletal muscle blood flow. When provided on a background of best medical therapy, revascularization of aortoiliac disease results in improved peak walking time and associated parameters of quality of life, and the magnitude of benefit is similar to that achieved with supervised exercise.

**Cellular and Molecular Pathophysiology of the Exercise Impairment in PAD**

Although the primary pathophysiology of the exercise limitation in PAD is initiated by the underlying arterial occlusive disease and associated reduction in exercise blood flow and oxygen delivery, the correlation is not strong between the flow limitation versus the observed decrease in exercise performance. Studies examining the resting ABI showed a weak correlation with peak treadmill exercise performance. In addition, interventions, such as exercise training, that lead to a large increase in peak exercise performance have little effect on skeletal muscle blood flow or ABI. Thus, although the occlusive atherosclerotic disease process is associated with a well-defined limitation of blood flow and oxygen delivery to exercising skeletal muscle, the absence of a strong correlation between the arterial obstruction and exercise performance indicates that factors other than changes in limb vascular resistance contribute to the exercise impairment in patients with PAD. Underlying mechanisms that may induce factors that are linked to the functional impairment include inflammation and oxidant stress with subsequent endothelial and microcirculatory dysfunction, skeletal muscle structural abnormalities, altered oxygen coupling and mitochondrial respiration, and skeletal muscle metabolic abnormalities. A better understanding of these pathways may help guide novel medical therapies for the treatment of PAD and improve functional outcomes in patients with claudication.

**Endothelial and Microcirculatory Dysfunction**

As described above, patients with PAD have significant abnormalities in endothelium-dependent vasodilation. A potential therapeutic strategy with respect to endothelial and microcirculatory dysfunction is antagonism of the release or action of endothelin. Endothelin can bind to either endothelin receptor A (ETA) or endothelin receptor B (ETB) subtypes 1 and 2. ETA receptors are located on the smooth muscle cells of vascular tissue, and endothelin binding causes vasoconstriction through agonist-induced receptor-mediated signaling. ETB receptor action is more complex, with ETB1 mediating vasodilation through nitric oxide release, whereas ETB2 causes vasoconstriction. Thus, antagonism of vasoconstriction mediated by ETA represents a potential therapeutic strategy to improve microcirculatory flow in PAD. However, the resultant vasodilator effect will be of benefit only if it improves microvascular flow to ischemic muscle and does not induce a steal phenomenon. Several selective ETA receptor antagonists are available for clinical testing. Ambrisentan is an ETA selective antagonist approved in the United States and Europe for the treatment of pulmonary arterial hypertension. Two other candidates for testing would be the ETA and ETB dual antagonists macitentan and bosentan, which are both also approved for the treatment of pulmonary artery hypertension. Macitentan represents an intermediate pharmacological profile between bosentan and ambrisentan, with a 50-fold selectivity for the ETA subtype compared with the ETB subtype. Given the high prevalence of diabetes mellitus in patients with PAD, it is interesting to note that studies have also suggested that pioglitazone could improve vascular function. The relevance of these changes to functional status in patients with PAD remains unknown.

**Inflammation and Oxidant Stress**

Inflammation is a key mediator of the atherosclerotic process and likely contributes to the limb manifestations of PAD as well. In addition, the mismatch between oxygen demand and oxygen delivery during exercise in patients with PAD induces an inflammatory response. In PAD, exercise is associated with an increase in plasma levels of numerous inflammatory mediators, including thiobarbituric acid–reactive substances (formed as a byproduct of lipid peroxidation), thromboxane, interleukin-8, tumor necrosis factor-α, soluble intercellular adhesion molecule-1, vascular cell adhesion molecule-1, von Willebrand factor, E-selectin, and thrombomodulin. Inflammatory mediators can aggravate endothelial dysfunction, and markers, such as interleukin-6, are inversely
correlated with maximum treadmill performance.94 When exercise impairment was evaluated by a series of walking tests, higher serum C-reactive protein and serum amyloid A concentrations were associated with reductions in 6-minute-walk distance, 4-meter walking velocity, and a summary performance score that combined performance in walking speed, standing balance, and time for 5 repeated chair rises.95 Elevated C-reactive protein levels were also associated with a greater annual decline in 6-minute walk performance for a period of 3 years and peak walking time.96,97

During ischemia, skeletal muscle mitochondria release free radicals, including superoxide and other reactive oxygen species, that are derived from the oxidation–reduction cascade.98 Reperfusion of ischemic muscle after exercise may also lead to an increase in oxidant stress.99,100 These reactive oxygen species have the potential to trigger numerous pathophysiologic pathways, including endothelial dysfunction and covalent modification of muscle macromolecules. Over time, this oxidant stress can also lead to mitochondrial DNA injury as demonstrated in patients with PAD.101,102 Mitochondrial DNA injury can also be detected in the less affected limbs of patients with unilateral PAD, suggesting that the mitochondrial injury may reflect systemic inflammation and not simply local skeletal muscle ischemia. Although exercise acutely induces oxidant stress in patients with PAD, exercise training has consistently been shown to improve symptoms among patients with PAD. Although this may seem to be contradictory, it is important to differentiate the effects of acute versus training exercise paradigms.103 For example, in model systems, exercise training has been shown to upregulate modulators of oxidative stress, including superoxide dismutase, inducible nitric oxide synthase, and thioredoxin.104–106 This modulation of oxidative stress defense mechanisms may be an important mechanism for the benefit of exercise training in patients with PAD.

Inflammatory mediators may also have proangiogenic and antiangiogenic effects, potentially modulating the endogenous response to ischemia.107 For example, circulating concentrations of vascular endothelial growth factor are decreased in patients with PAD when compared with controls with other comorbid conditions.93

Innovative anti-inflammatory therapies developed for other indications may have relevance for PAD. For example, intercellular adhesion molecules and vascular cell adhesion molecules play important roles in intracellular signaling and the immune and inflammatory responses and are elevated in patients with PAD.108 Antagonists of vascular cell adhesion molecule and intercellular adhesion molecule function have been developed that could be tested for efficacy in PAD. In particular, antagonists of very late antigen-4 have shown promise in treating inflammatory disorders in several animal models and could be advanced into clinical trials for this indication.109–112

Selectins, also elevated in PAD populations, are a set of cell adhesion molecules consisting of 3 subsets: E-selectins expressed on endothelial cells in skin microvessels after exposure to cytokines, L-selectins basally expressed on leukocytes, such as granulocytes and monocytes, and P-selectins basally expressed on platelets and endothelial cells. Recently, inclacumab, a potent and selective P-selectin–neutralizing antibody, has completed safety, tolerability, pharmacokinetic, and pharmacodynamic studies showing reduction in myocardial damage after percutaneous coronary intervention for non–ST-segment–elevation myocardial infarction.113 Whether modulating P-selectin in PAD has longer term clinical benefit has not yet been studied. In addition, inclacumab has been demonstrated to reduce elevated circulating platelet–leukocyte aggregate levels in patients with PAD, thus providing pharmacodynamic data useful for hypothesis generation.114

The angiotensin-converting enzyme inhibitor ramipril recently has been reported to improve placebo-corrected walking distance.115 However, the placebo group in this study did not show the usual walking performance improvements typically seen in these trials, which may have inflated the net benefit of the drug over placebo. Potential mechanisms of ramipril-mediated improvement in walking performance include associations with an increase in angiogenic biomarkers and reduction in the markers of thrombosis, inflammation, and leukocyte adhesion.116 These biomarker changes may reflect modulation of more fundamental processes. As a meta-analysis of angiotensin-converting inhibition did not find any benefit on exercise performance recently,117 it seems unlikely that renin–angiotensin–aldosterone system pathway intervention provides consistent functional benefit.

Metabolic agents, such as propionyl-l-carnitine, have also shown a signal of benefit but have not been fully developed as an approved pharmacological therapy.118 Although several large studies have demonstrated the atherosclerotic disease–modifying benefit of statin treatment, which may include actions through anti-inflammatory pathways, inconsistent benefits on exercise performance have been observed in PAD.119–121

Skeletal Muscle Structural Abnormalities
Numerous pathological changes have been identified in the skeletal muscle of patients with PAD, including muscle apoptosis and atrophy, increased fiber type switching, altered myosin heavy-chain expression, and muscle fiber denervation.122–125 These structural changes may be mediated, in part, by higher levels of inflammatory mediators in PAD.126 On the basis of biopsy specimens from the gastrocnemius muscles of patients with PAD, ≈4% of gastrocnemius cells are apoptotic, and caspase-3 levels are twice as high as in patients without PAD.125 Selective fiber type switching from type I (aerobic) to type II (glycolytic) fibers may impair skeletal muscle performance and has been associated with decreased exercise tolerance.123 Decreased expression of myosin heavy-chain isoform II in patients with PAD may also result in altered muscle contraction kinetics and decreased cellular efficiency.127 The observation of muscle fiber denervation among patients with PAD has also led to the hypothesis that arterial insufficiency coexists with a distal motor neuron neuropathy that worsens muscle weakness independent of arterial flow.128 Consistent with this hypothesis, impaired peroneal nerve conduction velocity was associated with decreased calf muscle area and lower 6-minute walk distance among patients with PAD.129

Numerous studies have correlated changes in calf muscle ultrastructure and overall muscle strength with subsequent cardiovascular outcomes in patients with claudication.
tomographic measurements of the content of muscle and fat in the calf have shown that calf muscle content is inversely associated with loss of mobility, even after adjusting for other risk factors and comorbidities.130 During long-term follow-up, a reduced calf muscle content was associated with increased all-cause and cardiovascular mortality independent of the ABI.131 Overall leg strength has also been shown to associate with mortality in men, but not women, with PAD.132 Taken together, these findings suggest that the morphological changes in calf muscle predispose to significant functional decline and may be an additional marker for increased risk among patients with symptomatic PAD.

One approach for modulating muscle apoptosis and atrophy is through exploitation of the myostatin pathway. Multiple new pharmacological agents that target this pathway are already in clinical development, including the soluble ActRIIB-Fc fusion decoy receptor ramatercept,133,134 antimyostatin antibody MYO-029, and the ActRIIB antibody BYM338. However, previous studies have shown that although endurance training improves function in patients with PAD, strength training does not result in the same degree of improvement. This discordance between the strong predictive value of endogenous muscle mass and the poor response to intervention may mean that endogenous muscle mass is an integrated marker of the complex limb changes occurring with chronic PAD. Thus, the likelihood of success of a muscle mass–based strategy alone may be low.74 Furthermore, this strategy is likely to be associated with systemic adverse events as exemplified by the epistaxis, gum bleeding, and skin vessel dilation observed in patients treated with ramatercept.133

Alterations in Oxygen Coupling and Mitochondrial Respiration

There is a tight coupling between pulmonary oxygen uptake, circulatory delivery of oxygen, and muscle mitochondrial respiration in healthy subjects during steady-state exercise. The transition from rest-to-exercise causes a rapid increase in the cellular degradation of ATP to ADP to support muscle contraction. Regeneration of ATP is initially supported by nonoxidative mechanisms, including a rapid depletion of phosphocreatine. Oxidative resynthesis of ATP occurs more slowly and requires delivery of oxygen to exercising skeletal muscle. The delay in oxidative adjustment to a new metabolic demand has been termed metabolic inertia.138

The transition to increased oxygen consumption can be characterized by several noninvasive measurements, including the kinetics of pulmonary oxygen consumption at the onset of exercise and the rate of hemoglobin desaturation in exercising skeletal muscle. In healthy subjects at the onset of exercise, these kinetic assessments are an attempt to characterize rates of mitochondrial oxidative metabolism rather than overall oxygen delivery.138 In patients with PAD, a profound prolongation of the kinetic rates of pulmonary oxygen consumption and tissue hemoglobin desaturation have been described at the onset of exercise relative to healthy, age-matched controls.139,140 This prolonged kinetic response associated with the reduced exercise performance in subjects with PAD suggests that alterations in skeletal muscle mitochondrial respiration may contribute to the decreased exercise performance in PAD.141 Studies have also been performed using 31P magnetic resonance spectroscopy in PAD. When assessed at the onset of exercise, changes in muscle pH and phosphocreatine concentration suggested inefficient oxidative metabolism necessitating a higher rate of ATP turnover for given power output as observed in control muscle.142 Taken together, these observations demonstrate increased metabolic inertia in patients with PAD and provide evidence of impaired muscle metabolic function in PAD.

Other evidence supports impaired mitochondrial function as an important factor in functional impairment in PAD. The electron transport chain in mitochondria oxidizes nicotinamide adenine dinucleotide in a series of enzymatic steps that transfer electrons to oxygen (Figure 2). Key mitochondrial pathways, including the electron transport chain, are vulnerable to free-radical injury.143 Skeletal muscle from the affected leg in patients with PAD has reduced the activities of mitochondrial nicotinamide adenine dinucleotide dehydrogenase of complex I and ubiquinol-cytochrome c oxidoreductase (complex III).144 The impairment in complex III of the electron transport chain may result in a higher redox potential to maintain electron flux, which may in turn lead to free-radical leakage in the mitochondria. Catalase and superoxide dismutase minimize the deleterious effects of hydrogen peroxide and superoxide, respectively, by catalyzing the conversion of these species into oxygen and hydrogen peroxide, which is subsequently converted to water. Damage ensues when reactive oxygen species levels exceed the reductive capacity of the cell.145 These observations suggest that electron transport chain activity is impaired, likely due to the ischemia-reperfusion injury in PAD, which may contribute to propagation of oxidative injury and metabolic dysfunction. Future strategies to modulate mitochondrial respiratory function and efficiency could represent novel therapeutic targets for patients with PAD.

Skeletal Muscle Metabolic Abnormalities

Numerous alterations in metabolic pathways have been studied in the skeletal muscle of patients with PAD. A commonly used experimental paradigm has been used to identify patients with predominant evidence of hemodynamic abnormalities in one leg with no ABI abnormality or claudication symptoms in the contralateral leg. This allowed comparisons between the affected leg, an apparently unaffected leg in the same patient, and an age-matched control population. These studies have identified many key metabolic abnormalities in PAD.

Early observations focused on alterations in muscle carnitine metabolism. Under normal metabolic conditions, fuel substrates, such as fatty acids, proteins, and carbohydrates, are converted into acyl-CoAs as intermediates in their complete oxidation. These coenzyme A–coupled intermediates are linked to the cellular carnitine pool through the reversible transfer of acyl groups between carnitine and coenzyme A.146 One of the functions of carnitine is to serve as a buffer for the acyl-CoA pool through the formation of acylcarnitines. Under conditions of metabolic stress, incomplete oxidation or incomplete use of acyl-CoAs leads to their accumulation. Transfer of the acyl group to carnitine results in the accumulation of the corresponding acylcarnitine, which can be measured.147 Thus, an increase in the concentration of acylcarnitines in plasma or
muscle in PAD is consistent with abnormal, incomplete oxidative metabolism of fuel substrates.

In patients with PAD, initial observations demonstrated accumulation of short-chain acylcarnitines in plasma that was inversely correlated with exercise performance. Subsequent studies evaluated changes in muscle carnitine metabolism using gastrocnemius biopsy specimens. These studies confirmed the accumulation of acylcarnitines in the affected skeletal muscle with no evidence of accumulation in the unaffected muscle. Acylcarnitine accumulation was also associated with reduced peak treadmill exercise performance and was a stronger predictor of exercise performance than the ABI. Muscle lactate levels are also significantly increased in the skeletal muscle of patients with PAD as a result of incomplete oxidation of glucose. Lactate accumulation likely reflects high nicotinamide adenine dinucleotide content (because of impaired entry into the electron transport chain) and decreased pyruvate dehydrogenase activity.

Pharmacological approaches to restoring mitochondrial function have been reviewed recently. Peroxisome proliferator–activated receptor (PPAR)-α and PPAR-δ both modulate mitochondrial expression, and their levels correlate well with type I fiber levels and endurance training and capacity. Although agonists of PPAR-δ, such as L165041 and GW501516, have not advanced substantially through clinical development because of concerns of neoplastic risk, the PPAR-α agonist fibrates have been marketed for many years. Fibrates could be used to test the therapeutic hypothesis that a PPAR-α agonist in patients with PAD would improve skeletal muscle in type I oxidative capacity and that this enrichment will translate into improved walking performance in PAD.

Several molecular interventions that modulate a subset of targets for mitochondrial biogenesis beyond the PPAR family are in preclinical development. These include the sirtuins and 5′ AMP-activated protein kinase pathway, estrogen related receptor (ERR)-α, estrogen related receptor (ERR)-γ, and nuclear respiratory factor 1. Although the use of the 5′ AMP-activated protein kinase activator 5-aminoimidazole-4-carboxamide ribonucleotide for doping in cycling and other professional sports is suspect, clinical trial results assessing its effect on performance have been generally disappointing. However, lack of benefit in healthy subjects may not exclude effectiveness in patients with PAD. The indirect 5′ AMP-activated protein kinase activator R118 has improved exercise performance and vascular insufficiency in high-fat fed mice. Although the overlapping metabolic functions of
Likewise, no facile methods to clinically modulate the import-
modulation available for use in the near future. ERR
only small molecules associated with the unclear benefit/risk
useful for experimental medicine hypothesis testing, leaving
modulators likely to be
minimal literature reporting ERR
γ
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Thus, careful choices of pharmacological agents will need to
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factor 1 exist at this time. Thus, both basic research and drug
suggestion for potential benefit for patients with
PAD, breast cancer association studies suggest that ERRα and ERRγ have differing effects on cancer progression, with
high levels of ERRα associated with expression of ErbB2, in-
creased risk of recurrence, and adverse clinical outcomes.61,62
Thus, careful choices of pharmacological agents will need to be
made if mitochondrial biogenesis in patients with PAD will be
attempted through ERR modulation. At this time, there is
minimal literature reporting ERRγ modulators likely to be useful for experimental medicine hypothesis testing, leaving only small molecules associated with the unclear benefit/risk ratio of ERRα modulation available for use in the near future. Likewise, no facile methods to clinically modulate the important regulator of mitochondrial expression nuclear respiratory factor 1 exist at this time. Thus, both basic research and drug discovery in the area of mitochondrial biogenesis are newly needed to generate hypotheses and new targets for clinical testing.

Conclusions
PAD represents a complex pathophysiology that results in significant functional limitations in exercise performance. A dominant mechanism in the initiation of the exercise limitation is a reduction in exercise blood flow and oxygen delivery to exercising skeletal muscle in the lower extremity. This flow limitation is also associated with skeletal muscle ischemia with walking exercise and reperfusion with rest that set up a cascade of pathophysiologic responses leading to further tissue injury, endothelial dysfunction, muscle metabolic abnormalities, and other changes that further reduce exercise capacity. Understanding these sequelae offers insights into mechanisms underlying the reduced exercise performance in these patients and potential new targets for therapeutic interventions.

Disclosures
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