# OXYGEN UPTAKE KINETICS IN PERIPHERAL ARTERIAL DISEASE

**by**

**TIMOTHY ALAN BAUER**

**B.S., Colorado State University, 1992**
**M.S., Colorado State University, 1995**

**AN ABSTRACT OF A DISSERTATION**

Submitted in partial fulfillment of the

requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Anatomy and Physiology
College of Veterinary Medicine

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## ABSTRACT

Peripheral arterial disease (PAD) is a manifestation of the systemic disease of atherosclerosis that results in arterial stenoses of the lower extremities. Patients with PAD demonstrate slowed dynamics of pulmonary oxygen uptake ($\dot{V}O_2$ kinetics) following the onset of exercise and a profound reduction in peak oxygen uptake and work capacity. However, whereas the primary pathophysiology of PAD results from the lower extremity hemodynamic limitation, there are abnormalities distal to the arterial stenoses in PAD-affected skeletal muscle that may also contribute to the impaired exercise responses. Thus, the potential contributions of abnormal muscle metabolism versus local circulatory defects in the PAD exercise impairment remains unclear. In this context, the purpose of the dissertation was to advance our understanding of the abnormal pulmonary $\dot{V}O_2$ kinetics in PAD and characterize the local muscle deoxygenation responses during the rest-exercise transition exercise in health and PAD. The present series of investigations were designed to: 1. localize the abnormal pulmonary $\dot{V}O_2$ kinetics in PAD to the affected lower extremities, 2. characterize the kinetics of calf muscle deoxygenation during walking in PAD and healthy subjects, 3. describe muscle deoxygenation kinetics in relation to exercise work rate and blood flow in PAD and health, and 4. evaluate the effect of arterial revascularization on pulmonary $\dot{V}O_2$ kinetics in PAD. These investigations revealed a persistent abnormality in muscle oxygen utilization in PAD-affected skeletal muscle that was not associated with the severity of hemodynamic compromise. In particular, we observed slowed pulmonary $\dot{V}O_2$ kinetics in PAD only during exercise of the PAD-affected skeletal muscles. Moreover, muscle deoxygenation kinetics following the onset of walking and lower intensity calf exercise

were prolonged in PAD subjects while leg blood flow responses were normal. However, at higher work rates, PAD muscle deoxygenation kinetics accelerated, demonstrating a work rate and presumably blood flow dependence. Lastly, arterial revascularization tended to improve, but not consistently normalize, pulmonary $\dot{V}O_2$ kinetics in PAD subjects. Thus, these investigations demonstrate abnormal oxygen uptake kinetics in PAD and provide evidence that local abnormalities of the affected skeletal muscle may contribute to the abnormal $\dot{V}O_2$ kinetics and exercise intolerance of patients with PAD.

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**Chapter 1**.

## Peripheral Arterial Disease: Pathophysiology and Response to Exercise

### *Introduction*

Peripheral arterial disease (PAD) is a manifestation of the systemic disease of atherosclerosis that results in arterial stenoses in the lower extremity. Peripheral arterial disease has an estimated prevalence of 12% in the general United States population affecting nearly 20% of those over the age of 70 years (Criqui et al. 1985). Importantly, the presence of PAD confers significant cardiovascular morbidity and mortality roughly equivalent to people with prior myocardial infarction or stroke (Leng et al. 1996). The characteristic symptom of PAD is intermittent claudication: a cramping leg pain that occurs in the affected limbs during exercise and that causes the patient to stop and rest until the pain subsides. Secondary to intermittent claudication, PAD is associated with a significant functional impairment, and peak exercise capacity is reduced approximately 50% compared with healthy subjects (Hiatt et al. 1994). Thus, PAD is associated with serious health consequences related to reduced exercise tolerance and increased risk for cardiovascular events.

The pathophysiology of PAD is associated with the lower extremity hemodynamic compromise. During exercise arterial stenoses constrain increases in lower limb blood flow resulting in reversible muscle ischemia. However, whereas the primary pathophysiology of PAD results from the lower extremity hemodynamic limitation, there are a spectrum of abnormalities distal to the arterial stenoses in PAD-affected skeletal muscle that may also contribute to the impaired exercise responses (Hiatt et al. 1992, Bhat et al. 1999, Brass et al. 2001). For example, there is an inverse

correlation between the accumulation of metabolic intermediates (e.g. short-chain [acylcarnitine]) in PAD-affected skeletal muscle and peak exercise performance in PAD suggesting abnormal oxidative metabolism (Hiatt et al. 1992). This contrasts with the poor correlations observed between performance and hemodynamic measures (Dahllof et al. 1976, Ekroth et al. 1978, Hiatt et al. 1987, Steinacker et al. 2000). Additionally, there are specific defects in mitochondrial electron chain complexes I and III (Brass et al. 2001), and altered control of mitochondrial respiration during exercise that may impair skeletal muscle oxygen utilization (Kemp et al. 1995, Kemp et al. 2001). Thus, it has been suggested that a portion of the PAD exercise impairment may be related to abnormal oxidative metabolism as well as the local circulatory defects in PAD-affected skeletal muscle (Brass and Hiatt 2000).

Insight into the PAD exercise impairment may be gained from investigations that examine the dynamic increase of oxygen uptake (pulmonary oxygen uptake kinetics, $\dot{V}O_2$ kinetics) during the transition from rest-to-constant work rate exercise. Accounting for a circulatory transit delay from exercising muscle to lung, pulmonary $\dot{V}O_2$ kinetics following the onset of exercise closely resembles the time course of oxygen utilization measured at the level of the exercising muscle (Grassi et al. 1996). Thus, pulmonary $\dot{V}O_2$ kinetics provides a window into the dynamics of muscle $\dot{V}O_2$ that are ultimately dependent upon the complex interaction between oxygen delivery, blood flow distribution, oxygen diffusion, and the rate of mitochondrial oxygen utilization. Previous investigations have described slowed pulmonary $\dot{V}O_2$ kinetics in a variety of cardiovascular diseases, including PAD (Sietsema et al. 1986, Koike et al. 1994, Sietsema et al. 1994, Bauer et al. 1999). The significance of slowed $\dot{V}O_2$ kinetics in

disease rests with a greater perturbation of intracellular metabolism and prolonged adjustments to increases in work rate such as those encountered in daily life. As a result, slowed $\dot{V}O_2$ kinetics may be related to the exercise intolerance observed in diseased populations. For example, in some cardiovascular diseases the slowed $\dot{V}O_2$ kinetics may result from a central impairment of oxygen delivery (e.g. cyanotic congenital heart disease (Sietsema et al. 1986)) whereas others may result from combined impairments of both cardiac output and peripheral skeletal muscle metabolic function (e.g. chronic heart failure) (Sietsema et al. 1994, Hepple et al. 1999). In PAD, early observations of slowed $\dot{V}O_2$ kinetics were attributed to the local mechanisms of blood flow limitation (Auchincloss et al. 1980, Haouzi et al. 1997) and/or to their abnormal skeletal muscle metabolism (Bauer et al. 1999, Barker et al. 2003). However, there remain potential systemic abnormalities secondary to atherosclerosis in addition to the local circulatory and metabolic abnormalities associated with PAD that could not be entirely excluded. Thus, the relative contributions of impaired limb blood flow and abnormal muscle oxidative metabolism to the impaired oxygen uptake responses in PAD have not been systematically addressed.

In this context, the following series of investigations sought to further characterize the systemic oxygen uptake kinetics during exercise in patients with intermittent claudication secondary to PAD and extend these observations to local muscle deoxygenation (e.g. oxygen extraction) measured at the level of the PAD-affected exercising muscle. Localization of the PAD-associated oxygen uptake abnormalities and evaluation of the dynamic responses of muscle deoxygenation in the transition from rest

to exercise would provide further insight into the pathophysiology of exercise intolerance and functional disability in patients with peripheral arterial disease.

The aims of these investigations were four-fold:

1. Evaluate whether the abnormal pulmonary $\dot{V}O_2$ kinetics in PAD are reflective of the systemic sequelae of atherosclerosis or can be attributed to peripheral factors local to the affected limbs in the PAD condition.
2. Characterize the dynamics of calf muscle deoxygenation across the rest-exercise transition during treadmill walking in PAD and healthy control subjects.
3. Evaluate muscle deoxygenation dynamics during single-leg, isolated calf muscle exercise in PAD and control subjects in relation to exercise work rate and limb blood flow responses.
4. Describe the effects of revascularization and the resulting improvement in peripheral blood flow capacity on the abnormal pulmonary $\dot{V}O_2$ kinetics and peak exercise response in patients with PAD.

**Background and Review of Literature:**

1.1 *Peripheral Arterial Disease: History and Prevalence.*

Peripheral arterial occlusive disease (PAD) refers to a common atherosclerotic condition that impairs arterial blood flow to the lower extremities. The primary symptom of PAD, intermittent claudication described as a cramping leg pain that occurs during exercise, has been recognized since the time of the Roman Empire. However, it wasn't until the early 19th century that the etiology of chronic peripheral arterial disease and the clinical symptoms of claudication were more clearly understood (Vascular Surgery: A Comprehensive Review1998). Indeed, the term 'claudication' is derived from the Latin word 'claudicatio', meaning *to limp,* which accurately describes the gait pattern of patients affected by PAD.

The first medical description of intermittent claudication involved carriage horses and was made by Jean-Francois Bouley jeune in 1831 in an oral report regarding claudication and subsequent collapse of a trotting horse (Sugar 1994). The autopsy revealed the "obliteration of the femoral arteries" caused by fibrinous clots and led to Bouley jeune's interpretation that muscle perfusion, even through the numerous anastomoses, was insufficient to support the demands of exercise (Bollinger et al. 2000). However, the modern characterization of intermittent claudication in human patients may well be credited to Jean-Martin Charcot in his 1858 report on the arterial obstruction and intermittent claudication in horse and man (Charcot 1858). Charcot's first human patient was a former soldier of the French conquest of Algeria who suffered an abdominal bullet wound that became embedded in his right iliac artery. The patient presented with loss of peripheral arterial pulses, cool extremity, and exercise intolerance resulting from the

reduced arterial lumen. This initial description of arterial stenosis, although of traumatic origin, formed the basis for what he and his students would later realize could also be caused by peripheral atherosclerosis (Bollinger et al. 2000). Charcot's characterization of the etiology of intermittent claudication in human patients remains accurate to the present day.

Interest into the surgical management of PAD soon followed, and new surgical techniques were developed to address the hemodynamics of acute and chronic peripheral arterial problems. The hemodynamics of PAD were characterized using both invasive and non-invasive methods, and by the late 20$^{th}$ century, evidence of the perturbed morphological and metabolic status of the PAD-affected skeletal muscle had been described. More recently, descriptions of PAD-induced tissue injury and in vivo muscle bioenergetic abnormalities have spread research interest into the potential mechanisms of functional limitation in PAD and potential for non-surgical alternatives in the treatment of patients with claudication.

Today, the prevalence of PAD comprises a significant health concern affecting an estimated 10-15 million adults in the general United States population and nearly 20% of those aged 70 years or greater (Criqui et al. 1985). While the natural history of PAD is generally stable, up to 25% of patients may have progression of symptoms (Weitz et al. 1996) and less than 5% will progress to the level requiring lower limb amputation (Leng et al. 1996). Symptomatic PAD causes a significant functional impairment (Regensteiner et al. 1990), and patients report an adverse effect of PAD on health-related quality of life (Arfvidsson et al. 1993). The presence of PAD is equally distributed between women and men, and importantly, the presence of PAD confers a similar level of cardiovascular

morbidity and mortality due to cardiovascular events as people with prior myocardial infarction or stroke (Leng et al. 1996). The increased risk of cardiovascular events extends to those patients with PAD but who remain asymptomatic (e.g. experiencing no claudication symptoms or pain at rest). Thus, PAD confers a significant functional impairment due to the symptoms of intermittent claudication and, whether symptomatic or not, is associated with serious cardiovascular consequences.

1.2 *Atherosclerosis of the lower limbs*

Peripheral arterial disease is a common manifestation of atherosclerosis that causes narrowing and stenoses of the large and medium sized arteries of the body. The risk factors for PAD are similar to those for other atherosclerotic diseases including: hypertension, hyperlipidemia, history of smoking, and elevated blood fibrinogen (Murabito et al. 2002). Due to its systemic nature, atherosclerosis is rarely confined to a specific circulatory region, and may often be present in the coronary, cerebral as well as the lower extremity peripheral arterial circulations in patients with PAD (Hertzer et al. 1984, Ness and Aronow 1999). Central to the disease process of atherosclerosis is the injury and dysfunction of the vascular endothelium. Patients with atherosclerosis (including PAD) have a systemic abnormality in endothelial function that is associated with impaired flow-mediated vasodilation, reduced vascular reactivity, and enhanced platelet aggregation (Anderson et al. 1995, Lieberman et al. 1996). A primary mediator of endothelial injury is considered to be oxidant stress from the generation of superoxide anion, an oxygen-derived free radical (Ohara et al. 1993, Tsao et al. 1995). Indeed, elevated markers of oxidant stress and endothelial dysfunction are observed systemically in PAD and increase acutely with claudication-producing exercises (Liao et al. 1991,

Hickman et al. 1994, Belch et al. 1995, Brendle et al. 2001). Importantly, this is consistent with the increased oxidative stress found in models of ischemia and ischemia-reperfusion (Karmazyn 1991, Turrens et al. 1991). Thus, in PAD systemic oxidant stress is elevated due to both the local muscle ischemic episodes occurring with exercise and the systemic disease process of atherosclerosis.

The pathogenesis of atherosclerosis relates to the alterations in the vascular biology of the endothelium, such as the effects of oxidant stress and decreased bioactivity of nitric oxide (NO) and its synthesis (Tesfamariam and Cohen 1992, Tsao et al. 1995). Atherosclerotic arterial stenoses develop as a consequence of the local injury and inflammation of the affected endothelium. Monocytes, attracted to the area of endothelial injury, infiltrate the sub-endothelial layers becoming engorged with oxidized low density lipoproteins (LDL) trapped below the endothelial surface (Ross 1997). The local immune and inflammatory responses promote further accumulation of macrophages and release reactive oxygen species that increase regional oxidative stress. Over time, the accumulations of fatty macrophages (foam cells) form fatty streaks and ultimately atherosclerotic plaques in the arteries that may impinge upon the lumen and effectively reduce arterial diameter. In PAD, atherosclerotic plaques form stenoses in the arterial circulation supplying the lower extremities, generally occurring in the femoral-popliteal arteries and aortoiliac arteries although distal tibial and pedal arteries may also be involved.

### 1.3 *Arterial hemodynamics and leg blood flow in peripheral arterial disease*

The narrowing of arterial diameter due to atherosclerotic lesions alters arterial hemodynamics. The changes in arterial hemodynamics can be described by Pouseille's Law:

$$P_1 - P_2 = \bar{V} \cdot 8L0/r^2 = Q \cdot 8Ln/Br^4 \qquad (1)$$

where $P_1 - P_2$ represents the change in potential energy (e.g. mean arterial pressure) between two points separated by a distance, L, and where Q is blood flow, $\bar{V}$ is the mean blood velocity along a tube (or vessel) with an inside radius of r and n represents the coefficient of blood viscosity. From this equation, it is clear that the internal radius of the artery plays a dominating role in determining arterial hemodynamics as blood flow and vessel resistance, R, are inversely proportional to the fourth power of the radius of the arterial vessel. Resistance is described as :

$$R = P_1 - P_2/Q \text{ and } R \propto Ln/r^4 \qquad (2)$$

This has important effects on the lower extremity arterial hemodynamics in patients with PAD. For example, over time as the radius of the artery is gradually reduced by an arterial stenosis, there is little change in the pressure gradient and blood flow across a stenosis until at least a 50% reduction in arterial diameter is attained (Vascular Surgery: A Comprehensive Review1998). With further narrowing of the artery, the pressure gradient across the stenosis increases and blood flow may only be maintained as long as the peripheral resistance of distal vascular beds can continue to fall. Once peripheral resistance can no longer compensate, further decreases in arterial diameter cause flow and mean arterial pressure distal to the stenosis to decline towards zero. The degree of stenosis at which mean arterial pressure and flow initially become affected is termed the critical stenosis. In peripheral, high resistance circulations (such as

the resting lower limbs), a 90% stenosis may be required to observe a significant decrease in mean arterial pressure and blood flow at rest. However, an increased velocity of blood flow such as with exercise may lower the threshold for a critical stenosis resulting from the loss of energy and apparent increase in resistance across the stenosis. For example, a single low grade iliac stenosis may have no effect on resting limb blood flow at low resting blood velocities because of a compensatory decrease in peripheral resistance. However with the increased blood velocities during exercise, resistance increases across the stenosis requiring a greater drop in mean distal peripheral resistance to maintain and/or increase flow. Thus, under the condition of high blood velocity, the same (non-critical) resting stenosis may become hemodynamically limiting. A decrease in mean arterial pressure distal to the stenosis with exercise may thus reflect the failure of blood flow to adequately increase to match the fall in peripheral resistance.

The hemodynamic changes in the affected lower limbs of patients with PAD provide a useful index to assess hemodynamic severity. Measured in the supine position using Doppler ultrasonography, the ankle-brachial index (ABI) is calculated as the ratio of systolic blood pressure of the artery in the ankle (dorsalis pedis or posterior tibialis) to that of the upper arm (Stoffers et al. 1996). In healthy subjects the mean arterial pressure from the heart to the ankle decreases by only a few millimeters of mercury. As the pulse travels distally from the heart, the pulse pressure widens and systolic pressure increases due to the reflection of waves from the high resistance arteriolar beds. Thus, at rest in normal subjects, the ankle systolic pressure will generally exceed that of the arm yielding an ABI of 1.0 or greater. As discussed above, arterial stenoses require a drop in peripheral resistance to maintain a normal resting limb blood flow. The main result is the

reduction of systolic pressure distal to the stenosis (e.g. ankle arteries). An ABI of 0.90 or less is diagnostic for PAD. Importantly, the hemodynamic severity of peripheral arterial disease is correlated with the maximal increase in limb blood flow following exercise (Sumner and Strandness, Jr. 1969).

The hemodynamics of the lower limb in patients with intermittent claudication disease are altered. At rest, patients with intermittent claudication have normal lower limb blood flow and no ischemic symptoms (Hillestad 1963a, Hillestad 1963b, Folse 1965, Hlavova et al. 1965, Hlavova et al. 1966, Pernow and Zetterquist 1968, Wahren et al. 1973, Pernow et al. 1975, Sorlie and Myhre 1978, Bernink et al. 1982, Pentecost 2003, Strandell and Wain 2003). Resting blood flow is maintained due to the compensatory decrease in peripheral resistance distal to the arterial occlusions. With exercise and the demand for an increase in leg blood flow, intramuscular arterioles dilate in the working muscles causing a further decrease in peripheral resistance and a proportional increase in leg blood flow (Sumner and Strandness, Jr. 1970). Thus, patients with intermittent claudication may demonstrate a normal increase in leg blood flow with initial step increases in work rate (Folse 1965, Hlavova et al. 1965, Hlavova et al. 1966, Pernow and Zetterquist 1968, Wahren et al. 1973, Pernow et al. 1975, Sorlie and Myhre 1978, Bernink et al. 1982, Pentecost 2003). However, with further increases in exercise work rate, leg blood flow may reach a plateau (Folse 1965, Hlavova et al. 1965, Wahren et al. 1973, Pernow et al. 1975, Sorlie and Myhre 1978) secondary to the failure of peripheral resistance to further decrease. With additional increases in exercise work rate, the blood flow limitation results in a supply-demand mismatch of oxygen delivery to meet the metabolic demands of the working muscle. This mismatch results in muscle ischemia

(defined by a reduction in blood flow and oxygen delivery), the symptoms of claudication pain, and presumably the abnormally low peak exercise capacity characteristic of this patient population.

### 1.4 S*keletal muscle histology in peripheral arterial disease*

Distal to the arterial stenoses, the skeletal muscle of patients with PAD is associated with a spectrum of histological changes compared with healthy, normal muscle. Grossly, skeletal muscle fibers in patients with PAD are generally smaller (e.g. have a smaller fiber area) than in healthy subjects (Teravainen and Makitie 1977, Henriksson et al. 1980, Clyne et al. 1982, Clyne et al. 1985, Regensteiner et al. 1993, McGuigan et al. 2001), and muscle capillarization (number of capillaries per fiber) is often increased in PAD-affected skeletal muscle (Makitie 1977, Hammarsten et al. 1980, Henriksson et al. 1980, Clyne et al. 1985, McGuigan et al. 2001). In severe ischemic disease, inflammation, necrosis, fiber atrophy, and fiber regeneration have been observed in affected skeletal muscle (Henriksson et al. 1980, Clyne et al. 1985). These observations are consistent with the pathology of muscle damage and denervation due to chronic ischemia (Sjostrom et al. 1980). Similar but less severe abnormalities have been described in the affected skeletal muscles of patients with moderate ischemic disease. Sjostrom et al. (1980) described a shift of nuclei from peripheral to central locations within skeletal muscle (Sjostrom et al. 1980). There may also be infiltration of inflammatory cells into the affected skeletal muscle of patients with PAD (Sjostrom et al. 1980). The skeletal muscles of patients with PAD have a greater proportion of angular muscle fibers than healthy muscle suggesting fiber denervation, and large groupings of muscle fibers by fiber type suggesting the subsequent re-innervation of denervated

muscle in the affected legs (Teravainen and Makitie 1977, Sjostrom et al. 1980, Regensteiner et al. 1993). Within the muscle cells, myofibrillar abnormalities (e.g. Z-band deviations) have been observed (Sjostrom et al. 1980). Additionally, mitochondrial content is often increased (Elander et al. 1985, Jansson et al. 1988, Lundgren et al. 1989, Hiatt et al. 1996) but mitochondria may appear abnormal (Teravainen and Makitie 1977, Sjostrom et al. 1980), and the arrangement of the sarcoplasmic reticulum may be poorly organized (Sjostrom et al. 1980). Indeed, based upon the histological changes alone, PAD skeletal muscle has been termed an *arteriovasculo-occlusive myopathy* (Sjostrom et al. 1980).

Human skeletal muscles are heterogeneously composed of fibers with distinct twitch (slow versus fast) and metabolic (oxidative versus glycolytic) characteristics. The classical description of skeletal muscle divides muscle fibers into two primary types defined histochemically by ATPase activity or by immunohistochemistry specific for distinct myosin heavy chain isoforms. Type I muscle fibers are slowly contracting, non-fatigable muscle fibers with a high content of mitochondria and oxidative enzymes. Type II fibers are fast contracting, fatigable muscle fibers with a high content of glycolytic enzymes. Type II fibers can be further divided into at least two subclasses: type IIa, a fast, oxidative fiber with modest to high mitochondrial content and type IIb, a fast, primarily glycolytic fiber with very low mitochondrial content. More recently, a third type II muscle fiber, IIx, has been described with an intermediate oxidative capacity to IIa and IIb fibers and a distinct myosin heavy chain isoform (Smerdu et al. 1994). Early studies of fiber type populations and proportions in PAD muscle are confusing but indicate similar relative percentages of type I and type II muscle fibers in PAD-affected

muscle compared with control (Hammarsten et al. 1980, Henriksson et al. 1980, Sjostrom et al. 1980). With increasing hemodynamic severity, the percentage of type I fibers and expression of the myosin heavy chain I isoform may become reduced (McGuigan et al. 2001) although this has not been universally observed (Makitie and Teravainen 1977, Steinacker et al. 2000). Reports of the proportion of type II fibers in PAD muscle are conflicting with observations of an increased (Henriksson et al. 1980) or decreased (Hammarsten et al. 1980) percentage of type IIb fibers. These inconsistencies likely result from the diverse hemodynamic severity and small numbers of patients typically studied. Steinacker et al. (2000) described no differences in myosin heavy chain isoform expression between patients with mild PAD (e.g. ABI ~ 0.82) compared with control subjects. However, there was a reduced percentage of the IIb myosin heavy chain isoform in patients with moderate PAD (ABI ~ 0.59) and reductions in both IIa and IIb isoforms in cases of severe PAD (e.g. chronic leg ischemia, ABI ~ 0.36) (Steinacker et al. 2000). In a larger trial, McGuigan et al. (2001) found an increased percentage of type IIx fibers in the muscle of intermittent claudicants (ABI ~ 0.62) compared with controls (McGuigan et al. 2001).

Though unresolved, these data suggest that type I and II fibers are generally reduced in size and the expression of myosin heavy chain IIx isoform increases with the presence of PAD. Further, the skeletal muscles of intermittent claudicants show greater capillarization compared with control muscle, and there is a spectrum of other histological abnormalities consistent with significant skeletal muscle damage. Whether these observations indicate a general shift in the relative proportions of type I to type II fibers or within type II fiber subpopulations in PAD-affected skeletal muscle (Henriksson

et al. 1980, Clyne et al. 1985, Regensteiner et al. 1993, McGuigan et al. 2001) remains to be determined. Fiber atrophy, ischemic injury, and disuse secondary to the sedentary nature of patients with intermittent claudication remain significant confounders to the questions of skeletal muscle damage and histological changes in patients with PAD.

### 1.5 *Skeletal muscle metabolism in intermittent claudication*

Secondary to the arterial occlusions and ischemic episodes with exercise in patients with PAD are abnormalities in the metabolism of affected skeletal muscle. As a general observation, there appear to be increases in the activities of important individual enzymes of glycolysis, the citric acid cycle, and oxidative phosphorylation with mild to moderate peripheral arterial disease. For example, hexokinase tends to be elevated (Pastoris et al. 1996), and the rate limiting enzymes of glycolysis, phosphofructokinase and pyruvate kinase, have higher activities in PAD-affected muscle compared with controls (Bylund et al. 1976, Clyne et al. 1985). Lactate dehydrogenase is also elevated in PAD muscle (Clyne et al. 1985, Schweiger 1991, Pastoris et al. 1996). In the citric acid cycle, the rate limiting enzymes of citrate synthase and isocitrate dehydrogenase are also elevated in PAD-affected skeletal muscle compared with healthy, sedentary subjects (Bylund et al. 1976, Elander et al. 1985, Jansson et al. 1988, Lundgren et al. 1989, Schweiger 1991, Schersten et al. 2001) as are other non-rate limiting enzymes (fumarase and malate dehydrogenase) (Schweiger 1991). Cytochrome c oxidase, a key enzyme in the oxidation of NADH and FADH by electron transport, is also elevated in PAD skeletal muscle (Bylund et al. 1976, Elander et al. 1985, Lundgren et al. 1989, Schweiger 1991, Schersten et al. 2001). Additionally, increases in enzyme activities associated with beta-oxidation (3 OH, acyl-Coenzyme A dehydrogenase) and amino acid metabolism (alanine

amino transferase) are observed in PAD (Bylund et al. 1976, Lundgren et al. 1989, Schweiger 1991). Thus, the capacity for glycolytic and oxidative reactions generally appears to be elevated in the skeletal muscle of patients with PAD.

However, there is also evidence that several key enzyme activities appear to be reduced in PAD. The pyruvate dehydrogenase (PDH) complex converts pyruvate into acetyl-Coenzyme A for subsequent metabolism by the citric acid cycle. The control of this enzyme is complicated, being inhibited directly by the accumulation of its products (NADH and acetyl-Coenzyme A) and well as by phosphorylation by individual enzymes loosely bound to it's core (PDH phosphatase, and PDH kinase)(Voet and Voet 1995). In resting PAD muscle, the activity of the pyruvate dehydrogenase complex may be reduced (Barker et al. 2003) suggesting a potential abnormality in regulating the entry of glucose subunits into the citric acid cycle. Moreover, there are abnormalities in electron transport chain complexes I and III in skeletal muscle (Brass et al. 2001). Normalized to mitochondrial content, the activities of NADH dehydrogenase (complex I) and ubiquinol-cytochrome c oxidoreductase (complex III) are reduced in PAD muscle compared with control subjects. Thus, there appear to be potential differences from normal muscle in the regulation of key enzymes and activity of the fundamental electron transport processes necessary to convert the free energy conserved from upstream reactions to ATP in PAD skeletal muscle.

These changes may explain the accumulation of intermediates of metabolism in the affected skeletal muscle in PAD. At rest, lactate concentration is higher in the skeletal muscle and the venous effluent of the affected limbs (Hiatt et al. 1992, Pastoris et al. 1996, Maass and Alexander 2001). This could result from an ischemic state due to a

low oxygen delivery or a preferential shunt away from pyruvate oxidation secondary to an inactive PDH activation state. Acylcarnitine concentrations are also increased in the affected skeletal muscle and plasma of PAD patients (Hiatt et al. 1987, Hiatt et al. 1992). Because acylcarnitine concentration is in equilibrium with acyl-Coenzyme A concentrations (via the reversible acyl-transferase reaction), this accumulation indicates incomplete substrate oxidation, and particularly that of acetyl-Coenzyme A. Importantly, the accumulation of acylcarnitines in PAD skeletal muscle is inversely related to peak exercise capacity suggesting that the abnormal metabolism was related to the observed limitation in PAD exercise performance (Hiatt et al. 1992). Indeed, acetyl-Coenzyme A tends to be increased in PAD skeletal muscle (Barker et al. 2003) a factor which could independently reduce PDH activity. As acetyl-Coenzyme A groups form the common substrate for entry into the citric acid cycle, this reflects a downstream obstruction in substrate oxidation. This is consistent with the observed increase in concentration of the citric acid cycle substrate, alpha-ketoglutarate, in PAD skeletal muscle (Pastoris et al. 1996). Alpha-ketoglutarate is the intermediate product of two rate limiting citric acid cycle enzymes: isocitrate dehydrogenase and alpha-ketoglutarate dehydrogenase. The accumulation of these products could suggest a common mechanism of altered enzymatic control in PAD skeletal muscle. For example, ischemia as well as an independent impairment in electron transport can abnormally increase the cellular redox potential (NADH/NAD+). Increases in the redox potential inhibit the activity of pyruvate dehydrogenase, isocitrate dehydrogenase, as well as alpha-ketoglutarate dehydrogenase. As a result, the substrates of these reactions increase. Thus, the accumulation of

metabolic intermediates are suggestive that alterations in enzymatic control and function in PAD muscle may have functional metabolic consequences.

As mentioned earlier and consistent with the observations of elevated activities of some individual oxidative enzymes, the mitochondrial content of PAD-affected skeletal muscle is elevated compared with control muscle (Elander et al. 1985). In healthy subjects, mitochondrial content and oxidative enzyme activities positively correlate with peak exercise capacity (Wang et al. 1999). As increases in mitochondrial activities are associated with exercise training and become reduced following detraining or bed rest, there is a relationship between muscle oxidative capacity and exercise performance in healthy individuals (Holloszy and Coyle 1984, Wibom et al. 1992). This relationship is absent in patients with intermittent claudication who have paradoxically increased activities of oxidative enzymes (Bylund et al. 1976, Elander et al. 1985, Gerdle et al. 1986, Lundgren et al. 1989, Schweiger 1991, Schersten et al. 2001) despite their limited walking ability and sedentary behavior. Because the increased mitochondrial expression is proportionate to the severity of impaired leg hemodynamics in mild to moderate (but not severe) PAD patients (Jansson et al. 1988), these changes have been interpreted as compensatory adaptations to the primary exercise impairment in oxygen delivery and resulting low muscle $PO_2$ in patients (Bylund et al. 1976, Bylund-Fellenius et al. 1984a, Bylund-Fellenius et al. 1984b, Elander et al. 1985, Jansson et al. 1988). Thus, the increased skeletal muscle mitochondria and expression of oxidative enzymes of these patients appear to reflect the severity of the lower limb hemodynamic impairment. The metabolic significance of these increases with PAD may be attributed to improved control of oxidative ATP synthesis. For example, an increased mitochondrial content and

oxidative capacity would require less of an increase in the potential controllers of mitochondrial respiration (e.g. free [ADP]) to maintain a particular rate of oxidative ATP production (Elander et al. 1985, Wang et al. 1999). Thus, an increase in mitochondrial content and oxidative enzyme activities in PAD muscle could minimize the perturbation of intracellular energy stores and improve the control of oxidative ATP generation. Indeed, some studies of isolated mitochondria from PAD-affected skeletal muscle have suggested normal oxidative function in PAD (Elander et al. 1985, Hou et al. 2002). However, normal oxidative function in PAD affected skeletal muscle measured in vivo is not universally observed (Hands et al. 1986, Hands et al. 1990, Pipinos et al. 2000). Whether these conflicting data are due to differences between in vivo versus in vitro preparations remains unclear. However, it should be noted that neither total mitochondrial content nor in vitro oxidative function predicts functional exercise capacity in patients with intermittent claudication. Moreover, there is a methodological concern of mitochondria isolation techniques particularly regarding the isolation of mitochondrial sub-populations that further confuse these data. Thus, at present there is no compelling evidence that changes in total mitochondrial content or maximal oxidative enzyme activities are directly related to the functional exercise limitation in patients with PAD.

Increases in mitochondrial content and oxidative enzymes could also reflect a compensatory mechanism for an intrinsic abnormality in mitochondrial oxidative capacity (DiMauro 2004). The production of proteins destined for oxidative synthesis from mitochondrial and nuclear sources are not well coordinated (Duborjal et al. 2002). Thus, increases in the activity of nuclear versus mitochondrial encoded oxidative enzymes are a classic characteristic of mitochondrial diseases. Moreover, there is

evidence that mitochondria are the target of oxidative injury in PAD patients. For example, there is an accumulation of somatic mitochondrial DNA deletions in the affected skeletal muscle of PAD patients compared with age-matched control muscle (Bhat et al. 1999). The mitochondrial DNA encodes for 13 peptide subunits involved in oxidative phosphorylation: seven subunits for complex I, one for complex III, three for complex IV, and two for the ATPase of complex V. Thus, one basis for the development of an acquired mitochondrial myopathy may reside with the deletion of important mitochondrial DNA base pairs. These deletions can adversely affect the transcription of peptide subunits necessary for normal enzyme synthesis and oxidative phosphorylation with functional consequences (DiMauro 2004). In PAD, the documented abnormalities in electron transport function, particularly of NADH dehydrogenase of complex I and ubiquinol-cytochrome c oxidoreductase of complex III in PAD-affected muscle (Brass et al. 2001), are consistent with the mitochondrial DNA injury and electron transport dysfunction observed in ischemic myocardium (Rouslin 1983, Rouslin and Ranganathan 1983). Because mitochondria are also a major producer of oxygen free radicals, this injury may lead to further generation of reactive oxygen species resulting in a feed-forward system of oxidative injury and mitochondrial dysfunction. Collectively, mitochondrial DNA deletions, abnormal electron transport function, as well as the pronounced increase in activities of nuclear encoded oxidative enzymes are characteristic of heteroplasmic and inherited mitochondrial diseases (e.g. mitochondrial myopathy)(DiMauro 2004). Thus, the changes in mitochondrial content and oxidative enzymes may reflect a partial compensation for an acquired mitochondrial myopathy that could impair oxidative function in patients with PAD.

1.6 *Response to exercise in intermittent claudication*

Systemic measures of exercise performance are generally assessed by oxygen consumption measured non-invasively at the level of the mouth (e.g. pulmonary $\dot{V}O_2$) which encompasses the total changes in oxygen uptake by exercising muscle and non-active tissues. Because pulmonary $\dot{V}O_2$ is coupled to tissue oxygen uptake via the circulation (and providing pulmonary gas exchange is not limited), increases in pulmonary $\dot{V}O_2$ during exercise predominantly reflect the increases in oxygen uptake occurring at the level of the working skeletal muscles. Thus, peak $\dot{V}O_2$ can be calculated as the product of systemic oxygen extraction (a-$vO_2$ difference) and cardiac output (Q, blood flow) at peak exercise as described by the Fick principle:

$$\dot{V}O_2 = Q \cdot \text{a-}vO_2 \text{ difference} \qquad (4)$$

Exercise tolerance is profoundly reduced secondary to claudication symptoms in patients with peripheral arterial disease. With the onset of claudication symptoms walking ability is reduced in PAD, limiting exercise performance to merely a few city blocks in the typical patient. This free-living exercise impairment can be reproducibly demonstrated in the laboratory during graded exercise testing in patients with claudication. For example, peak $\dot{V}O_2$ is reduced during incremental treadmill exercise by approximately 50% in PAD patients compared with healthy subjects of similar age (Hiatt et al. 1987, Hiatt et al. 1988, Hiatt et al. 1992, Regensteiner et al. 1993, Bauer et al. 1999, Askew et al. 2002). Because patients with PAD stop exercising prematurely due to the local symptoms of claudication pain rather than from generalized fatigue, peak heart rate, blood pressure, and respiratory exchange ratio rarely approach the peak values observed in healthy control subjects (Eldridge and Hossack 1987, Hiatt et al. 1988, Bauer

et al. 1999). Moreover, PAD patients do not generally demonstrate evidence of a systemic lactate threshold by estimates from pulmonary gas exchange (V-slope) or from measures of venous lactate (Hiatt et al. 1988, Bauer et al. 1999, Maass and Alexander 2001). Thus, the primary hemodynamic compromise leading to muscle ischemia and claudication pain during incremental exercise in PAD is a likely determinant to their peak exercise impairment.

In healthy subjects, blood flow is a major determinant of peak $\dot{V}O_2$, particularly during exercise of exercise-trained muscle (Andersen and Saltin 1985, Richardson et al. 1995, Wagner 1996, Richardson et al. 1998, Richardson et al. 1999a). In patients with PAD, many histological (increased capillarization and mitochondrial content) and metabolic (increased activities of most oxidative enzyme) changes that occur in PAD-affected skeletal muscle are similar to that observed in exercise-trained healthy muscle. Thus, the simple conclusion is that the lower limb hemodynamic limitation is the primary determinant of exercise performance. However, as muscle oxygen extraction at peak exercise may approach a diffusion-limited maximum in PAD (Zetterquist 1970), the capacity for oxygen extraction at peak exercise may be an equally important determinant (Pernow et al. 1975). This may explain how exercise blood flow sometimes (Alpert et al. 1969, Green 2002), but not always (Pernow and Zetterquist 1968, Zetterquist 1970, Hiatt et al. 1992, Gardner et al. 1992) correlates with peak exercise performance. In contrast to hemodynamic measures, there is a significant inverse correlation between the abnormal metabolism of affected skeletal muscle (accumulation of acylcarnitines) and peak exercise capacity (Hiatt et al. 1992). This fact emphasizes the relative importance of *both* impaired leg blood flow responses and muscle metabolism in the determination of PAD

exercise performance. Thus, while the primary pathophysiology of patients with intermittent claudication is the hemodynamic compromise that occurs with exercise, abnormal muscle oxidative metabolism may also explain a portion of the exercise impairment in patients with peripheral arterial disease.

### 1.7 *Exercise Bioenergetics*

Following the onset of exercise, total muscle ATP turnover increases with negligible delay relative to the ATP demand of the skeletal muscle contractile elements. The re-generation of ATP to sustain the near-invariant intracellular [ATP] with exercise initially arises from the depletion of phosphocreatine (PCr) via the Lohman reaction and from glycolysis. Oxidative resynthesis of ATP increases following exercise onset but follows a slower and more finite time course dictated by the control of mitochondrial respiration in the physiological range of intracellular pH and $PO_2$ (Whipp and Mahler M. 1980, Barstow et al. 1994, Kemp et al. 2002, Kemp 2005). While the precise determinants in the control of oxidative metabolism remain unclear, oxidative resynthesis of ATP appears to follow a monoexponential rise that may be related to changes in [PCr], free[ADP], [Pi], and the free energy change of [ATP] as described by first order Michaelis-Menten kinetics (Barstow et al. 1994, Behnke et al. 2002, Grassi et al. 2002).

In healthy subjects, following the onset of moderate exercise, the decrease in [PCr] can be described by an exponential function without a delay to a new steady state (Barstow et al. 1994, Rossiter et al. 1999). The depletion of [PCr] acts as a buffer (and perhaps a signal) for the maintenance (via oxidative resynthesis) of cellular [ATP] such that the temporary mismatch between oxidative ATP synthesis and ATP use is reflected by the change in [PCr]. The time course and magnitude of [PCr] decrease following

exercise onset thus reflect the relationship between the acceleration of oxidative metabolism and ATP utilization. Thus, the intramuscular processes (or limitations) that control the rate of increase of mitochondrial oxygen utilization may be expressed in the dynamic increase of muscle $\dot{V}O_2$ (e.g. the accumulation/action of potential controllers of mitochondrial respiration and $O_2$ availability).

Following the onset of claudication-producing exercise in patients with PAD, [PCr] decreases to a greater extent and with a slower time course (time constant) compared with control subjects at a similar exercise work rate (G.J. Kemp, personal communication 12/2004)(Hands et al. 1986, Kemp et al. 1995, Kemp et al. 2001). The change in [PCr] is associated with an increased [ADP] needed to sustain a given increase in oxidative ATP resynthesis at end-exercise (Kemp et al. 1993). A greater fall in [PCr] during ischemia-producing exercise in PAD is also observed in healthy humans during exercise while breathing a hypoxic mixture (Haseler et al. 1998, Haseler et al. 2004), which is consistent with the changes in [PCr] during ischemia and hypoxemia in contracting canine skeletal muscle (Hogan et al. 1992). However, it should be noted that in healthy skeletal muscle the greater fall of [PCr] under hypoxic conditions is not associated with a slower time course of [PCr] depletion (Haseler et al. 2004). In PAD, the slowed dynamics of [PCr] depletion may be related with a slowed time course of the rise in oxidative ATP synthesis requiring a greater contribution of ATP synthesis from glycolysis and glycogenolysis early in the exercise transition, thus reflecting a potential abnormality in the control of or function of oxidative metabolism in exercising PAD skeletal muscle (Kemp et al. 2002). The slow increase of oxidative metabolism in PAD muscle may cause the adverse result of a greater perturbation of intracellular homeostasis

by the alterations in [PCr], free [ADP] and increasing H+ secondary to the increased reliance on glycolytic ATP production. Thus, the intracellular accumulation of metabolites and depletion of preferred metabolic substrates (e.g. glycogen) associated with slowed dynamics of oxidative metabolism may contribute to muscle dysfunction and the exercise intolerance observed in PAD.

The recovery from exercise is also abnormal in patients with PAD. Because the recovery of [PCr] is almost entirely dependent upon the oxidative resynthesis of ATP, slowed recovery dynamics of [PCr] and calculated free [ADP] are indicative of abnormal mitochondrial respiration (Hands et al. 1986). In PAD, the time course of [PCr] recovery following claudication-producing exercise is slowed compared with healthy muscle (Hands et al. 1986, Kemp et al. 1993, Kemp et al. 1995, Kemp et al. 2001) and is related to the slowed dynamics of muscle re-oxygenation (and thus presumably blood flow) due to the arterial occlusions (Kemp et al. 2001). However, even when exercise is not associated with a decrease in muscle pH or the development of claudication pain, [PCr] recovery remains altered (Pipinos et al. 2000) suggesting an intrinsic impairment in mitochondrial function that may be independent of the muscle blood flow limitation (Pipinos et al. 2000, Kemp 2005). This latter view is supported by the findings that [PCr] recovery following exercise remains abnormal after revascularization despite an acute improvement in limb hemodynamics (Zatina et al. 1986). These data suggest the impairment in blood flow may explain a portion of the mitochondrial dysfunction observed in recovery from ischemia-producing exercise. However, the altered dynamics of [PCr] and free [ADP] following exercise onset and during recovery from non-ischemic exercise as well as after post-revascularization are most consistent with an impairment in

mitochondrial function in PAD skeletal muscle even when a limitation in limb blood flow and oxygen delivery may not be manifest. These changes ultimately have important influences on the expression and dynamics of skeletal muscle $\dot{V}O_2$ in the rest-to-exercise transition.

### 1.8 *Pulmonary $\dot{V}O_2$ kinetics*

In the transition from rest to exercise, the rate of increase in muscle oxygen consumption ($\dot{V}O_2$ kinetics) is not instantaneous, but rather follows an exponential course determined by the dynamics of increasing oxidative metabolism (e.g. $O_2$ utilization) and possibly the delivery of oxygen. Further, the increase in muscle $\dot{V}O_2$ is coupled to that of pulmonary $\dot{V}O_2$ through predictable interactions of blood volume, gas stores, and circulatory transit delays such that dynamic increases in pulmonary $\dot{V}O_2$ may reflect those occurring at the level of the exercising muscle (Barstow et al. 1990, Grassi et al. 1996, Rossiter et al. 1999). Thus, measurement of pulmonary $\dot{V}O_2$ in the transition from rest to exercise provides a useful, non-invasive method for evaluation of the integration of cardiovascular, pulmonary, and muscle systems in health and disease.

Classically, pulmonary $\dot{V}O_2$ kinetics are characterized by three temporal phases described using modeling techniques with distinct exponential components:

$$\begin{aligned} \dot{V}O_2(t) = \dot{V}O_2(b) &+ A_1(1 - e^{-(t-TD1)/\tau 1}) && \text{phase I} \\ &+ A_2[1 - e^{-(t-TD2)/\tau 2}] && \text{phase II} \\ &+ A_3[1 - e^{-(t-TD3)/\tau 3}] && \text{slow component} \end{aligned} \quad (5)$$

The model provides estimates of the baseline $\dot{V}O_2$ ($\dot{V}O_2(b)$), amplitudes of the increase in $\dot{V}O_2$ for the individual exponential components ($A_1$, $A_2$, $A_3$), independent time delays for the onset of each exponential phase ($TD_1$, $TD_2$, $TD_3$), and time constants

of the respective exponential responses ($\tau_1$, $\tau_2$, $\tau_3$). With little delay following the onset of exercise, phase I reflects a rapid increase in pulmonary $\dot{V}O_2$ above baseline resulting from an abrupt increase in cardiac output and pulmonary gas exchange (Casaburi et al. 1989). This phase is typically of short duration lasting approximately 15-20 seconds during cycle exercise and with a time course that roughly corresponds to a transit delay for blood arriving from the exercising limbs (Barstow and Mole 1987). Thus, phase I represents an immediate increase in pulmonary $\dot{V}O_2$ that is largely independent of increases in muscle $\dot{V}O_2$ (Grassi et al. 1996). Upon arrival of desaturated venous blood from the exercising muscles, phase II begins and pulmonary $\dot{V}O_2$ increases with an exponential time course similar to that of muscle $\dot{V}O_2$ and [PCr] depletion (Whipp and Mahler M. 1980, Barstow and Mole 1987, Barstow et al. 1990, Grassi et al. 1996, Rossiter et al. 1999). Phase III reflects the steady state of oxygen consumption for exercise moderate intensity work rates defined as work rate below the individual's lactate threshold. However, for work rates above the individual's lactate threshold, phase III may demonstrate further increases secondary to a slowly developing additional oxygen consumption above that predicted from the $\dot{V}O_2$/WR relationship from moderate intensity work rates (Whipp and Mahler M. 1980). The phase III increase in oxygen consumption (e.g. the $\dot{V}O_2$ slow component) is derived predominantly from the exercising legs (Poole et al. 1991) possibly reflecting an increase in the recruitment of 'lower efficiency' type II muscle fibers with sustained heavy intensity exercise or a difference in the relative composition of type II to type I fibers in the skeletal muscle. The precise mechanisms of which remain to be more fully elucidated.

As noted above, pulmonary $\dot{V}O_2$ kinetics during phase II are closely matched with the dynamic increase in muscle $\dot{V}O_2$. The evidence for this relationship comes primarily from the work of several groups. Using modeling simulations, Barstow and colleagues (1987 & 1990) suggested that muscle $\dot{V}O_2$ dynamics could be predictably described in the expression of phase II pulmonary $\dot{V}O_2$ kinetics (Barstow and Mole 1987, Barstow et al. 1990). More directly, Grassi and colleagues (1996) measured muscle $\dot{V}O_2$ kinetics invasively using catheterization techniques and found similar time constants with the phase II pulmonary $\dot{V}O_2$ responses during exercise in human subjects (Grassi et al. 1996). Rossiter et al. (1999) made simultaneous measurements of [PCr] depletion and pulmonary $\dot{V}O_2$ kinetics that allowed the description of a close temporal time course between muscle bioenergetics and phase II pulmonary $\dot{V}O_2$ responses during moderate exercise (Rossiter et al. 1999). This was consistent with previous studies where simultaneous measurements could not be obtained (Barstow et al. 1994, Grassi et al. 1996, McCreary et al. 1996, Chilibeck et al. 1998). Thus, at least in healthy subjects during moderate exercise transitions, these observations have led to the conclusion that muscle $\dot{V}O_2$ kinetics are closely matched to the expression of pulmonary $\dot{V}O_2$ measured at the level of the mouth.

Considerable debate has been centered on the primary determinants of the $\dot{V}O_2$ kinetic response to exercise. One view contends that $\dot{V}O_2$ kinetics are determined by the dynamics of blood flow and oxygen delivery. Thus, a shortfall in oxygen supply constrains the dynamics of oxygen uptake following the onset of exercise (Hughson 1990). This view is largely supported by observations where $\dot{V}O_2$ kinetics were slowed under conditions that might impair oxygen delivery. For example, $\dot{V}O_2$ kinetics are

slowed by the administration of beta-blockade in healthy subjects (Petersen et al. 1983, Hughson 1984). This finding was attributed to the effect of beta-blockade slowing cardiac output dynamics and thus bulk delivery of blood flow. Moreover, body position appears to affect $\dot{V}O_2$ kinetics such that $\dot{V}O_2$ kinetics during leg exercise are slower in the supine position compared to the upright position in healthy subjects (Hughson et al. 1991, Hughson et al. 1993, MacDonald et al. 1998). That the slowed $\dot{V}O_2$ kinetics were then speeded to values similar to that of upright exercise by the application of lower body negative pressure appears to support a blood flow limitation to the $\dot{V}O_2$ kinetic response, at least during supine exercise (Hughson et al. 1993). Similar support has come from an apparent slowing of $\dot{V}O_2$ kinetics during forearm exercise with the arm elevated above the heart compared to the arm in a dependent position (Hughson et al. 1996). These data have been interpreted to suggest that perfusion pressure to the exercising muscles may play an important role in the determination of $\dot{V}O_2$ kinetics, a factor that may become reduced in both the supine and elevated arm positions. Another factor in altering oxygen delivery is through the lowering of arterial oxygen content by breathing hypoxic gas mixtures. This has been shown to slow the $\dot{V}O_2$ kinetic response (Springer et al. 1991, Hughson and Kowalchuk 1995, Engelen et al. 1996) consistent with a reduction in oxygen diffusion due to a lowering of the capillary-mitochondrial $PO_2$ gradient (Koike et al. 1990). Thus, there is evidence that $\dot{V}O_2$ kinetics may be slowed under conditions of significant blood flow or oxygen delivery compromise.

An opposing view has suggested that muscle $\dot{V}O_2$ kinetics are determined by the intramuscular metabolic processes that affect muscle $O_2$ utilization (Whipp and Mahler M. 1980). This concept, termed ‘metabolic inertia’ (Grassi et al. 1996), is supported by a

series of experiments comparing the dynamics of blood flow and oxygen consumption. For example, the kinetics of cardiac output are normally faster than pulmonary $\dot{V}O_2$ (De et al. 1991, Yoshida and Whipp 1994). Non-invasive studies of limb blood flow dynamics using ultrasonic Doppler technology and pulmonary $\dot{V}O_2$ have demonstrated faster limb blood flow dynamics than pulmonary $\dot{V}O_2$ kinetics during the transition from rest to moderate exercise (Hughson et al. 1996, MacDonald et al. 1998). Invasive studies confirm faster increases in directly measured leg blood flow than leg oxygen uptake during moderate and intense exercise (Grassi et al. 1996, Bangsbo et al. 2000). These studies also suggested that following the onset of contractions, muscle oxygen delivery may transiently exceed muscle oxygen uptake resulting in a decrease (Grassi et al. 1996) or delay (Bangsbo et al. 2000, Behnke et al. 2001) in oxygen extraction following exercise onset. Moreover, as described above, the dynamics of a potential controller of muscle mitochondrial respiration ([PCr] kinetics) closely match the phase II pulmonary $\dot{V}O_2$ kinetic response (Rossiter et al. 1999). While these data are suggestive of an intrinsic metabolic limitation, more compelling evidence of an intrinsic muscle metabolic limitation to$\dot{V}O_2$ kinetics would come from experiments that tested whether $\dot{V}O_2$ kinetics could become faster under conditions of increased blood flow and oxygen delivery. For example, in patients who underwent heart transplantation, Grassi et al. (1997) found no difference in pulmonary $\dot{V}O_2$ kinetics during exercise transitions after a "priming" bout of prior exercise (Grassi et al. 1997). The significance of this experiment was that an elevated cardiac output (resulting from the first bout of exercise) prior to and during the exercise transient of the second bout did not cause an appreciable speeding of pulmonary $\dot{V}O_2$ kinetics. Further, using animal preparations of isolated canine muscle,

Grassi et al. (1998a) were able to increase blood flow to exercise levels prior to beginning exercise contractions (Grassi et al. 1998a). In this experiment, faster oxygen delivery caused no discernable speeding of muscle $\dot{V}O_2$ kinetics compared to the non-adapted blood flow condition (Grassi et al. 1998a) similar to the results found in heart transplant patients. In a subsequent experiment, neither hyperoxia nor hyperoxia + pharmacological right-shifting of the oxygen-hemoglobin dissociation relationship (to favor oxygen unloading from hemoglobin, e.g. Bohr effect) speeded the muscle $\dot{V}O_2$ kinetic response (Grassi et al. 1998b). In contrast to studies demonstrating slowed $\dot{V}O_2$ kinetics when exercise blood flow and oxygen delivery is significantly reduced, these experiments suggested that $\dot{V}O_2$ kinetics are normally determined by an intrinsic limitation within the exercising skeletal muscle. Thus, it is generally accepted that in healthy individuals during the transition from rest to moderate exercise, the acceleration of muscle oxygen uptake following the onset of exercise is set by factors within the exercising muscle that affect the rate of increase in muscle oxygen utilization ( e.g. metabolic inertia). However, the precise location of this inertia remains to be elucidated.

Several lines of investigation have attempted to identify the location of metabolic inertia during the exercise transition. Nitric oxide, the product of nitric oxide synthase and L-arginine, is involved in an incredibly wide array of physiological reactions that include the endothelial regulation of arterial vasodilation and skeletal muscle mitochondrial respiration (Stamler and Meissner 2001). Nitric oxide synthase is also expressed in skeletal muscles, and its product, nitric oxide, reversibly binds with cytochrome –c oxidase inhibiting a key enzyme of the electron transport system (Stamler and Meissner 2001). Thus, its endogenous activity on mitochondrial respiration has been

suggested as a potential contributor to the metabolic inertia following the onset of exercise (Kindig et al. 2001). Kindig et al. (2001) were the first to demonstrate that inhibition of nitric oxide synthase with nitro-L-arginine methyl esther (L-NAME) speeded pulmonary $\dot{V}O_2$ kinetics during heavy and moderate exercise transitions in the horse (Kindig et al. 2001, Kindig et al. 2002). Their results suggested that inhibition of nitric oxide synthase resulted in reduced availibility of nitric oxide and thus may have removed the inhibitory effects of nitric oxide on cytochrome-c oxidase. Consistent with this notion, subsequent investigations in exercising humans using nitric oxide synthase inhibitors demonstrated faster pulmonary phase II $\dot{V}O_2$ kinetics during moderate (Jones et al. 2003) and heavy exercise transitions (Jones et al. 2004b). Thus, a portion of the intrinsic metabolic inertia following the onset of moderate and intense exercise in healthy subjects may be related to the inhibitory effects of nitric oxide on cytochrome-c oxidase and mitochondrial respiration.

Another possible location of metabolic inertia is in the activity of the pyruvate dehydrogenase complex and provision of its metabolic substrate, acetyl-Coenzyme A, for oxidation in the citric acid cycle. The control of pyruvate dehydrogenase is complex relating to its inhibition by accumulation of its products and to phosphorylation by complex associated components. Timmons et al. (1998) have proposed that the basal activation state of pyruvate dehydrogenase, and thus availability of muscle acetyl-Coenzyme A, may be related to the delay (e.g. metabolic inertia) of mitochondrial oxidative metabolism following the onset of exercise (Timmons et al. 1998a). Their postulate rests upon the notion that there is an inherent delay in the activation of pyruvate dehydrogenase and acetyl-Coenzyme A provision following exercise onset. This results

in an initial limitation in providing adequate acetyl-Coenzyme A groups to the citric acid cycle for oxidation causing an acetyl group 'deficit' or shortfall following exercise onset. Thus, a transient lag of acetyl-Coenzyme A production may limit the oxidation of acetyl groups affecting the generation of high energy equivalents (NADH) and oxidation by the electron transport chain. A method to test this hypothesis is through the administration of dichloroacetate, an agent that blocks the kinase that inhibits pyruvate dehydrogenase activity (e.g resulting in a greater proportion of the active fraction of pyruvate dehydrogenase). As a result, dichloroacetate administration increases acetyl group formation and availability for oxidation by the citric acid cycle thereby reducing substrate level phosphorylation (e.g. oxygen independent routes of ATP synthesis: [PCr] depletion and glycolysis resulting in the formation of lactate) following the onset of exercise. Muscle biopsy studies suggested that activation of the pyruvate dehydrogenase complex by dichloroacetate prior to exercise elevated resting acetylcarnitine concentration (a marker of acetyl-Coenzyme A accumulation) and decreased the magnitude of [PCr] fall and muscle [lactate] increase during exercise compared to the control condition (Timmons et al. 1998a). This was particularly evident during muscle contractions in animal models of partial ischemia (Timmons et al. 1996, Timmons et al. 1997, Roberts et al. 2002) and a model of human peripheral ischemia using lower body positive pressure (Timmons et al. 1998b). Indeed, during ischemic exercise in canine muscle, a transient decrease followed by an increase in acetylcarnitine levels was observed during the first minute of exercise contractions in the control condition and that was not apparent during ischemic exercise contractions with dichloroacetate (Roberts et al. 2002). These observations were consistent with the hypothesis that an acetyl group deficit and thus the

kinetics of pyruvate dehydrogenase activation may have regulatory effects on the oxidative production of ATP in the transition from rest to exercise. However, a series of investigations comparing the effects of pyruvate dehydrogenase activation on muscle $\dot{V}O_2$ kinetics during exercise yielded contrasting results. Bangsbo et al. (2002) found no difference in muscle $\dot{V}O_2$ or [PCr] dynamics with dichloroacetate administration during supra-maximal exercise transitions in humans despite increased pyruvate dehydrogenase activity at rest and throughout exercise (Bangsbo et al. 2002). Grassi et al. (2002) found no speeding of muscle $\dot{V}O_2$ kinetics in exercising canine muscle contracting at 60-70% of peak $\dot{V}O_2$ with dichloroacetate despite an elevation in muscle acetyl group concentration (Grassi et al. 200a). Similar findings of unaltered pulmonary phase II $\dot{V}O_2$ kinetics with dichloroacetate administration have been observed during moderate (Koppo et al. 2004) and heavy exercise in human subjects (Rossiter et al. 2003, Jones et al. 2004a). Using simultaneous $^{31}$P-NMR and pulmonary $\dot{V}O_2$, Rossiter et al. (2003) observed no change in pulmonary phase II $\dot{V}O_2$ kinetics or [PCr] depletion kinetics with dichloroacetate administration compared to the control condition (Rossiter et al. 2003). Despite this, dichloroacetate administration significantly reduced end-exercise [lactate] and the magnitude of [PCr] depletion versus the control condition (Rossiter et al. 2003). Although consistent with the energetic effects of reducing substrate level phosphorylation, pre-exercise activation of pyruvate dehydrogenase with dichloroacetate did not result in a speeding of the phase II $\dot{V}O_2$ kinetic response, and by inference, of muscle $\dot{V}O_2$ kinetics. Thus, these results suggest that in healthy subjects the acetyl group deficit does not likely represent a functional intramuscular limitation to $\dot{V}O_2$ kinetics following the onset of exercise. However, whether the mechanism of acetyl group deficit

plays a role in determining muscle $\dot{V}O_2$ kinetics following the onset of ischemic exercise remains to be more fully characterized.

As discussed above in healthy subjects, $\dot{V}O_2$ kinetics appear to be set by the metabolic characteristics controlling oxidative metabolism within the exercising muscle. However, as described in the previous section, $\dot{V}O_2$ kinetics may become slowed under conditions where blood flow and oxygen delivery are significantly compromised. Thus, under certain circumstances, pulmonary $\dot{V}O_2$ kinetics may be dominated by the dynamics of $O_2$ delivery rather than local intramuscular mechanisms when blood flow adjustments (i.e. the time constant for $O_2$ delivery) or oxygen diffusion are impaired. This has particular relevance in the study of $\dot{V}O_2$ kinetics in human cardiovascular diseases.

One common factor to all cardiovascular diseases is the abnormal circulatory hemodynamics associated with the diseased condition. Indeed, understanding the influences of circulatory modulation on the expression of muscle $\dot{V}O_2$ kinetics at the level of the mouth is critical in evaluating the potential determinants of $\dot{V}O_2$ kinetics in cardiovascular diseases. Barstow et al. modeled the circulatory modulation of $\dot{V}O_2$ kinetics in healthy subjects using computer simulations that allowed the parameters of blood flow and $\dot{V}O_2$ to be manipulated independently (Barstow et al. 1990). Based upon these simulations, the authors made the following conclusions regarding the interaction between blood flow dynamics and $\dot{V}O_2$ kinetics: 1.) $O_2$ delivery is only limiting to muscle $\dot{V}O_2$ kinetics when the time course of blood flow (i.e. $O_2$ delivery) increase is substantially longer than that for muscle $\dot{V}O_2$ and 2.) Under normal circulatory (cardiac and muscle blood flow) and muscle $\dot{V}O_2$ dynamics, pulmonary phase II $\dot{V}O_2$ kinetics predominantly reflect muscle $\dot{V}O_2$ kinetics. Moreover, pulmonary phase II $\dot{V}O_2$ kinetics

are largely invariant across moderate work intensities and slowing circulatory dynamics (assuming normal muscle $\dot{V}O_2$ utilization) predictably lengthens the pulmonary phase 1 duration, decreases the phase 1 amplitude, and speeds pulmonary phase 2 $\dot{V}O_2$ kinetic time constant. Thus, these experimental data provide a substantial foundation for examining pulmonary $\dot{V}O_2$ kinetic responses and *indirectly,* the possible contributors to slowed $\dot{V}O_2$ kinetics and exercise intolerance in cardiovascular diseases.

Conceptually, slowed pulmonary $\dot{V}O_2$ kinetics in cardiovascular diseases are the expected result of a disruption of the cardiovascular system where modulation of the circulatory dynamics and oxygen delivery may delay the $\dot{V}O_2$ kinetic response to exercise. Indeed, patients with cardiovascular diseases have characteristically slowed pulmonary $\dot{V}O_2$ kinetics following the onset of exercise. For example, in cyanotic congenital heart disease, a falling arterial $O_2$ content following exercise onset secondary to a cardiac shunt (R to L) impairs the dynamic increase in $O_2$ delivery (via the fall in arterial oxygen content) resulting in profoundly slowed pulmonary $\dot{V}O_2$ kinetic responses (Sietsema et al. 1986). Constraining dynamic increases in cardiac output during exercise transitions slows pulmonary $\dot{V}O_2$ kinetics as observed in coronary heart disease (Koike et al. 1994, Koike et al. 1995). Moreover, abnormalities in the skeletal muscle may potentially serve as a secondary mechanism that could impair $\dot{V}O_2$ kinetics following the onset of exercise (e.g. chronic heart failure) (Sietsema et al. 1994, Hepple et al. 1999). Thus, deciphering the mechanisms of impaired $\dot{V}O_2$ kinetics in cardiovascular diseases requires consideration of whether the response is due to a primary reduction in oxygen delivery or a reduction in exercising skeletal muscle oxygen utilization, or both.

Previous studies have described slowed pulmonary $\dot{V}O_2$ kinetics in patients with intermittent claudication secondary to peripheral arterial disease (Auchincloss et al. 1980, Haouzi et al. 1997, Barker et al. 2003, Barker et al. 2004). Auchincloss et al. (1976) noted a lower oxygen uptake one-minute following the onset of walking exercise in patients with peripheral arterial disease using the Douglas bag technique (Auchincloss et al. 1976, Auchincloss et al. 1980). Haouzi et al. (1997), using breath–by-breath pulmonary $\dot{V}O_2$, found a significantly prolonged half-time of the total increase in pulmonary $\dot{V}O_2$ following the onset of treadmill exercise among a group of severe PAD patients (Haouzi et al. 1997). These early studies attributed the slowed pulmonary $\dot{V}O_2$ responses in peripheral arterial disease to the primary limitation in lower limb blood flow. In contrast, more recent observations have postulated that peripheral factors relating to abnormal skeletal muscle metabolism may also play a significant role in the impairment of peripheral arterial disease $\dot{V}O_2$ kinetics. For example, we previously described slowed phase II pulmonary $\dot{V}O_2$ kinetic responses in patients with intermittent claudication compared to controls and that was not correlated with the degree of hemodynamic severity (e.g. ABI) (Bauer et al. 1999). We interpreted these data to suggest that the altered skeletal muscle metabolism in patients with intermittent claudication may play a significant role in the peripheral arterial disease $\dot{V}O_2$ kinetic impairment, although the potential limb blood flow limitation could not be excluded. In order to evaluate a potential metabolic limitation in the $\dot{V}O_2$ kinetic response in peripheral arterial disease, Barker et al. (2003) examined pyruvate dehydrogenase activity and acetylcarnitine concentration from resting muscle along with pulmonary $\dot{V}O_2$ kinetics in patients with PAD (Barker et al. 2003). They found that in the patients, the

phase II $\dot{V}O_2$ kinetic time constants were inversely correlated with maximal walking time and peak $\dot{V}O_2$. They also observed that pyruvate dehydrogenase activity was ~32% lower and resting acetylcarnitine concentration was ~13% higher in the resting skeletal muscle of patients with intermittent claudication compared to controls; although these changes did not reach statistical significance. Interestingly, resting pyruvate dehydrogenase activity in patients with intermittent claudication tended to be inversely correlated with the phase II time constant. The authors interpreted these data to indicate that the impaired $\dot{V}O_2$ kinetics contributed to the exercise intolerance of patients with intermittent claudication and suggested that the impairment in pulmonary $\dot{V}O_2$ kinetics with peripheral arterial disease may be linked, in part, to changes in pyruvate dehydrogenase activity and alterations in carbohydrate metabolism (Barker et al. 2003). Thus, research has demonstrated that pulmonary $\dot{V}O_2$ kinetics are slowed in patients with PAD. However, it remains unresolved whether the slowed $\dot{V}O_2$ kinetics in patients are related to limitations in limb blood flow due to the peripheral arterial stenoses and/or to abnormalities of skeletal muscle metabolism. Moreover, there remain potential systemic abnormalities secondary to atherosclerosis that could also explain the impaired pulmonary $\dot{V}O_2$ kinetics in peripheral arterial disease. To clarify these issues, further research is needed to localize the observed $\dot{V}O_2$ kinetic defect to exercise of the affected lower limbs and evaluate parameters of oxygen delivery and oxygen utilization at the level of the affected exercising skeletal muscle.

### 1.9 *Muscle deoxygenation kinetics*

Continuous wavelength near-infrared spectroscopy (cwNIRS) provides a non-invasive method of evaluating tissue oxygenation in vivo. Near-infrared spectroscopy

utilizes light sources generating near-infrared wavelengths typically in the range of 650-950nm and is based on the principle that the amount of each wavelength reflected back to a detector is dependent upon the absorption by biological chromophores and effects of light scattering in tissue (e.g. skin, fat, muscle, bone) (Chance 1989). In biological tissues, only the heme groups of hemoglobin and myoglobin and the copper moiety of cytochrome– c oxidase are known to influence NIR absorption as a function of oxygen tension (Boushel and Piantadosi 2000). Thus, cwNIRS can follow changes in the heme absorption characteristics with alterations in oxygen tension, as may occur during muscular exercise or ischemia.

Most continuous wave NIRS devices use between two and six near-infrared wavelengths to assess changes in tissue concentrations of oxygenated and deoxygenated heme chromophores (e.g. hemoglobin and myoglobin) using the modified Beer-Lambert law (Cooper et al. 1996). The maximal penetration of light reflected to the detectors is approximately 50% of the distance between the light source and photo detector, and the NIRS signal is suggested to be dominated by hemoglobin within the microvasculature of the region of NIRS interrogation (Seiyama et al. 1988, Mancini et al. 1994). However, it should be noted that the optical spectra of heme groups for hemoglobin and myoglobin cannot be easily differentiated due to their spectral similarities. Thus, myoglobin may influence NIRS signals, and its contribution to the NIRS signal remains an important and controversial issue. For example, Seiyama et al. (1988) showed in an isolated rat hindlimb using fluorocarbon perfusion (e.g. hemoglobin-free tissue perfusion) that the contribution of myoglobin and cytochrome $aa_3$ to the overall NIRS signal in skeletal muscle totaled less than 10% (Seiyama et al. 1988). However, the influence of

myoglobin saturation on NIRS could be more important in muscle characterized by higher myoglobin contents, such as cardiac tissue, and may contribute as much as 46% to the NIRS signals (Nighswander-Rempel et al. 2005). The relevance of myoglobin saturation and its contribution to the NIRS signals during exercise has more recently been disputed. Tran et al. (1999) suggested that NIRS signals primarily reflect changes in myoglobin rather than hemoglobin saturation due to similarities between the desaturation kinetics of NIRS and $^{1}$H NMR-derived myoglobin during plantar flexion exercise (Tran et al. 1999). Indeed, myoglobin has been shown to become desaturated at moderate work rates (~50% maximum) (Mole et al. 1999, Richardson et al. 1999b) but may not show further decreases with increments in exercise intensity up to maximal exercise (Richardson et al. 2001). It should be noted that similar data by the same group of Tran et al. (1999) also describe the fall in NIRS signals to follow similar kinetics to that of hemoglobin desaturation (Jue et al. 1999). Unfortunately, the time course analysis by Tran et al. (1999) is confounded by an inability to detect low levels of deoxyhemoglobin signals using $^{1}$H NMR and a slow data acquisition rate that precludes more complete kinetic analysis (Tran et al. 1999). In this context, at present it can only be assumed that NIRS provides a composite view of tissue oxygenation, inclusive of myoglobin and hemoglobin oxygen saturation.

Another limitation of cwNIRS is that the quantification of absolute heme concentrations requires the direct knowledge of tissue scattering coefficients and light path lengths. Unfortunately, these parameters can only be assessed through the use of more sophisticated methods. Thus, cwNIRS is generally limited to the description of relative directional changes in oxygenated and deoxygenated heme concentrations.

The application of second derivative spectroscopy to multi-wavelength cwNIRS data has extended the utility of the NIRS to quantitatively determine hemoglobin (and more recently myoglobin) oxygen saturation in muscle (Cooper et al. 1996, Schenkman et al. 1997, Marcinek et al. 2004, Myers et al. 2005). The second derivative as a function of wavelength (Cooper et al. 1996, Schenkman et al. 1997, Harrison et al. 1998) or the ratio of two $2^{nd}$ derivative optical attenuation signals (Myers et al. 2005) can be empirically scaled to a calibration relationship for pure hemoglobin (or myoglobin) saturation. Thus, a non-invasive, quantitative measure of hemoglobin oxygen saturation in tissues ($StO_2$) can be evaluated without the explicit determination of tissue scattering, differential path lengths, or calculation of absolute hemoglobin concentration. This is a distinct advantage over traditional cwNIRS, and NIRS-derived $StO_2$ has been shown to be experimentally robust to tissue scattering, motion artifact, and changes in total hemoglobin concentration (Myers et al. 2005). Moreover, $StO_2$ is significantly correlated with weighted venous hemoglobin oxygen saturation among isolated muscle preparations in animal models and in humans (D.E. Myers, personal communication)(Myers et al. 2005).

Muscle oxygenation is defined by intracellular myoglobin oxygen saturation and microvascular oxygen contents and reflects the dynamic balance between local oxygen delivery and oxygen utilization (Behnke et al. 2001, Grassi et al. 2003, DeLorey et al. 2003). Recent work using traditional cwNIRS has characterized the kinetics of muscle deoxygenation (measured as relative [deoxyhemoglobin] changes) following the onset of exercise in healthy subjects (Chuang et al. 2002, Grassi et al. 2003, DeLorey et al. 2003). These studies unanimously showed that muscle oxygenation decreases after a short time

delay and with faster kinetics than for muscle $\dot{V}O_2$ as assessed by phase II pulmonary $\dot{V}O_2$ kinetics (Chuang et al. 2002, Grassi et al. 2003, DeLorey et al. 2003). Importantly, these findings are consistent with observations of microvascular $PO_2$ kinetics in animal models that indicated the presence of an initial delay prior to deoxygenation (Behnke et al. 2001). This observation may reflect an early matching or surplus of oxygen delivery relative to oxygen utilization following exercise onset. Moreover, because of the characteristics that define muscle oxygenation (e.g. hemoglobin and myoglobin concentrations and oxygen saturation), it has been suggested that the muscle deoxygenation following exercise onset may reflect the dynamics of oxygen extraction (Grassi et al. 2003). Indeed, the kinetics of muscle deoxygenation appear quite similar to that of directly measured venous effluent from exercising skeletal muscle (Grassi et al. 1996). Importantly, these data provide a basis for the evaluation of altered muscle deoxygenation kinetics in human diseases where initial changes in oxygen delivery or oxygen utilization following the onset of exercise may be impaired.

Previous studies with cwNIRS in peripheral arterial disease have described a greater decrease in muscle oxygenation during claudication-producing exercise in the affected legs of patients compared with healthy limbs exercising at the same absolute work rate (McCully et al. 1994, Kooijman et al. 1997, Egun et al. 2002, Comerota et al. 2003). These findings are consistent with invasive measures of lower intramuscular $PO_2$, and blood gases of the venous effluent in PAD patients compared with controls during equivalent exercise (Nissen et al. 1974, Holm and Bylund-Fellenius 1981, Bylund-Fellenius et al. 1981, Maass and Alexander 1983). Thus, during claudication-producing (e.g. ischemic) exercise there is an increase in oxygen extraction as would be predicted

for an equivalent oxygen demand (e.g. consumption) from the Fick principle. Moreover, muscle re-oxygenation is slowed following ischemic exercise in PAD consistent with the blood flow limitation that occurs during claudication-producing exercise and the prolonged recovery of oxygen consumption and blood flow when exercise ceases (McCully et al. 1994, Kooijman et al. 1997, Kemp et al. 2001, Egun et al. 2002, Komiyama et al. 2002, Comerota et al. 2003). Indeed, the kinetics of muscle oxygenation recovery following claudication-producing exercise is correlated with hemodynamic disease severity (McCully et al. 1994, McCully et al. 1997, Kooijman et al. 1997, Comerota et al. 2003). However, the recovery from exercise may represent a different pathophysiological event than the onset of exercise. For example, at the end of claudication-producing exercise, patients with PAD experience skeletal muscle ischemia and claudication pain. This contrasts with the situation at rest in patients with PAD, where limb blood flow and hemoglobin oxygen saturation are normal (Maass and Alexander 1983, Kooijman et al. 1997). During the early moments of exercise, limb blood flow responses may initially match the external work demand in PAD (Pernow et al. 1975, Bernink et al. 1982). Studies have not specifically addressed the muscle deoxygenation responses at exercise onset, but if initial blood flow is not limiting, then the early muscle deoxygenation responses may be driven by alterations in tissue oxygen diffusion or mitochondrial oxygen utilization. Thus, if patients with PAD have a normal or elevated capacity for $O_2$ diffusion and skeletal muscle oxygen utilization (e.g. increased muscle capillarization and oxidative enzyme capacities), then a limitation in $O_2$ delivery would be associated with normal or faster muscle deoxygenation kinetics compared with control subjects following the onset of exercise. In contrast, if oxygen

utilization is impaired secondary to a diffusive or oxidative metabolic limitation, there could be an associated slowing of the muscle deoxygenation kinetics in PAD during the transition to exercise. Only one study has examined muscle deoxygenation responses following the onset of exercise in patients with PAD. Kemp et al. (2001) in a study of $^{31}$P-NMR and cwNIRS showed the rate constant (e.g. total muscle deoxygenation/exercise duration) of muscle oxygenation during 50% and 75% MVC calf ergometer exercise was greater in patients with peripheral arterial disease compared with control subjects, however the time course (e.g. half-time) of muscle deoxygenation appeared prolonged (G.J. Kemp personal communication 12/2004) (Kemp et al. 2001). The greater rate constant suggested a significant oxygen delivery shortfall relative to oxygen demand (e.g. the greater oxygen utilization exceeds oxygen delivery, the greater rate of muscle deoxygenation). This is consistent with a primary blood flow limitation and resulting muscle ischemia in these patients. However, the prolonged half-time following exercise onset in PAD patients suggests that the dynamics of the balance between oxygen utilization and oxygen delivery was continually altered throughout ischemia-producing exercise. However, whether the slowed muscle deoxygenation kinetics in PAD reflects an impaired oxygen delivery or an intrinsic abnormality of oxidative metabolism, or both cannot be presently resolved. Further investigations are needed that specifically evaluate the muscle deoxygenation kinetics across the rest-exercise transition using systemic and local muscle exercises across a range of exercise intensities and levels of blood flow limitation in PAD. Abnormalities in PAD muscle deoxygenation kinetics at work rates associated with and without a blood flow impairment would provide valuable insight into the pathophysiology and potential

contributors to the abnormal $\dot{V}O_2$ kinetics and exercise intolerance of patients with peripheral arterial disease. This concept has not been previously examined in PAD.

### 1.10 *Summary*

Patients with peripheral arterial disease (PAD) have atherosclerotic arterial occlusions in the lower extremities that can constrain the blood flow response to exercise. However, reduced limb arterial pressures and impaired exercise blood flow responses may not entirely explain the exercise limitation in PAD patients. PAD is also associated with a spectrum of histological and metabolic changes in affected skeletal muscle that may suggest both adaptation and metabolic dysfunction. For example, many oxidative enzyme activities are increased in patients with intermittent claudication whereas the function or control of oxidative ATP production in PAD-affected skeletal muscle may be impaired. Slowed pulmonary oxygen uptake kinetics in patients with peripheral arterial disease suggest that oxygen delivery and/or muscle oxygen utilization may be impaired. Observations using $^{31}$P NMR have shown that the time course of phosphocreatine depletion (and inferred kinetics of muscle oxidative metabolism) following exercise onset in PAD may be slowed. Indeed, the large vessel blood flow limitation and intrinsic metabolic alterations of the affected skeletal muscle could likely contribute to the exercise impairment in peripheral arterial disease. The respective roles of these pathophysiological mechanisms have not been elucidated and a significant question remains regarding the relative contribution of impaired limb blood flow versus the metabolic abnormalities in the exercise impairment in PAD. Key steps to further understanding the pathophysiology of exercise limitation in PAD require localizing the oxygen uptake kinetic abnormality during exercise of the affected lower limbs in PAD.

This would include obtaining dynamic measurements of muscle deoxygenation directly at the level of the exercising skeletal muscles and assessing the acute effects of increased blood flow capacity on oxygen uptake kinetics following revascularization. A greater understanding of the PAD-associated processes that disrupt the normal dynamic responses of oxygen uptake in the transition from rest to exercise would provide further insight into the mechanisms of exercise intolerance and functional disability in patients with peripheral arterial disease.

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## Chapter 2

## Pulmonary $\dot{V}O_2$ Dynamics during Treadmill and Arm Exercise In Peripheral Arterial Disease [1]


### Abstract

Slowed pulmonary oxygen uptake kinetics ($\dot{V}O_2$ kinetics) in peripheral arterial disease (PAD) have been attributed to impaired limb blood flow and/or peripheral muscle metabolic abnormalities. While PAD results from atherosclerotic occlusive disease in the arteries to the lower extremities, systemic abnormalities affecting whole body oxygen delivery or vascular function in PAD could also partially explain the exercise impairment. To date, the effects of these systemic abnormalities have not been evaluated. In order to test the hypothesis that the slowed pulmonary $\dot{V}O_2$ kinetics in PAD reflects local and not systemic abnormalities, $\dot{V}O_2$ kinetics were evaluated following the onset of constant load exercise of the upper and lower limbs in PAD patients and healthy controls. Ten PAD patients and 10 control subjects (CON) without significant cardiopulmonary dysfunction performed multiple transitions from rest to moderate intensity arm ergometry and treadmill exercise to assess their $\dot{V}O_2$ kinetic responses. Reactive hyperemic (RH) blood flow was assessed in the arms and legs as a measure of endothelial function. As compared with controls, PAD $\dot{V}O_2$ kinetic phase 2 time constants were prolonged during treadmill exercise (PAD 34.3+9.2 sec. vs. CON 19.6+3.5 sec., $p<0.01$) but not arm exercise (PAD 38.5+7.5 sec. vs. CON 32.5+9.0 sec. $p>0.05$). RH blood flow was significantly reduced in the legs (PAD 20.7+8.3 vs. CON 46.1+17.1 ml/100ml/min, $p<0.01$) and arms of PAD subjects (PAD 34.0+8.6 vs. CON 50.8+12.2 ml/100ml/min,


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$p<0.01$) compared with controls, but RH limb flow was not correlated with arm or treadmill $\dot{V}O_2$ kinetic responses in either group. In summary, slowed pulmonary $\dot{V}O_2$ kinetics in PAD patients occur only with exercise of the lower limbs affected by the arterial occlusive disease process, and are not slowed with exercise of the unaffected upper extremities as compared with controls. Further, the slowed pulmonary$\dot{V}O_2$ kinetics of the lower extremity could not be explained by any abnormalities in resting cardiac or pulmonary function, and were not related to the magnitude of reduction in limb vascular reactivity.

**Introduction**

Patients with peripheral arterial disease (PAD) have atherosclerotic arterial occlusions that predominantly affect the lower limbs (Laroche et al. 1976, Welling et al. 1981). The principal symptom of PAD is claudication pain in the muscles of the lower extremity that results in a profound reduction in exercise tolerance and community-based walking ability (Hiatt et al. 1988, Hiatt et al. 1994).

Following the onset of walking exercise in PAD, pulmonary oxygen uptake kinetics ($\dot{V}O_2$ kinetics) are slowed indicating an impaired rate of oxygen uptake to meet the increased muscle metabolic demand of exercise (Auchincloss et al. 1980, Haouzi et al. 1997, Bauer et al. 1999, Barker et al. 2003). Previous observations of slowed $\dot{V}O_2$ kinetics in PAD have attributed the impaired kinetic response to the arterial occlusions of the lower extremity limiting $O_2$ delivery (Auchincloss et al. 1980) and/or to abnormalities of peripheral skeletal muscle metabolism (Bauer et al. 1999, Barker et al. 2003). However, it remains unresolved whether the observed pulmonary $\dot{V}O_2$ kinetic responses in PAD result from systemic *rather than* local abnormalities secondary to atherosclerosis

that could influence $O_2$ delivery. For example, abnormalities in cardiac or pulmonary function could slow pulmonary $\dot{V}O_2$ kinetics by altering systemic $O_2$ delivery via a central cardiopulmonary impairment as observed in patients with atherosclerotic coronary artery disease (Koike et al. 1995) or obstructive pulmonary disease (Somfay et al. 2002). Further, $O_2$ delivery in PAD could be influenced not only by the large artery occlusions, but also by reductions in *systemic* vascular reactivity that are observed in atherosclerotic diseases (Harris et al. 1995). Thus, given the complex pathophysiology of PAD, distinguishing between these potential systemic and regional influences on pulmonary $\dot{V}O_2$ kinetics would further clarify and localize potential causes of the exercise impairment in PAD.

The present investigation tested the hypothesis that the abnormal pulmonary $\dot{V}O_2$ kinetics in PAD reflect local and not systemic abnormalities. To differentiate the impact of systemic sequelae from atherosclerotic disease as a factor in the abnormal $\dot{V}O_2$ kinetics, pulmonary $\dot{V}O_2$ kinetics were measured during leg (affected by the arterial occlusive disease process) and arm (no gross evidence of arterial disease) exercise in PAD patients compared with healthy controls. Reactive hyperemic responses in the arms and legs were assessed to quantify the anticipated systemic dysfunction, and the relationships between the hyperemic response and $\dot{V}O_2$ kinetics were defined. Formal evaluations were conducted to ensure that no participants in the studies had significant cardiac or pulmonary dysfunction, thus partially excluding the influence of any central $O_2$ delivery impairment on the PAD $\dot{V}O_2$ kinetic responses.

**Materials and Methods**

*Subjects*. Ten patients with PAD and 10 healthy control subjects of similar ages were recruited for this investigation. The University of Colorado Multiple Institutional Review Board approved the study, and informed consent was obtained from all subjects prior to study participation.

All subjects underwent screening pulmonary function tests, resting echocardiography, and peak exercise testing with electrocardiograph (ECG) monitoring in order to exclude cardiopulmonary disease that could affect systemic $O_2$ delivery. Subjects were excluded from study if they exhibited: 1) a history of coronary artery disease, previous myocardial infarction or coronary revascularization, angina, stroke, congestive heart failure, or diabetes mellitus 2), hematology or chemistry laboratory values outside of normal limits, 3) evidence of impaired pulmonary function ($FEV_1/FVC < 0.70$ or $> 1.20$), 4) echocardiographic findings of impaired cardiac performance (resting EF < 50%, diastolic dysfunction, or any left ventricular wall motion abnormalities) or 5) evidence of ischemic ECG changes during graded maximal exercise testing.

Healthy, non-smoking, control subjects were studied who had no chronic medical diseases by medical history, and a normal physical examination. Healthy subjects had an ankle-brachial index (ABI) > 1.00 in both legs at rest, no history of PAD or other cardiovascular disease, and had no ischemic ECG changes at rest or with graded, maximal exercise testing. All subjects were sedentary as defined by not participating in a regular exercise program (< 1 episode of exercise/week) and having similar scores on the Low Level Physical Activity Recall (LOPAR) questionnaire with PAD patients (Table 1)(Regensteiner et al. 1996). Two healthy control subjects were treated with statin drugs, but the remaining healthy subjects were taking no medications.

Peripheral arterial disease was confirmed in patients by a resting ABI < 0.90, that fell at least 0.10 following peak exercise. All PAD patients exhibited symptoms of claudication during walking, defined as localized discomfort or cramping in the muscles of the affected legs that occurred only with exercise and that was completely relieved following ten minutes of rest. The absence of upper extremity occlusive arterial disease was confirmed by equal arm blood pressures and pulse examinations, and the absence of ischemic arm symptoms during arm ergometer testing. Patients with ischemic rest pain or ischemic ulceration in either leg were excluded from study. Patients with PAD included eight subjects with bilateral occlusive disease and two subjects with unilateral disease (defined as a reduced ABI and claudication symptoms in one leg, but no symptoms and an ABI greater than 0.90 at rest that did not decrease with exercise in the other leg). All patients with PAD were taking aspirin, seven were treated with statin drugs, four were treated with calcium channel blocking agents, two with diuretics, and one with an angiotensin-converting enzyme inhibitor. Since beta -adrenergic blocking drugs may alter the $\dot{V}O_2$ kinetic response to exercise (Petersen et al. 1983), subjects taking these medications were excluded from study.

*Protocol Design.* Each study participant visited the Vascular Research Laboratory at the University of Colorado-Health Sciences Center on seven occasions for evaluation. Subjects were instructed to avoid the consumption of alcohol, caffeine, and smoking (only 2 PAD patients and no control subjects were current smokers) within the 12 hours prior to each visit and to avoid food consumption within 4 hours before each visit. All exercise testing visits took place at the same time of day for each subject. The first visit was used to obtain initial screening measurements and for subject familiarization with the

exercise testing equipment. At the second visit, all subjects underwent a resting echocardiogram. The subsequent five visits consisted of peak treadmill and arm ergometry testing, arm and leg limb blood flow measurements, and constant work rate exercise tests for the analysis of the oxygen uptake kinetics.

*Graded Exercise Testing*. Subjects performed a single graded treadmill test and an incremental arm ergometry test on separate days for the determination of peak arm and peak treadmill exercise performance (peak $\dot{V}O_2$). Patients with PAD performed their graded treadmill test using the Gardner Protocol (speed constant at 2 mph, 2% increase in grade every 2 minutes) to maximal claudication pain that prevented any further walking (Gardner et al. 1992). Healthy subjects performed a standard Bruce protocol to maximal effort (Bruce et al. 1973). All treadmill tests were performed on a Quinton 4000 treadmill (Quinton Instruments, Seattle, WA). For determination of upper extremity peak $\dot{V}O_2$, subjects performed an incremental arm ergometry test (ramping function of 7-10 watts/minute) on an electrically braked cycle ergometer modified for this purpose (Lode Excalibur, The Netherlands). For all graded exercise tests, heart rate (HR) was measured continuously using 12-lead ECG recordings.

*Constant Work Rate (CWR) Exercise Testing.* On two separate days, subjects performed exercise transitions from rest to a constant work rate (CWR) of treadmill walking (2.0 mph, 4% grade) as previously described (Bauer et al. 1999). This particular work rate was selected because all subjects (including PAD) could sustain six-minutes of constant work rate exercise without stopping, and because the work rate was sufficient to elicit a measurable increase in oxygen consumption suitable for determination of the $\dot{V}O_2$ kinetic responses. Each exercise transition consisted of a resting baseline period to obtain gas

exchange data, followed by six-minutes of CWR walking exercise. On a different day, subjects performed three six-minute CWR arm exercise transitions at a moderate workload equal to approximately 90% of the individual subject's arm-specific lactate threshold (LT) by gas exchange criteria (i.e. 10% below the individual LT). Each arm transition was separated by 10 minutes of rest. Respiratory gas exchange measurements and heart rate data were recorded throughout the resting baseline, exercise, and recovery of each CWR bout.

*Reactive Hyperemia (RH) Blood Flow Measurements*. Limb blood flow was measured in the supine position using venous occlusion strain gauge plethysmography (D.E. Hokanson Inc. Issaquah, WA) at rest and during reactive hyperemia (RH) immediately after release of cuff occlusion as previously described (Hellige et al. 1979). The limb to be assessed was supported just above the level of the heart and a mercury-in-silastic strain gauge was placed around the widest part of the forearm or calf. A cuff distal to the strain gauge on the wrist or ankle was inflated to 50 mmHg above systolic pressure to eliminate hand or foot circulation from the measurement. A pneumatic cuff was placed on the arm or thigh and inflated to 30mmHg to achieve venous occlusion. The cuff occlusion was maintained for several (4-6) cardiac cycles to obtain resting blood flow measurements. Blood flow was expressed as ml of flow/100ml of tissue/minute. Resting blood flow was calculated as the average of 6 separate measurements in each limb. Peak RH blood flow was determined following limb ischemia induced by a proximal cuff that was inflated 50 mmHg above systolic blood pressure for 5 minutes. Post-occlusion RH blood flow measurements were made every few seconds and the highest value achieved was taken as the peak value. Resting blood flow, peak RH blood flow and the change in

blood flow from rest to peak RH (Δ blood flow, peak RH -resting blood flow) are presented as the sum of the individual values from both arms or both legs.

*Ankle Brachial Index.* The ABI was calculated in all subjects at rest and in PAD patients within one-minute following graded treadmill exercise as previously described (Bauer et al. 1999). The ratio of ankle-to-brachial systolic pressure was determined by taking the highest arm pressure divided into the higher of the two vessels in each ankle.

*Spirometry*. Lung volumes and flow rates were measured using the flow meter and pulmonary function software of a metabolic system (Medical Graphics Corporation, BreezEx, St. Paul, MN). Tidal volumes, Forced Vital Capacity (FVC), and Forced Expiratory Volume measured at 1.0 second ($FEV_1$) were assessed.

*Echocardiography.* A cardiologist blinded to study group assessed resting cardiac function using echocardiography (Sonos 5500, Philips Medical Systems, Andover, MA). Measurements of left ventricular size and wall thickness were determined in standard fashion as recommended by the American Society of Echocardiography (Schiller et al. 2003). Specifically, systolic function was assessed by visual inspection, fractional shortening, and measurement of left ventricular ejection fraction by the method of disks (Schiller et al. 2003). Diastolic function was assessed using left ventricular inflow Doppler and tissue Doppler measurements as previously described (Rakowski et al. 1996). Any regional wall motion abnormalities were noted and considered a disqualifying index of cardiac dysfunction.

*Measurement of Pulmonary Gas Exchange.* For all exercise tests, oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}E$), and other respiratory variables were measured and recorded breath-by-breath using a metabolic measurement

system (MedGraphics CPX/D, Medical Graphics Corp., St. Paul, MN, USA). The system $O_2$ and $CO_2$ analyzers were calibrated prior to each test using gases of known concentrations. Inspired and expired volumes were also calibrated using a syringe of known volume (3.0 L). All breath-by-breath data collected were stored to computer disk for analysis. During graded exercise, the highest $\dot{V}O_2$ averaged over 20-seconds was defined as peak $\dot{V}O_2$. The respiratory exchange ratio (RER) was calculated as the ratio of $\dot{V}CO_2/\dot{V}O_2$. Estimated lactate threshold (LT) was determined from graded exercise gas exchange data for each individual's arm and leg exercise using the V-slope method (point of nonlinear increase of $\dot{V}CO_2$ in relation to $\dot{V}O_2$) (Beaver et al. 1986). Individual lactate thresholds could be determined for all subjects (PAD and control) during graded arm exercise. However, no PAD patients demonstrated a measurable V-slope point of inflection during graded treadmill exercise and thus, a LT by gas exchange could not be determined.

*Data Analysis.* Breath-by-breath gas exchange data for each exercise transition were processed using a software program developed by our laboratory as previously described (Bauer et al. 1999). Breath-by-breath data for each exercise transition were time interpolated to 1-second intervals. The first constant work rate treadmill exercise transitions from each day of testing were time-aligned and averaged to provide a single $\dot{V}O_2$ kinetic response for each subject (e.g. average of 2 CWR transitions). In a similar fashion, the breath-by-breath data from 3 transitions of CWR arm exercise were processed to achieve a single kinetic response for arm ergometry exercise.
The pulmonary $\dot{V}O_2$ kinetic responses at the onset of CWR exercise were evaluated using 2- and 3- component exponential mathematical models (e.g. 1and 2).

$$\dot{V}O_2(t) = \dot{V}O_2(b) + A_1(1 - e^{-(t-TD1)/\tau 1}) \quad \text{phase 1}$$
$$+ A_2[1 - e^{-(t-TD2)/\tau 2}] \quad \text{phase 2} \qquad (1)$$

$$\dot{V}O_2(t) = \dot{V}O_2(b) + A_1(1 - e^{-(t-TD1)/\tau 1}) \quad \text{phase 1}$$
$$+ A_2[1 - e^{-(t-TD2)/\tau 2}] \quad \text{phase 2}$$
$$+ A_3[1 - e^{-(t-TD3)/\tau 3}] \quad \text{phase 3} \qquad (2)$$

The models provided estimates of the baseline $\dot{V}O_2$, amplitude of the individual exponential components ($A_1$, $A_2$, $A_3$), independent time delays for the onset of each exponential phase ($TD_1$, $TD_2$, $TD_3$), and time constants of the individual exponential components ($\tau_1$, $\tau_2$, $\tau_3$) using nonlinear regression (Sigmaplot 2001, SPSS, Inc., Chicago, IL) as previously described (Bauer et al. 1999). For each subject, the best-fit model (decision to include 2 versus 3 components) was determined across all exercise data points by an F-test and confirmed by examination of the residuals between 20 and 180 seconds (i.e. *phase 2* of the response). The latter criterion was included to ensure that data points within the likely period of phase 2 of the response were appropriately represented. All CWR arm exercise transitions were performed at an exercise intensity of 90% of arm LT (moderate exercise), and the pulmonary $\dot{V}O_2$ kinetics were best fit using a two component exponential model. The physiologically relevant amplitude of $\dot{V}O_2$ for each phase of the transition was computed from the individual kinetic parameter estimates:

$$A1' = A_1(1-e^{-(TD2/\tau 1)}), \qquad (3)$$

$$A_2' = A_1' + A_2, \qquad (4)$$

$$A_3' = A_3(1-e^{(-(ED-TD3)/\tau 3)}), \qquad (5)$$

$$Atot = A_2' + A_3' \qquad (6).$$

*Heart Rate Kinetics.* The heart rate half-time ($HR_{50}$) was calculated as the time for HR to achieve 50% of the change in HR from rest to end-CWR exercise.

*Statistical Analysis.* Unpaired student t-tests were used for comparisons between groups for all variables. Planned comparisons within groups for arm and leg variables were made using paired t-tests. The planned comparisons were PAD versus control for arm and leg responses and within PAD or control groups for arm versus leg responses with the primary endpoint of $\dot{V}O_2$ kinetics. The Pearson's R product was used to evaluate significant correlations. Statistical significance for all comparisons was declared at $p < 0.05$.

**Results**

*Subject Characterization.* Subject characteristics are presented in Table 1. PAD and control subjects were of similar age, weight and BMI. Two PAD subjects were current smokers and the PAD group had a significantly greater smoking history than controls as assessed by pack years ($p < 0.05$). No differences were observed between PAD and controls for measures of pulmonary function (FEV1/FVC), resting cardiac function (Ejection Fraction >56% in all subjects), or in habitual physical activity (Low Level Physical Activity Recall, LOPAR). Further, no subject demonstrated evidence of cardiac diastolic dysfunction or regional wall motion abnormalities. As expected, resting ABI values were lower in the PAD group than controls (2 unilateral and 8 bilateral subjects are combined in PAD group mean). Furthermore, following graded treadmill exercise, the ABI significantly decreased in both legs of bilateral subjects in the PAD group and in the affected leg of the two unilateral PAD subjects ($p < 0.05$).

*Peak Performance.* The peak lower and upper extremity exercise responses are presented in Table 2. As previously described in the PAD patient population, claudication-limited peak $\dot{V}O_2$ during graded treadmill exercise was reduced approximately 50% in PAD

subjects compared with age-matched healthy controls ($p < 0.01$) (Hiatt et al. 1988). This was associated with a reduced peak RER and peak HR in PAD patients as compared with controls during graded treadmill exercise ($p < 0.01$). In the control subjects, the $\dot{V}O_2$ at the lactate threshold was 19.3+ 2.7 ml/kg/min. However, no PAD patient demonstrated a measurable V-slope point of inflection during graded treadmill exercise, and therefore a LT by gas exchange could not be determined. During peak arm exercise, peak $\dot{V}O_2$ was similar between groups, and no PAD subject was limited by ischemic arm symptoms (i.e. arm muscle cramping or localized arm symptoms) during upper extremity exercise. There were also no differences with graded arm exercise in peak RER or peak HR between PAD and control groups. With arm exercise, PAD patients did demonstrate a LT by gas exchange criteria which occurred at a similar $\dot{V}O_2$ compared with control subjects. The only difference in arm exercise responses was that control subjects attained a greater power output at peak exercise than PAD subjects ($p<0.05$).

*Constant work rate exercise*. All PAD subjects experienced claudication pain during the CWR treadmill exercise with a mean onset at 142 seconds (Table 3). No PAD patient stopped exercise prior to completing 6-minutes of CWR exercise. Heart rate kinetics during lower extremity exercise, assessed by $HR_{50}$, was prolonged in PAD ($p < 0.01$), but the PAD group had a greater change in HR from onset to the end of 6-minutes of CWR treadmill exercise ($\Delta$HR) compared with control subjects. End-exercise oxygen consumption was similar between PAD and control groups during treadmill CWR walking exercise. However, the relative intensity of CWR treadmill exercise as a percentage of peak exercise $\dot{V}O_2$ was greater in the PAD group (83+17%) compared with controls (43+9%) ($p < 0.01$). Consistent with a high relative exercise intensity in PAD,

the respiratory exchange ratio was significantly greater in PAD subjects compared with controls at end treadmill CWR exercise (0.93 + 0.05 in PAD vs. 0.87 + 0.03 in controls, $p < 0.05$). In contrast, there were no differences in exercise characteristics during CWR arm exercise at an individual relative intensity of 90% of mode-specific lactate threshold in both PAD and control groups. Specifically, HR kinetics were similar between PAD and control groups during arm exercise.

*$O_2$ uptake kinetics.* Kinetic data from all control subjects and three of ten PAD subjects' treadmill CWR tests were best fit using a 2-component model, while seven PAD patients required 3-component modeling due to the presence of a slow, phase 3 component. Consistent with previous reports (Haouzi et al. 1997, Bauer et al. 1999), the pulmonary $\dot{V}O_2$ time constant for phase 2 ($\tau_2$) during treadmill CWR exercise was 75% longer in PAD patients than controls (Table 4, $p < 0.01$). In contrast, during CWR arm ergometry, the phase 2 $\dot{V}O_2$ time constant was similar in PAD patients and controls (Figure 1). Individual subject data are presented in Figure 2.

The amplitude parameter ($A_1'$) of $\dot{V}O_2$ during phase 1 was significantly lower in PAD subjects than in controls during both treadmill ($p < 0.05$) and arm CWR exercise ($p < 0.05$). The $\dot{V}O_2$ amplitude of phase 2 ($A_2'$) was also reduced in PAD during treadmill CWR exercise ($p < 0.01$) but not arm exercise. However, the total amplitude of $\dot{V}O_2$ (Atot) during treadmill and arm CWR exercise was similar between groups. In the seven PAD subjects whose $\dot{V}O_2$ kinetics were best fit with a 3-component model, the magnitude of the slow component (phase 3) accounting for approximately 20% of the total increase in end-exercise $\dot{V}O_2$.

*Limb Hemodynamics.* Resting blood flow measurements of the upper and lower limbs were not different between groups (Table 5). Peak RH Δ blood flow was reduced over 50% in the legs of PAD patients compared with control subjects ($p < 0.01$). In PAD, the upper extremity Δ blood flow was reduced 33% compared with control subjects ($p < 0.01$) despite equal brachial systolic pressures across the upper extremities in PAD patients. Whereas there were no differences in RH blood flow responses between the arms and legs of control subjects, patients with PAD had reduced RH blood flow responses in the legs compared with the arms ($p < 0.05$).

*Relationships between exercise and hemodynamic parameters.* There were no significant relationships between RH blood flow responses and the phase 2 ($\tau_2$) time constants for arm or treadmill CWR exercise within either group. Whereas there were slowed heart rate kinetics ($HR_{50}$) during CWR treadmill exercise in PAD patients, this was not correlated with the prolonged phase 2 time constants ($\tau_2$). Consistent with previous reports, no relationships between the ABI and exercise performance parameters were observed. However, a significant relationship was observed between the lower extremity Δ RH blood flow and treadmill peak $\dot{V}O_2$ in patients with PAD ($y= 0.22x + 10.98$, $R = 0.70$, $p < 0.03$). This relationship was not observed in the control group.

**Discussion**

Consistent with our hypothesis, as compared with healthy subjects, PAD patients with no cardiac or pulmonary dysfunction demonstrated a specific defect (slowed time constant) in pulmonary $\dot{V}O_2$ kinetics during leg but not arm exercise. A systemic abnormality in vascular reactivity (reduced RH blood flow responses) was demonstrated in both the arms and legs of PAD patients compared with control subjects. However, the

RH blood flow abnormality in PAD was not correlated with the $\dot{V}O_2$ kinetic responses of either the upper or lower extremity. These data suggest that the slowed PAD pulmonary $\dot{V}O_2$ kinetic responses appear localized to the lower extremities directly affected by the arterial occlusive disease process, and cannot be explained by a systemic defect in endothelial function (RH blood flow) nor by any systemic impairment in cardiac or pulmonary function. Thus, the altered $\dot{V}O_2$ kinetic responses to leg exercise are likely related to the peripheral impairment in $O_2$ delivery or oxygen utilization in the PAD-affected lower limbs.

Previous studies have described prolonged pulmonary $\dot{V}O_2$ kinetics in PAD patients during CWR treadmill walking (Auchincloss et al. 1980, Haouzi et al. 1997, Bauer et al. 1999, Barker et al. 2003). Auchincloss et al. attributed a lower one-minute $\dot{V}O_2$ in PAD to the limitation of lower limb blood flow (Auchincloss et al. 1980) while more recently we and others have suggested that peripheral muscle metabolic abnormalities may play a significant role in the $\dot{V}O_2$ kinetic impairment (Bauer et al. 1999, Barker et al. 2003). However, these studies did not evaluate the potential confounders of impairments in systemic $O_2$ delivery from cardiac or pulmonary disease, or the potential contributions of altered vascular function (reduced vascular reactivity), that occur in patients with atherosclerotic disease. The present study demonstrates that the abnormal pulmonary $\dot{V}O_2$ kinetics in PAD is specific to exercise of the lower limbs affected by the arterial occlusive disease process. Oxygen uptake kinetics of the exercising muscles reflect the inter-related influences of local muscle oxygen delivery, oxygen diffusion and mitochondrial oxygen consumption. As described by Barstow et al., impairment of $O_2$ delivery may influence the expression of pulmonary $\dot{V}O_2$ kinetics

(Barstow et al. 1990). However, in these modeling experiments (where muscle oxygen uptake was assumed to be normal), a reduction in $O_2$ delivery was associated with a paradoxical speeding of the primary exponential phase of pulmonary $\dot{V}O_2$ kinetics (phase 2) as $O_2$ extraction increases across the 'exercising' muscle (Barstow et al. 1990). Thus, our findings of slowed phase 2 time constants in PAD as compared with controls during treadmill exercise may not be consistent with a simple reduction in the rate of oxygen delivery but could also indicate a localized defect in oxygen diffusion or mitochondrial oxygen uptake that was not observed during arm exercise. Indeed, using magnetic resonance spectroscopy, Kemp and colleagues have previously suggested a significant impairment in muscle oxidative metabolism during calf-muscle exercise that could alter the kinetics of mitochondrial respiration in PAD (Kemp et al. 1995, Kemp et al. 2001). Taking into account these considerations, our findings confirm that the defect of slowed pulmonary $\dot{V}O_2$ kinetics during treadmill walking reflects the disease-associated abnormalities specific to the lower extremities in PAD.

Heart rate dynamics ($HR_{50}$) were slowed during treadmill walking exercise in patients with PAD. Moreover, although resting heart rates were similar between groups, PAD patients had a greater absolute increase in HR during fixed-rate treadmill walking than did control subjects. In contrast, the HR responses ($HR_{50}$ and $\Delta HR$) during arm exercise were not different between groups. In consideration that the $HR_{50}$ was not correlated with pulmonary $\dot{V}O_2$ kinetics during arm or leg exercise, it is unlikely that any alterations in the heart rate component of cardiac output affected the slowed $\dot{V}O_2$ kinetics during leg exercise. Rather, the slowed $HR_{50}$ during PAD leg exercise may have been related to their greater $\Delta HR$, or other factors.

Limb blood flow during reactive hyperemia increased above baseline in the arms and legs of PAD patients following supra-systolic cuff occlusion. Thus, all PAD patients demonstrated a functional limb blood flow reserve. However, the RH blood flow responses were reduced 33% in the arms and by 50% in the legs of PAD patients compared with control subjects. Impaired vascular reactivity, measured as flow-mediated vasodilation or reactive hyperemic blood flow, has been demonstrated in the lower (occluded) and upper (non-occluded) arterial circulations in PAD patients indicating a systemic defect in endothelial vasodilator function (Yataco et al. 1999, Brendle et al. 2001, Brevetti et al. 2003). Presumably, the reduction in arm reactive hyperemia response in PAD patients was not the result of arterial occlusive disease as systolic pressures were equal between the arms in the PAD patients. Moreover, no patient experienced muscle cramping or other evidence of muscle ischemia during arm exercise. Thus, we conclude that the reduction in the arm reactive hyperemic responses in the present PAD patients most likely reflected a systemic non-occlusive limitation in limb vascular reactivity related to their endothelial dysfunction.

The reductions in reactive hyperemic blood flow were not correlated with arm or leg pulmonary $\dot{V}O_2$ kinetics in either group. In the upper extremity of PAD patients, the lack of a direct correlation between RH blood flow and pulmonary $\dot{V}O_2$ kinetics suggests that these changes in vascular function alone were insufficient to compromise (i.e. slow) the arm $\dot{V}O_2$ kinetic response. Thus, in the present study we conclude that the PAD-associated systemic reduction in vascular reactivity alone does not significantly impair the pulmonary $\dot{V}O_2$ kinetic responses in these highly selected PAD patients.

Despite the inferences above, we cannot exclude the possibility that the arterial occlusions in PAD combined with the impaired vascular reactivity contributed to the slowed lower extremity $\dot{V}O_2$ kinetics. Dorigo and Bartoli previously described that reactive hyperemia responses in PAD are quantitatively less than the blood flow response immediately following exercise (Bartoli et al. 1979). Thus, our measure of reactive hyperemia likely does not elicit the same level of hyperemia achieved during exercise and could partially explain the lack of correlation with $\dot{V}O_2$ kinetic and peak arm responses. However, the PAD lower extremity reactive hyperemia responses did correlate with claudication-limited peak exercise performance. This result emphasizes the significance of limb atherosclerosis and impaired arterial flow in contributing to the limitation of peak exercise function in PAD (Green 2002). Moreover, the reactive hyperemic blood flow response may not only reflect the severity of the arterial obstruction, but also may be a surrogate for the magnitude of oxidative perturbation and altered regulatory processes that occur distal to the arterial obstruction in PAD. This could include metabolic dysfunction of the affected skeletal muscle mitochondria, which may relate to a portion of the mechanism of exercise limitation and claudication symptoms in PAD (Walker 1991). Thus, the correlation between reactive hyperemic measures and peak exercise performance in PAD may also suggest that the PAD exercise impairment is more than simply a flow limited phenomenon. This could explain why reactive hyperemia, but not resting blood flow or resting ABI measurements are correlated with peak exercise function. Clearly, the mechanism of exercise limitation in PAD is multifactorial, with contributions from both the blood flow limitation and the distal responses to the ischemic condition.

*Study limitations*.

Treadmill walking was employed in the current study to assess lower extremity pulmonary $\dot{V}O_2$ kinetics because walking is the mode of exercise that predominantly produces the symptoms of claudication pain and exercise intolerance in PAD. Whereas the arm CWR transitions were of low exercise intensity (below the individual's lactate threshold) for all subjects, the treadmill CWR exercise represented a higher relative percent of peak exercise performance in PAD (83% of claudication -limited peak $\dot{V}O_2$) than in control subjects (43% of peak $\dot{V}O_2$) at the same absolute treadmill work rate. This resulted in a heterogeneous exercise response during treadmill CWR exercise in PAD patients such that seven patients demonstrated an apparent $\dot{V}O_2$ slow component, consistent in healthy subjects with exercise in the heavy domain (i.e. > LT). Although there is evidence that heavy exercise may alter the phase 2 time constants from that observed during moderate exercise due to a potential $O_2$ delivery limitation in healthy subjects (Gerbino et al. 1996, Macdonald et al. 1997), others have described invariant or faster phase 2 time constants using similar modeling methods during heavy or severe exercise (Hughson et al. 2000, Ozyener et al. 2001, Scheuermann et al. 2003). From the present data, we cannot directly evaluate the influence of exercise intensity on the pulmonary $\dot{V}O_2$ kinetic responses in PAD. However, consistent with previous observations (Haouzi et al. 1997, Bauer et al. 1999), the phase 2 time constants during treadmill CWR exercise were slowed in PAD as compared with controls irrespective of modeling procedure. Moreover, the phase 2 time constants were similar in PAD patients that demonstrated a $\dot{V}O_2$ slow component (n=7) to those without a phase 3 $\dot{V}O_2$ increase (n=3) (subject means of 35.9 s. vs. 36.9 s, respectively).

A second limitation of the study is that we did not conduct imaging studies of the upper extremity circulation to exclude potential upper extremity occlusive disease. However, previous studies have suggested that significant arterial occlusive involvement occurs approximately 20 times less frequently in the arms than the legs in the PAD patient population (Laroche et al. 1976, Welling et al. 1981). Thus, our findings of preserved peak arm exercise capacity along with the absence of any symptoms of muscle ischemia during arm exercise, suggest that the arm exercise comparison with controls may provide a valid basis for evaluating potential systemic influences on pulmonary $\dot{V}O_2$ kinetics in PAD.

In conclusion, the present results show that in PAD patients without apparent cardiopulmonary disease, pulmonary $\dot{V}O_2$ kinetics are slowed during exercise of the affected lower extremities but not during exercise of the unaffected upper extremities compared with controls. That the leg pulmonary $\dot{V}O_2$ kinetic responses were slowed in these highly selected PAD patients supports the presence of a significant peripheral $\dot{V}O_2$ impairment that is localized to the PAD lower extremity and not related to their abnormal vascular reactivity. Local abnormalities distal to the lower extremity arterial occlusions in patients with PAD offer putative explanations for their slowed pulmonary oxygen uptake kinetics.

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**Table 1: Subject Characteristics**

| | Control | Range | *PAD* | Range |
|---|---|---|---|---|
| N | 10 | | 10 | |
| Age (years) | 63.1 ± 5.5 | [53-70] | 57.5 ±12.4 | [40-76] |
| Weight (kg) | 82.1 ± 12.5 | [70-100] | 80.3 ± 16.0 | [69-114] |
| BMI ($kg/m^2$) | 26.55 ± 4.06 | [22-31.7] | 26.53 ± 3.75 | [20-35.2] |
| Pack-years | 4.6 ± 9.6 | [0-15] | 41.1 ± 20.2** | [20-86] |
| Current Smokers | 0 | | 2 | |
| $FEV_1/FVC$ (%) | 79 ± 4 | [73-84] | 78 ± 7 | [70-92] |
| Ejection Fraction (%) | 67 ± 6 | [57-75] | 63 ± 7 | [56-71] |
| LOPAR (MET hrs/wk) | 264 ± 42 | [213-279] | 254 ± 26 | [212-289] |
| ABI | | | | |
| Best leg resting | 1.20 ± 0.09 | 1.09-1.23 | 0.89 ± 0.18 ** | 0.56-1.09 |
| - post-exercise | - | | 0.54 ± 0.33 § | |
| Worse leg resting | 1.17 ± 0.10 | 1.09-1.25 | 0.66 ± 0.12 ** | 0.51-0.93 |
| - post-exercise | - | | 0.32 ± 0.16 § | |

Values are mean± SD [Range]. PAD, peripheral arterial disease. LOPAR, Low Level Physical Activity Recall. ABI, Ankle-Brachial Index. ABI data include 2 unilateral subjects in PAD group. ** $p < 0.01$ for PAD vs. Control; § $p < 0.01$ for difference in ABI from resting to post-exercise.

**Table 2: Peak Exercise Characteristics**

| | Control | Range | PAD | Range |
|---|---|---|---|---|
| **Treadmill** | | | | |
| $\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$) | 30.1 ± 4.8 † | [22.7 - 35.6] | 15.6 ± 2.6 ** | [12.4 – 20.2] |
| RER | 1.14 ± 0.09 | | 1.00 ± 0.07 ** | |
| HR ($beats \cdot min^{-1}$) | 164 ± 12 | | 113 ± 18 ** | |
| $\dot{V}O_2$ at LT ($ml \cdot kg^{-1} \cdot min^{-1}$) | 19.3 ± 2.7 | | - | |
| ICT (seconds) | - | | 113 ± 63 | |
| ACT (seconds) | - | | 471 ± 201 | |
| **Arm Ergometry** | | | | |
| $\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$) | 18.2 ± 3.4 | [13.3 – 23.9] | 15.1 ± 4.1 | [10.0 – 21.4] |
| RER | 1.21 ± 0.09 | | 1.23 ± 0.09 | |
| HR (bpm) | 144 ± 17 | | 129 ± 16 | |
| $\dot{V}O_2$ at LT ($ml \cdot kg^{-1} \cdot min^{-1}$) | 10.7± 2.1 | | 9.7± 3.2 | |
| Peak Workload (w) | 97 ± 22 | | 73 ± 23 * | |

Values are mean ± SD [Range]. LT, lactate threshol; ICT, Time to onset of claudication symptoms; ACT, Claudication limited total walking time. * $p < 0.05$, ** $p < 0.0l$ for PAD vs. Control; † $p < 0.05$ for arm vs. leg.

**Table 3: Constant Work Rate (CWR) Exercise Characteristics**

| | Control | PAD |
|---|---|---|
| **Treadmill** | | |
| ICT (seconds) | - | 142 ± 72 |
| $HR_{50}$ time (seconds) | 19 ± 12 | 72 ± 41 ** |
| Δ HR (bpm) | 12 ± 9 | 20 ± 6 * |
| End Exercise $\dot{V}O_2$ ($ml \cdot min^{-1}$) | 1042 ± 228 | 1108 ± 197 |
| End Exercise $\dot{V}O_2$ (% of peak) | 43 ± 7 | 83 ± 14 ** |
| End Exercise RER | 0.87± 0.03 | 0.93 ± 0.05 ** |
| ***Arm Ergometry*** | | |
| Workload (watts) | 32 ± 8 | 26 ± 10 |
| $HR_{50}$ time (seconds) | 31 ± 17 | 49 ± 26 |
| Δ HR (bpm) | 19 ± 7 | 21 ± 7 |
| End Exercise $\dot{V}O_2$ ($ml \cdot min^{-1}$) | 830 ± 106 | 777 ± 147 |
| End Exercise RER | 0.98 ± 0.03 | 0.98 ± 0.06 |

Values are mean ± SD. All subjects exercised for 6-minutes. CWR treadmill exercise was performed at 2.0 mph, 4% grade. CWR arm ergometry was performed at 90% of lactate threshold. All PAD subjects experienced claudication symptoms during CWR treadmill testing. ICT, Time to onset of claudication symptoms during CWR treadmill walking. $HR_{50}$ time is time to achieve 50% of the heart rate response (ΔHR). ΔHR, 6-minute heart rate minus resting baseline. * $p < 0.05$, ** $p < 0.01$ for PAD vs. Control.

**Table 4: $\dot{V}O_2$ Kinetic Parameters for Constant Work Rate Exercise**

| | Treadmill | | Arm Ergometry | |
|---|---|---|---|---|
| | Control | PAD | Control | PAD |
| Resting $\dot{V}O_2$ (ml/min) | 342 ± 72 | 348 ± 68 | 305 ± 59 | 282 ± 50 |
| Phase 1 | | | | |
| n | 10 | 10 | 10 | 10 |
| $\tau_1$ (s) | 1.0 ± 1.0 | 1.3 ± 1.0 | 0.8 ± 0.9 | 0.9 ± 1.4 |
| $TD_1$ (s) | 1.1 ± 2.2 | 2.9 ± 3.5 | 0.5 ± 1.2 | 1.7 ± 2.5 |
| $A_1$' (ml/min) | 380 ± 117 | 258 ± 89 * | 198 ± 33 | 159 ± 47 * |
| Phase 2 | | | | |
| n | 10 | 10 | 10 | 10 |
| $\tau_2$ (sec) | 19.6 ± 3.5 † | 34.3 ± 9.2 ** | 32.5 ± 9.0 | 38.5 ± 7.5 |
| $TD_2$ (s) | 28.9 ± 9.2 | 34.9 ± 6.9 | 31.5 ± 8.3 | 32.7 ± 6.7 |
| $A_2$' (ml/min) | 746 ± 185 | 599 ± 176 ** | 511 ± 68 | 498 ± 126 |
| Phase 3 | | | | |
| n | | 7 | | |
| $\tau_3$ (s) | - | 329.9 ± 715 | - | - |
| $TD_3$ (s) | - | 156.4 ± 42.1 | - | - |
| $A_3$' (ml/min) | - | 117 ± 89 | - | - |
| Atot (ml/min) | 746 ± 185 | 694 ± 151 | 511 ± 68 | 498 ± 126 |

Values are mean ± SD. Refer to Figure 1 for definitions. τ Exponential time constant. TD, time delay. A', Amplitude of $\dot{V}O_2$ response. Phase 3 data are presented from 7 PAD patients that demonstrated a slow component of $\dot{V}O_2$. * $p < 0.05$, ** $p < 0.01$ for PAD vs. Control,; † $p < 0.05$ for arm vs. leg.

**Table 5: Limb Blood Flow**

| | Control | PAD |
|---|---|---|
| **Leg blood flow (ml/100cc tissue/min)** | | |
| Resting | 7.01 ± 1.57 | 5.86 ± 0.98 |
| Peak | 58.17 ± 11.73 | 26.60 ± 8.37 ** |
| Δ blood flow | 46.12 ± 17.08 | 20.73 + 8.31 **,† |
| **Arm blood flow (ml/100cc tissue/min)** | | |
| Resting | 9.24 ± 3.04 | 9.17 ± 1.73 |
| Peak | 60.01 ± 12.57 | 43.17 ± 9.33 ** |
| Δ blood flow | 50.79 ± 12.23 | 34.01 ± 8.57 ** |

Values are mean ± SD. Measurements were made at rest and during reactive hypermia. Rest, Peak and Δ blood flow are presented as sum of right and left limbs. Δ blood flow, (peak – resting flow). Data include 2 unilateral subjects in the PAD group mean. ** $p < 0.0l$ for PAD vs. Control; † $p < 0.05$ for arm vs. leg.

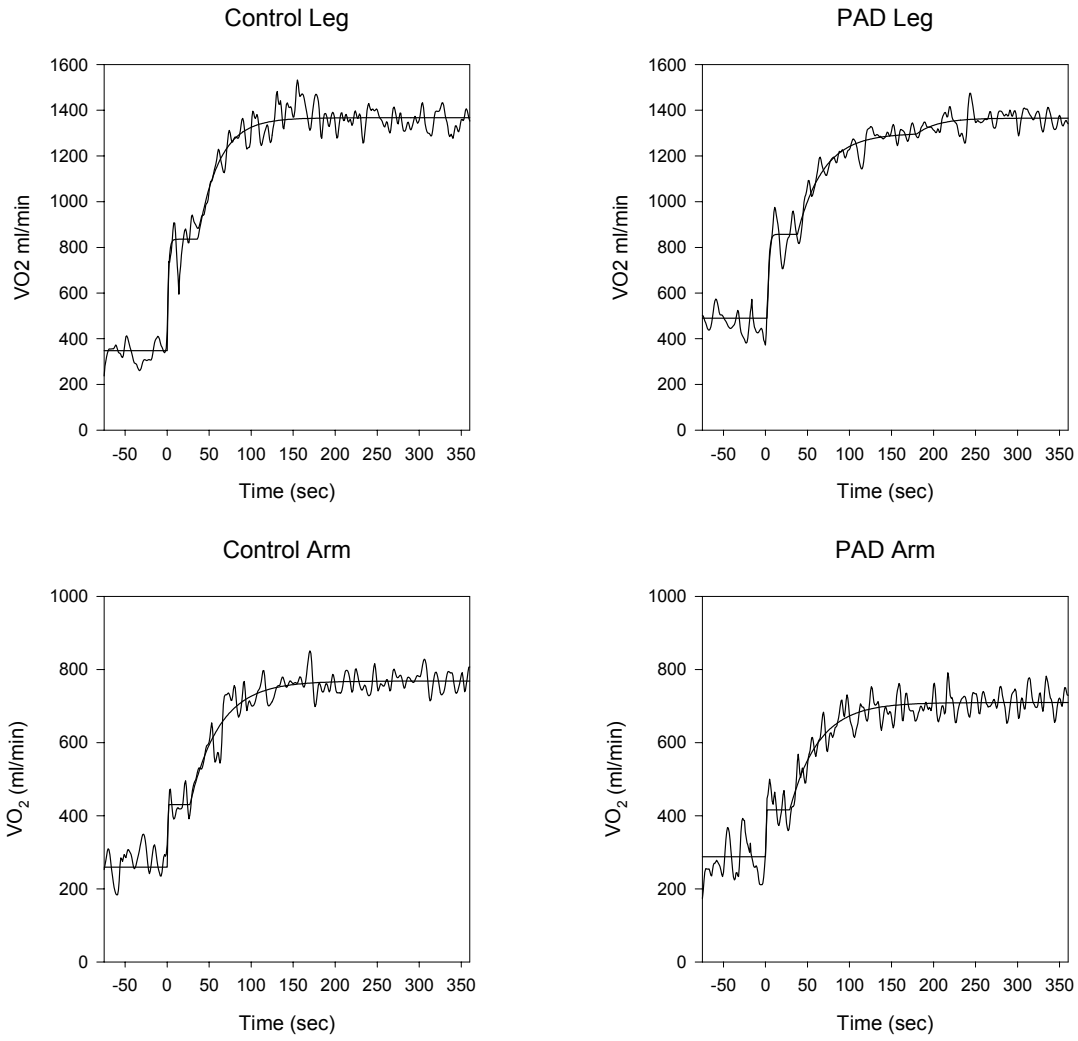


**Figure 1**. Comparison of pulmonary $\dot{V}O_2$ kinetic responses from a representative PAD patient and control subject during the transition from rest to treadmill (upper panels) and arm ergometry (lower panels) constant work rate (CWR) exercise. Treadmill exercise was performed at 2.0 mph, 4% grade, and arm ergometry was performed at 90% of individual LT. Exercise was initiated at time 0. Note presence of $\dot{V}O_2$ slow component and modeling of third component during PAD CWR treadmill exercise.

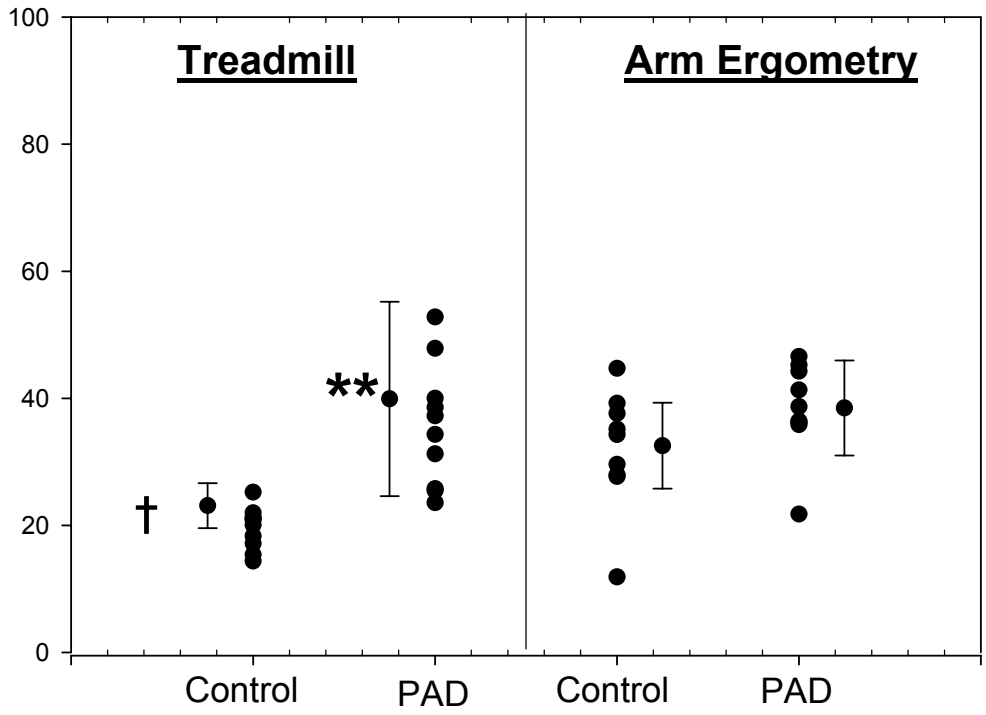


**Figure 2.** Group means (± SD) and individual data of pulmonary phase 2 $\dot{V}O_2$ time constants during the transition from rest to constant work rate exercise. **, $p < .01$ PAD vs. Control; †, $p < 0.05$ Arm vs. leg.

## Chapter 3

## Impaired Muscle Oxygen Use at the Onset of Exercise in Peripheral Arterial Disease [2]


### Abstract

In patients with peripheral arterial disease (PAD), abnormal muscle metabolism and impaired oxygen delivery distal to the arterial occlusions may contribute to the exercise limitation observed in this population. Muscle tissue hemoglobin saturation ($StO_2$), measured using near-infrared spectroscopy, reflects the relative contributions of oxygen delivery and oxygen utilization. Thus, differences in the kinetics of $StO_2$ in response to exercise may yield important insight into the potential mechanisms associated with the PAD exercise impairment. The purposes of this study were to (1) characterize the muscle oxygenation responses in patients with PAD and healthy controls at the onset of exercise and (2) compare the kinetics of $StO_2$ desaturation. We hypothesized that at the onset of exercise, the kinetics of $StO_2$ desaturation would be slowed in PAD compared with control responses. Six patients with PAD and 6 healthy controls from a university center were evaluated in a prospective cross sectional analysis evaluating the desaturation kinetics of $StO_2$ at the onset of walking exercise. On separate visits, subjects performed graded treadmill exercise and three constant work rate (CWR) treadmill tests equivalent to ~60% (Low), ~80% (Medium), and 100% (Peak) of their peak exercise work rate. Gastrocnemious muscle $StO_2$ response profiles (Inspectra tissue spectrometer) were measured at rest and across the rest to exercise transition. Muscle $StO_2$ responses were characterized by an exponential mathematical model. The endpoint value was taken as the time constant of $StO_2$ desaturation following the onset of exercise (e.g equivalent to


[2] Journal of Vascular Surgery 40:488-493, 2004.

the time to reach approximately 63% of the $StO_2$ decrease). The PAD patients and controls subjects were of similar age and activity level. The qualitative patterns of $StO_2$ responses at the onset of exercise were also similar between PAD and controls at all work rates. However, the kinetic time constants of $StO_2$ desaturation were prolonged in PAD versus controls (averaged time constant across all work rates, 21.9±9.4 seconds versus 4.9±2.2 seconds respectively, $p<.01$). The slowed muscle $StO_2$ kinetics in PAD are consistent with an impairment in muscle $O_2$ utilization at the onset of walking exercise. Impaired muscle metabolism may contribute to the altered physiologic responses to exercise and the exercise impairment in patients with PAD.

**Introduction:**

Patients with peripheral arterial disease (PAD) and claudication have a 50% reduction in peak exercise performance that limits daily walking activities and functional capacity (Hiatt et al. 1988). In PAD, atherosclerotic arterial occlusions limit blood flow and oxygen delivery to the working muscles of the leg during exercise. However, patients with PAD also have increased oxidative stress and a spectrum of muscle metabolic abnormalities which may have functional implications (Hiatt et al. 1992, Hickman et al. 1994, Brass et al. 2001).

Near-infrared spectroscopy (NIRS) has utility in monitoring local muscle hemoglobin oxygen saturation ($StO_2$) at rest and during exercise and recovery from exercise (Grassi et al. 2003). Because muscle $StO_2$ reflects the relative contributions of oxygen delivery and oxygen utilization, differences in the dynamic $StO_2$ response across transitions from rest to exercise may provide insight into the pathophysiology of the exercise limitation in PAD. For example, in many PAD patients, resting blood flow and the initial increment in blood flow with low levels of exercise is not limited (Sorlie et al.

1978). Thus, in the absence of a defect in tissue oxygen utilization, the dynamic $StO_2$ responses immediately following the onset of exercise should be similar between PAD and controls. However at higher work rates, patients with PAD cannot deliver sufficient blood flow and oxygen to meet muscle metabolic demand. Under these conditions, muscle metabolism would require a very rapid desaturation response as oxygen extraction increased in the absence of an adequate increase in oxygen delivery. In contrast, if PAD was associated with an impaired ability of muscle to utilize oxygen, the rate of hemoglobin desaturation at the onset of exercise would be slowed. Thus, quantitative differences in $StO_2$ kinetics at the onset of exercise would identify whether a defect in metabolic oxygen utilization contributes to the exercise pathophysiology of PAD.

**Methods:**

*Subjects:* Six men with PAD and six healthy men of similar age and body mass index were included in this study, which was approved by the Colorado Multiple Institutional Review Board, and all subjects gave informed consent. Patients with PAD were defined by resting ankle-brachial index (ABI) < 0.90 determined as the ratio of highest ankle systolic blood pressure (in each leg) to the highest brachial systolic pressure. The index leg was defined by the lowest ABI value in PAD subjects, and the dominant leg in controls. All PAD patients experienced the symptoms of claudication (pain or cramping in the muscles of the affected legs) that was the only limiting symptom during graded treadmill exercise. Subjects with diabetes or patients who were taking β-adrenergic antagonists were excluded as exercise responses are known to be altered by these factors (Petersen et al. 1983, Regensteiner et al. 1995). PAD patients on other medications were

not excluded, and the six studied PAD patients included five taking a statin, and all six were taking aspirin and antihypertensive drugs.

*Exercise Protocols:* Subjects performed a graded treadmill exercise test (Astrand Protocol or Gardner Protocol) to maximally tolerated workloads for the identification of peak exercise capacity (Q-stress, Quinton Instruments, Seattle, WA) (Astrand et al. 1977, Gardner et al. 1992). On subsequent days, subjects performed six-minute constant work rate (CWR) treadmill tests at workloads equal to approximately 60% (Low), 80% (Medium), and 100% (Peak) of their peak exercise work rate. Thus, the CWR exercise tests were individualized to induce a similar exercise intensity (relative to peak exercise capacity) and thus a measurable $StO_2$ desaturation response. Systemic pulmonary gas exchange measurements of oxygen consumption ($VO_2$), carbon dioxide production and minute ventilation (Medical Graphics, CPX/D, St. Paul, MN) were measured to quantify the relative exercise work rate of each CWR bout as previously described.(Bauer et al. 1999) Arterial hemoglobin saturation was monitored and recorded using pulse oximetry during all exercise tests (Ohmed Corp., Louisville, CO).

*Skeletal muscle hemoglobin saturation:* Non-invasive muscle tissue oxygen saturation ($StO_2$) was measured using a continuous-wave, near-infrared spectrometer (InSpectra$^{TM}$ Model 325, Hutchinson Technology, Inc, Hutchinson, MN). The NIRS signal is derived from the hemoglobin in the microvasculature (precapillary, capillary, and postcapillary) of the tissue sampled, although contribution from intracellular myoglobin cannot be definitively excluded. Thus, NIRS can be considered to reflect local tissue oxygenation. The InSpectra Tissue Spectrometer was modified for rapid sample measurement and data acquisition (6 Hz). Prior to each study, the InSpectra device was calibrated using a single

light scattering standard and validated against standard references equivalent to 38% and 90% hemoglobin saturation. Tissue (muscle) oxygen saturation ($StO_2$) was determined utilizing the second derivative of the absorbance as a function of wavelength at near infra-red wavelengths associated with changes in oxy- and deoxy-hemoglobin/myoglobin concentrations. The ratio of this second derivative at 720nm and 760nm has been empirically scaled to hemoglobin saturation, and is used by the instrument to calculate the reported $StO_2$ values (Hutchinson Technology, Inc., User and Service Manual, 2004). The optical data were acquired using a 25mm NIRS probe that was attached to the skin over the lateral gastrocnemious muscle of the index limb by an adhesive patch.

*Analysis of $StO_2$ kinetics***.** The 6 Hz $StO_2$ data for each individual CWR test were time-bin averaged to yield 1 Hz data files. Using a statistical graphing program (Sigmaplot, 2002), a two component mathematical curve-fitting model was employed to describe the $StO_2$ kinetic response using non-linear regression techniques.

$$StO_2(t) = StO_2(b) + A_1(1 - e^{-t-TD1/\tau1}) \quad (1)$$

$$+ A_2[1- e^{-(-t-TD2)/\tau2}] \quad (2)$$

In each curve fit (see Figure 1, panel B for graphical representation), $StO_2(b)$ was the resting baseline value. Following the onset of exercise, there was an initial time delay (TD1) followed by a primary desaturation in $StO_2$ (below baseline) that was described with an exponential time constant ($\tau1$) representing the time to reach ~63% of the desaturation response. The difference between the resting $StO_2$ and the nadir of the primary $StO_2$ desaturation was calculated as $A_1$. Where a subsequent $StO_2$ increase was observed, a second exponential function (equation 2) was employed such that the overall model would allow a best fit of the primary $StO_2$ desaturation response. Data for $A_2$,

TD2, and $\tau 2$, are not presented but contributed to the model which yielded the reported values for $A_1$, TD1, and $\tau 1$.

**Results:**

Patients with PAD were of similar age (66±7 years) as compared with controls (65±7 years) but had a lower ABI in the index leg (0.62±0.13 versus 1.24±0.08 in controls, $P<0.01$) and reduced peak exercise oxygen uptake (16.4±4.2 ml/kg/min) as compared with controls (26.0±4.2 ml/kg/min, $P<0.01$). No subject demonstrated arterial hemoglobin desaturation during any of the exercise testing.

The qualitative patterns of the initial $StO_2$ response were similar between groups during Low, Medium, and Peak CWR exercise (Figure 1). At the Low CWR, three healthy subjects and one PAD patient demonstrated an $StO_2$ profile with no desaturation below baseline levels (Figure 1. Panel A and Panel D). One control subject did not desaturate below baseline levels during Medium CWR exercise. The remaining control and PAD subjects exhibited a desaturation response at all work rates. Following the onset of exercise (for example, Figure 1, Panel B), there was an initial increase in $StO_2$ (characterized by TD1) which was followed by a rapid decrease in $StO_2$ to a plateau below baseline ($StO_2$ nadir). In control subjects, the nadir of $StO_2$ desaturation typically occurred within 40 seconds, followed by a slow phase of increase in $StO_2$. In contrast, the initial $StO_2$ desaturation was slower in patients with PAD, reaching a nadir at approximately 100 seconds into exercise (Figure 1. Panels E and F). The nadir of $STO_2$ was similar at the same relative work rates between PAD and control subjects, with the total decrease in $STO_2$ proportionate to increases in exercise intensity. However as previously reported, the magnitude of $STO_2$ desaturation at similar absolute work rates

was greater in PAD as compared with control subjects (Comerota et al. 2003). Specifically, the $StO_2$ desaturation with Peak CWR exercise in PAD patients was greater than observed during Low CWR exercise in controls (Table I) despite similar absolute work rates performed (1330±105 ml/min vs. 1354±219 ml/min PAD and Controls, respectively).

Control subjects had a short time delay and rapid $StO_2$ time constant ($\tau 1$) that was consistent between subjects and independent of work intensity (Table I). In contrast, PAD patients had a longer time delay and slowed $StO_2$ desaturation kinetics that displayed considerable heterogeneity between PAD subjects. Nonetheless, within the PAD cohort as a whole, or when analyzed on an individual subject basis, there was no relationship between $\tau 1$ and workload. The $StO_2$ time constant ($\tau 1$) was statistically different between PAD and controls at Low and Medium CWR ($P<0.05$) and tended to separate at Peak CWR ($P=0.14$). As the time constant did not vary as a function of increasing workload in individual subjects, the time constants for all CWR tests performed by an individual were averaged to yield the best estimate for this parameter for each subject. Analyzed in this manner, the time constant average was 4.9±2.2 seconds for controls and 21.9±9.4 seconds for PAD subjects ($n=6$ per group, $P<0.01$).

**Discussion:**

This study demonstrated that while muscle $StO_2$ responses at the onset of treadmill exercise were qualitatively similar between PAD and control subjects, the kinetics of $StO_2$ desaturation were markedly slowed in PAD patients. In both groups, a low relative work rate could be identified in selected subjects that was not associated with $StO_2$ desaturation below resting values. Thus, workloads could be identified in which

PAD patients appeared to have increases in blood flow which matched oxygen demand, resulting in no $StO_2$ desaturation. At higher relative work rates, all subjects had $StO_2$ responses that were characterized by an initial increase in $StO_2$ saturation, followed by a primary decrease in $StO_2$, the magnitude of which increased with increasing relative exercise intensity. In contrast, the $StO_2$ kinetic responses (time constants) in both groups appeared independent of the relative work rate and remained slowed even at similar absolute work rates, suggesting that the observed differences in $StO_2$ kinetic responses could not be explained solely on the basis of a difference in absolute work performed. Thus, whether the groups were compared at the same absolute or relative work rates, the time constants were clearly different, supporting the conclusion that the differences in $StO_2$ kinetics observed reflected the PAD population and not the absolute workloads evaluated.

Previous investigations have described a greater drop in muscle oxygenation during incremental exercise and slowed $StO_2$ dynamics in the recovery from exercise in PAD, attributing these differences from healthy subjects to the impaired hemodynamics of the PAD condition (McCully et al. 1994, Comerota et al. 2003). The current study confirms the greater decrement in $StO_2$ observed in PAD subjects at similar absolute work rates. However, the analysis of metabolic processes during recovery from exercise are complicated by the clear differences in blood flow between PAD and control subjects provoked by higher workloads, and the accumulation of lactate and other metabolic intermediates that can modulate cellular metabolism associated with the exercise bout. In contrast, the present study of the events associated with the onset of exercise begin with the near normal blood flow status of the resting muscle in the two groups. Thus, our approach provided a unique perspective on the interaction between the metabolic and

hemodynamic factors at the onset of exercise that may contribute to the exercise limitation in PAD.

Patients with intermittent claudication have relatively normal resting lower limb blood flow, and blood flow can increase with exercise (Sorlie et al. 1978). However as exercise continues, arterial occlusions restrict the exercise hyperemia, and oxygen delivery reaches a plateau (Sorlie et al. 1978). When increases in metabolic demand exceed increases in oxygen delivery, muscle oxygen extraction increases as reflected by a decrease in $StO_2$. Thus in PAD, a limitation in oxygen delivery at the onset of exercise would be expected to accelerate the rate of $StO_2$ desaturation within the muscle, provided that oxygen diffusion and mitochondrial function were normal. This response was not observed in this cohort of PAD patients (Figure 1, Table 1) despite reports of increased muscle capillary density, and thus potentially enhanced microvascular oxygen diffusion in this patient population (Hammarsten et al. 1980). In contrast, the slowed rate of $StO_2$ desaturation following the onset of treadmill exercise in PAD may be explained by the abnormalities of muscle oxidative metabolism previously described in this patient group. For example, patients with PAD have alterations in the regulation of oxidative ATP generation, abnormalities of electron transport chain enzyme activities, and an accumulation of short chain acylcarnitines in affected skeletal muscle that are inversely correlated with exercise performance (Hiatt et al. 1992, Brass et al. 2001, Kemp et al. 2001). The inherent delay to increase muscle oxidative metabolism in the face of increasing ATP requirements has been termed 'metabolic inertia' and has been hypothesized to be an important physiologic characteristic of the exercise response (Grassi et al. 1998, Greenhaff et al. 2004). These observations support the concept that patients with PAD develop a mitochondrial myopathy. Moreover, these findings support

previous reports of slowed pulmonary oxygen uptake kinetics in PAD (Bauer et al. 1999, Barker et al. 2004) and extend these observations to suggest that metabolic abnormalities specific to the affected skeletal muscle in PAD may contribute, in part, to the observed exercise limitation of this patient population.

Factors other than the balance between oxygen delivery and oxygen utilization may influence the integrated $StO_2$ measurement at the onset of exercise. Although the $StO_2$ signal is empirically scaled to hemoglobin saturation, the contribution of myoglobin cannot be definitively excluded and its proportion to the overall NIRS signal remains a topic of controversy. Without entering into this debate, the NIRS measurements can be considered to reflect local tissue oxygenation inclusive of hemoglobin and myoglobin. Thus, we consider our measurements to reflect a general view of muscle tissue oxygen saturation and cannot address interactions between microvascular and intracellular oxygen stores. The $StO_2$ measurement may also be influenced by the re-distribution of oxy- and deoxy-hemoglobin within the sample window representing a mixture of capillary and venuolar blood with different saturations (McCully et al. 2000). For example, at the onset of exercise, activation of the muscle pump was associated with a rapid decrease in the total absorbance of hemoglobin (tHb) (data not shown) and thus may, in part, explain the transient increase in muscle $StO_2$ observed immediately following exercise onset.

In patients with PAD, $StO_2$ desaturation kinetics were slowed at the onset of treadmill exercise, consistent with impaired muscle $O_2$ utilization. These findings provide further evidence of the interplay between the metabolic and hemodynamic factors that may contribute to the exercise impairment in patients with PAD. Muscle

metabolic function may be an important therapeutic target in PAD (Brass 1996, Greenhaff et al. 2004).

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**Table 1. Constant work rate $StO_2$ characteristics**

| | Control | | PAD | |
|---|---|---|---|---|
| **Low** | n=3 | | n=5 | |
| Work intensity (%peak $\dot{V}O_2$) | 58±15 | [43-73] | 68±17 | [48-90] |
| $StO_2$(b) | 50±10 | [38-57] | 53±10 | [42-60] |
| τ1 (s) | 4.0±0.9 | [2.9-4.6] | 24.0±2.9 † | [19.9-28.1] |
| TD1 (s) | 13.9±3.1 | [11.0-17.2] | 27.6±9.4* | [14.9-37.0] |
| A1 (%) | -18±5 | [13-23] | -22±3 | [14-26] |
| **Med** | n=5 | | n=6 | |
| Work intensity (%peak $\dot{V}O_2$) | 86±5 | [78-93] | 77±13 | [56-93] |
| $StO_2$(b) | 63±9 | [52-70] | 54±6 | [46-59] |
| τ1 (s) | 5.2±2.8 | [1.8-9.5] | 20.0±12* | [9.3-42.4] |
| TD1 (s) | 8.7±3.4 | [3.7-11.8] | 21.6±10.7* | [10.5-39.7] |
| A1 (%) | -38±10 | [20-48] | -32±14 | [17-56] |
| **Peak** | n=6 | | n=6 | |
| (100% peak $\dot{V}O_2$) | | | | |
| $StO_2$(b) | 66±14 | [50-89] | 55±10 | [37-64] |
| τ1 (s) | 5.7±3.1 | [2.4-11.0] | 19.2±18.6 ‡ | [6.7-53.5] |
| TD1 (s) | 8.3±4.0 | [5.3-15.5] | 13.2±11.2 | [0.0-29.9] |
| A1 (%) | -41±9 | [29-57] | -44±17 | [20-65] |

Values are mean±SD, [Range]. Three control subjects and one PAD subject had no desaturation during Low constant work rate exercise, and thus no kinetic parameters were calculated. $StO_2$(b), resting muscle oxygenation (%). τ1, $StO_2$ time constant. TD1, time delay. A1, amplitude of $StO_2$ response. * $p<0.05$, † $p<0.01$, ‡ $p=0.14$ for PAD vs. Control.

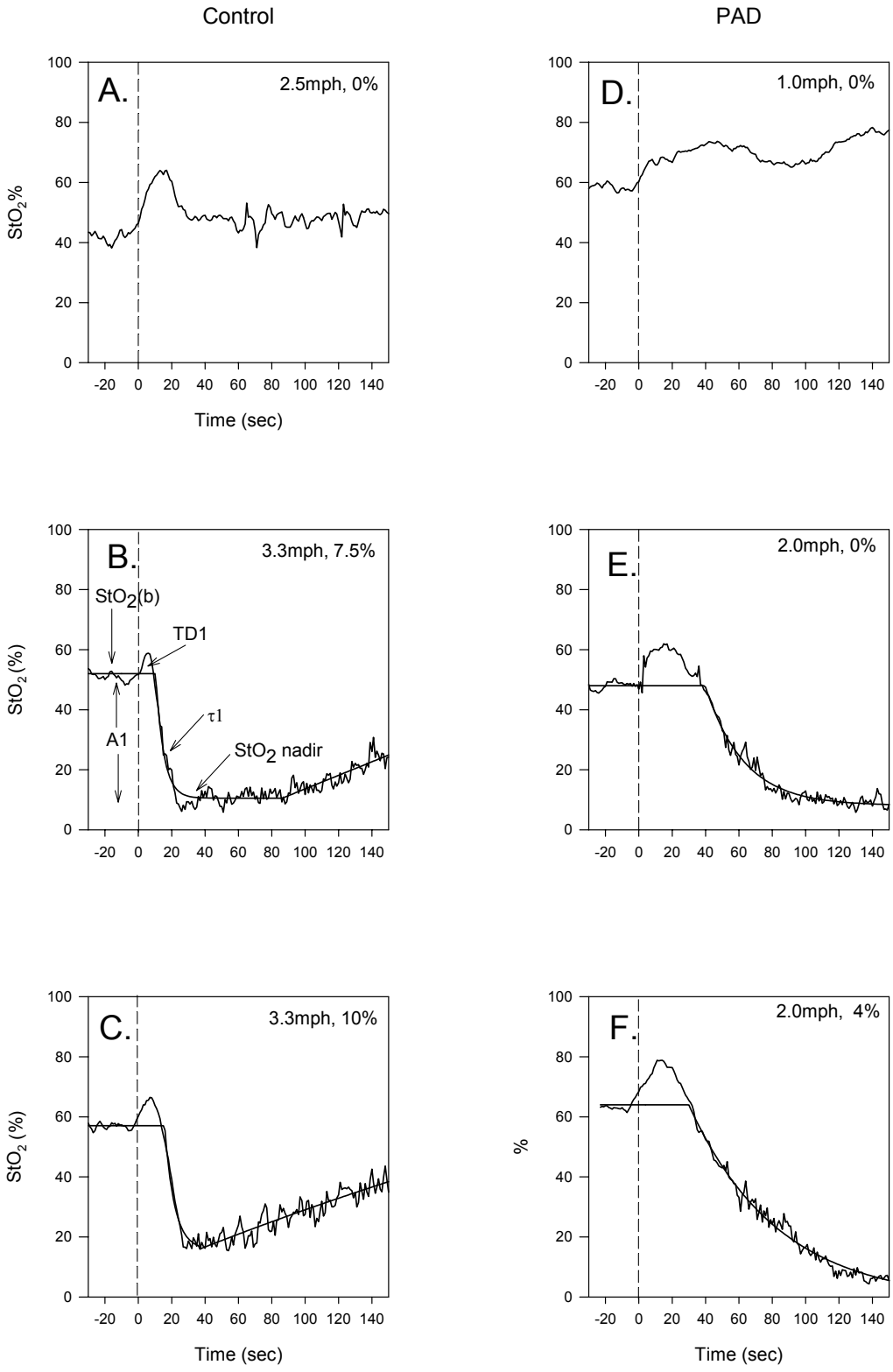


**Figure 1**. Muscle oxygen saturation responses ($StO_2$) from a representative PAD and control subject during Low (A, D), Medium (B,E) and Peak (C,F) constant work rate (CWR) exercise. Exercise was initiated at time 0 (vertical dashed line). During Low

CWR exercise, there was no decrease in $StO_2$. With the higher work rates, there was an initial increase, followed by a rapid decrease in $StO_2$. $StO_2$(b)=baseline, TD1=time delay (seconds) during the period of increase in $StO_2$, $A_1$=difference between the baseline and nadir of initial $StO_2$ desaturation, $\tau 1$=time constant of $StO_2$ desaturation.

## Chapter 4

## Muscle $StO_2$ Kinetics are Work Rate Dependent in Peripheral Arterial Disease[3]


### Abstract

In peripheral arterial disease (PAD), the relative influences of oxygen delivery limitation versus abnormal muscle metabolism on the impaired PAD exercise responses remain unresolved. Using near infrared spectroscopy, we tested the hypothesis that the kinetics of muscle hemoglobin desaturation ($StO_2$) are slowed following the onset of dynamic calf plantar flexion exercise across a range of work rates and end-exercise blood flow responses. Eight subjects with PAD and eight healthy subjects performed five constant work rate tests (1, 5, 10, 30, 50% of MVC). Muscle $StO_2$ was assessed continuously during exercise, and limb blood flow was measured at rest and immediately post-exercise using venous plethysmography. Absolute work rates across the range of exercise transitions were similar between groups. In PAD, the $StO_2$ time constants were slower than controls during 5% MVC (13.5±1.7 vs. 6.9±1.2) and 10% MVC work rates (14.5 ±2.7s vs. 6.8 ±1.1s). However the $StO_2$ time constants became faster in PAD subjects during 30% and 50% MVC work rates and did not differ from control subjects. Post-exercise calf blood flows were similar between groups for 1%, 5%, and 10% MVC exercise, but were significantly lower in PAD subjects compared with controls for 30% and 50% MVC exercises. An inverse correlation was found between the 10% MVC $StO_2$ time constant ($\tau 1$) and peak oxygen uptake during graded treadmill exercise in PAD subjects ($R=-0.88$, $p< 0.01$). In summary, muscle $StO_2$ kinetics demonstrate a work rate dependence following the onset of calf exercise in PAD compared with healthy older


[3] Submitted for publication

subjects. Whereas muscle $StO_2$ kinetics are slowed during lower intensity exercise in PAD, the muscle $StO_2$ responses were faster during higher intensity exercise where blood flow limitation was manifest. These data suggest that the abnormal dynamic relationship between muscle oxygen utilization and oxygen delivery observed at low work rates in PAD may be modifiable with higher work intensity.

**Introduction**

The onset of exercise is associated with an immediate demand for energy which is initially met by pre-formed high-energy phosphates (creatine phosphate and ATP). However, sustained exercise requires the production of ATP via oxidative phosphorylation. The increase in oxidative phosphorylation (and hence oxygen consumption) does not meet the ATP demand instantaneously, but is accelerated as metabolic intermediates (including free ADP, creatine, and inorganic phosphate) accumulate during the earliest phase of the rest-to-exercise transition (Whipp and Mahler M. 1980, Barstow et al. 1994, Rossiter et al. 1999). The time course of accelerating aerobic ATP production and oxygen consumption following the onset of exercise has been associated with a 'metabolic inertia' that is determined, at least in part, by the inherent activation, sensitivity, and capacitance of the muscle mitochondria (Whipp and Mahler M. 1980, Meyer 1988, Grassi et al. 1996). However, once initiated, maintaining optimal rates of oxidative phosphorylation with continued exercise will be dependent on adequate oxygen delivery via enhanced exercise blood flow.

In patients with peripheral arterial disease (PAD), blood flow limitations during exercise due to lower extremity atherosclerotic arterial stenoses and occlusions are of primary pathophysiologic importance. However, there are also alterations in skeletal muscle mitochondrial function secondary to chronic, repetitive ischemic insults that

appear to be of functional significance (Hiatt et al. 1992, Brass et al. 2001). Thus, the mitochondrial dysfunction of PAD skeletal muscle may promote a greater degree of metabolic inertia during exercise transitions. As metabolic inertia has been hypothesized to be a major determinant of the muscle oxygen utilization kinetics following the onset of moderate exercise in healthy subjects (Whipp and Mahler M. 1980, Grassi et al. 1996), an alteration in the magnitude of metabolic inertia in PAD would provide insight into the functional consequences of the described metabolic abnormalities in PAD-affected muscle (Hiatt et al. 1992, Kemp et al. 1993, Brass et al. 2001)

One approach to address this question is through the dynamic assessment of hemoglobin desaturation in skeletal muscle during the transition from rest to exercise using Near Infrared Spectroscopy (NIRS) (Shoemaker et al. 1997, Grassi et al. 2003, DeLorey et al. 2003). As hemoglobin saturation in perfused muscle ($StO_2$) is dependent upon the balance between local oxygen delivery and oxygen utilization, it has been suggested that NIRS-derived $StO_2$ may reflect muscle oxygen extraction as described by the Fick principle (Grassi et al. 2003). Thus, the kinetics of $StO_2$ following the onset of muscle contractions may provide a unique perspective into the kinetics of muscle oxygen utilization (and magnitude of metabolic inertia) relative to blood flow adjustments in health and cardiovascular diseases.

Consistent with these concepts, previous work has shown that both pulmonary oxygen uptake kinetics and calf muscle hemoglobin desaturation kinetics are slowed following the onset of treadmill exercise in patients with PAD compared with control subjects (Bauer et al. 1999, Bauer et al. 2004a). However, others have reported an accelerated rate of hemoglobin desaturation associated with PAD, which could suggest that oxygen utilization responses in the muscle are preserved (Kemp et al. 2001). To

date, the relative influences of oxygen delivery limitation versus abnormal muscle metabolism on the altered NIRS and impaired oxygen uptake responses in PAD remain unresolved (Brass et al. 2004).

The current study was designed to test the hypothesis that hemoglobin desaturation kinetics are slowed in PAD patients using a well-defined exercise protocol that functionally isolates the gastrocnemius muscle. The protocol allowed $StO_2$ kinetics to be assessed as a function of muscle work rate, and also allowed post-exercise blood flow to be quantified. The results confirmed that hemoglobin desaturation kinetics are slowed in patients with PAD performing lower intensity exercise, but that the kinetics were unexpectedly work rate dependent. At high work rates, $StO_2$ kinetics were not different between PAD and control subjects, suggesting the possibility that the greater degree of metabolic inertia associated with PAD could be overcome at high intensity work rates.

**Methods**

*Subjects.* Eight male subjects with PAD and eight healthy men without clinical cardiovascular disease were enrolled in this study. All subjects provided informed consent, and the study was approved by the University of Colorado Multiple Institutional Review Board in accordance with the Declaration of Helsinki.

For all subjects, the following exclusion criteria were followed: 1) a history of coronary artery disease, previous myocardial infarction or coronary revascularization, angina, stroke, congestive heart failure, or diabetes mellitus 2), hematology or blood chemistry laboratory values outside of normal limits, 3) evidence of impaired pulmonary function ($FEV_1/FVC < 0.70$), or 4) evidence of ECG changes suggestive of cardiac ischemia during graded exercise testing. Additionally, subjects with skin and

subcutaneous thickness greater than 15mm (by ultrasonography) over the muscle region of interest were excluded from the study, as increased skin and non-muscle tissue thickness may influence NIRS signals (van Beekvelt et al. 2001). Subjects taking β-adrenergic antagonists were excluded as exercise responses are known to be altered by these medications (Petersen et al. 1983).

Peripheral arterial disease was defined by resting ankle-brachial index (ABI) < 0.90 defined as the ratio of the highest ankle systolic blood pressure (in each leg) to the highest brachial systolic pressure. The index leg in PAD patients was individually defined as the leg with the lowest ABI. All PAD subjects experienced the symptoms of claudication (pain or cramping in the muscles of the affected legs) that was the only limiting symptom during graded treadmill exercise. Healthy, similarly-aged control subjects had a normal physical examination without signs of cardiovascular disease, a resting ABI > 1.00 in each leg at rest, and no history of PAD. All control subjects were sedentary, as defined by not participating in a regular exercise program (< 1 episode of exercise/week). The dominant leg was taken as the index leg for control subjects.

*Exercise Protocol*: Subjects reported to the laboratory on three different occasions. At the first visit, subjects were familiarized with single leg plantar flexion exercise using a dynamometer (KinCom, Chattanooga, TN), and each individual's maximal voluntary contraction (MVC) was assessed in the supine position (Newtons). Following a rest period, subjects performed a graded treadmill exercise test (Astrand or Gardner protocol, control and PAD respectively) to maximal voluntary exhaustion for the determination of peak exercise capacity (peak oxygen uptake) as previously described (Bauer et al. 2004b).

On a subsequent visit, subjects performed three, 3-minute, single leg plantar flexion exercise transitions from rest to constant work rate exercise corresponding to 1%, 5% and 30% of the individual's maximal voluntary contraction. On a separate visit, subjects performed two exercise transitions from rest to plantar flexion exercise of 10 and 50% of MVC. The dynamometer was adjusted for isotonic contractions of the posterior calf muscles from 0 to 15º of plantar flexion. All constant work rate transitions were performed in the supine position with the knee of the index leg at full extension. Each constant work rate transition consisted of a resting period to establish a stable baseline $StO_2$ and calf blood flow, followed by 3-minutes of contractions at the pre-determined load force (%MVC). Contraction frequency was maintained at 0.5 Hz with the aid of an audible metronome. Immediately following the last contraction of each 3-minute transition, post-exercise calf blood flow was measured and continually repeated throughout recovery using strain gauge plethysmography. A resting recovery period of at least 25 minutes separated each constant work rate bout and subsequent exercise was only performed when calf blood flow and $StO_2$ had returned to pre-exercise values.

*Tissue oxygen saturation ($StO_2$).* Tissue oxygen saturation was measured using a continuous-wave, near-infrared, four wavelength (680, 720, 760, and 800nm) spectrometer (InSpectraTM Model 325, Hutchinson Technology, Inc, Hutchinson, MN) as described previously (Bauer et al. 2004a). The $StO_2$ signal is primarily derived from hemoglobin in the microvasculature (pre-capillary, capillary, and post-capillary) of the tissue sampled, although the contribution from intracellular myoglobin in the region of optical interrogation cannot be definitively excluded. Thus, $StO_2$ is considered to reflect local tissue oxygenation without the ability to discern microvascular vs. intracellular oxygen stores.

The optical data were acquired with a probe positioned over the lateral gastrocnemius muscle of the index leg. Anatomic localization of the $StO_2$ probe was determined prior to study and was marked for identical placement for all subsequent visits. The $StO_2$ probe was designed with a fixed distance from the LED light source to photodetectors of 25mm. The probe was firmly attached to the skin over the tissue region for optical interrogation by an adhesive patch that eliminated contamination by ambient light. The spacing between the light source and photodetectors provided light attenuation measurements of tissue depth averaging approximately 12mm. Because substantial subcutaneous tissue may influence near infrared light absorption of the skeletal muscle (van Beekvelt et al. 2001), muscle tissue depth was measured by 2D ultrasonography (VingMed, General Electric, Milwaukee, WI) using digital calipers from the surface of the skin to the superficial muscle fascia border.

The $StO_2$ measurement was calculated as percent oxygen saturation from the measured attenuation of four near infrared wavelengths (680, 720, 760 and 800nm) as previously described in detail (Myers et al. 2005). Briefly, the second derivative attenuation as a function of wavelength was calculated to correct for baseline differences, and the ratio of second derivative attenuations at 720 and 760 nm ($2D_{720}/2D_{760}$) was empirically scaled with pure blood hemoglobin oxygen saturation from a tabled calibration relationship as previously described (Myers et al. 2005). This method offers an advantage over traditional near infrared spectroscopy systems (Runman, OMRON) in that quantitative tissue oxygen saturation (%) can be assessed without the determination of tissue scattering coefficients, absolute changes in oxy- and deoxyhemoglobin concentrations, or differential path lengths. Further, the method is experimentally robust

to changes in tissue blood volume and has been validated with human hemoglobin oxygen saturation *in vivo* during limb ischemia and exercise (Myers et al. 2005). Prior to each visit, the InSpectra device was calibrated using a single light scattering standard and the $StO_2$ measurement validated by optical references equivalent to 38% and 90% hemoglobin saturation. The InSpectra Tissue Spectrometer was modified for rapid sample measurement and data acquisition (6 Hz) to a dedicated computer.

*Calf blood flow and blood pressure measurements.* Calf blood flow was measured in the supine position using venous occlusion strain gauge plethysmography (D.E. Hokanson Inc. Issaquah, WA) at rest, immediately post-exercise, and during recovery as described (Dorigo et al. 1980, Bauer et al. 1999). Briefly, a mercury-in-silastic strain gauge was placed around the widest part of the calf and a rapid inflating pneumatic cuff was placed just above the knee. Venous occlusion was achieved by pneumatic cuff inflation to ~35mmHg and maintained for several (4-6) cardiac cycles to obtain blood flow measurements. Calf blood flow is expressed as ml of flow/100ml of tissue/minute. Resting blood flow was calculated as the average of 5 separate measurements. Immediate post-exercise and recovery blood flows are presented as individual readings, with peak flow determined as the highest flow post-exercise. Ankle and arm systolic blood pressures were determined using a Doppler ultrasonic instrument (model 841, Parks Medical Electronics, Beaverton, OR) as described previously (Bauer et al. 1999).

*Pulmonary gas exchange, heart rate, and arterial saturation.* Pulmonary gas exchange (oxygen uptake and carbon dioxide production) was measured during graded treadmill exercise testing using a metabolic measurement system (CPX/D, Medical Graphics Corporation, St. Paul, MN) as previously detailed (Bauer et al. 1999). The highest 20

second-averaged value for pulmonary oxygen uptake was taken as peak oxygen uptake. Heart rate data was continuously recorded from an integrated electrocardiography system (Q-stress, Quinton Instruments, Seattle, WA). Arterial hemoglobin saturation was monitored and recorded every 30 seconds during rest, exercise, and recovery of all experiments (graded and constant work rate testing) by an oximeter placed on the index finger of the dominant hand (Ohmeda Corp., Louisville, CO).

*Data Analysis.* The 6 Hz sampled $StO_2$ data for each constant work rate test were averaged into 1s time bins using a proprietary software program (NIRPro, University of Colorado Health Sciences Center, Denver, CO). Using a statistical graphing program (Sigmaplot, 2002), an exponential mathematical model was fit from baseline to the $StO_2$ nadir using non-linear regression techniques as described previously (Bauer et al. 2004a).

$$StO_2(t) = StO_2(b) + A_1(1 - e^{-(t-TD1)/\tau 1}) \quad (1)$$

For each individual exercise response, $StO_2(b)$ was the resting baseline value. Following the onset of exercise, there was an initial time delay (TD1) followed by the primary fall in $StO_2$ that was described by the time constant ($\tau 1$). The difference between the resting $StO_2$ and the nadir of the $StO_2$ decrease was calculated as $A_1$. The mean response time (MRT) was calculated as the sum of the $StO_2$ time constant ($\tau 1$) and time delay (TD1).

*Statistical Analysis.* Subject characteristics were compared using Student's t-tests. Repeated-measures ANOVA was employed for comparisons between and within groups for all variables obtained during constant work rate testing (NCSS 2000, Kaysville, UT). Where a significant F statistic was observed, Tukey-Kramer multiple comparison analyses were performed to determine the pairing of significant differences. Linear regression was performed for the determination of the blood flow/power output

relationships. The Pearson's R product was used to evaluate significant correlations. Statistical significance for all comparisons was declared at $p < 0.05$. Data are presented as mean ± S.E.M.

**Results**

There were no differences between control and PAD subjects in age or absolute MVC force (Table 1). Typical of subjects with intermittent claudication, the ABI of the index leg and treadmill peak pulmonary oxygen uptake in PAD subjects were reduced compared with controls ($p < 0.01$) (Hiatt et al. 1988). All PAD subjects were taking medications indicated for the treatment of cardiovascular disease, and four PAD subjects were taking medications indicated for the treatment of hypertension. Two control subjects were treated with statin medications for hyperlipidemia. Mean skin and subcutaneous tissue thickness of the region of interest for PAD and control subjects were 5.76± 0.82mm and 5.72±1.16mm respectively. The measurement of arterial hemoglobin saturation revealed no changes in any subject during graded or constant work rate exercise testing.

*$StO_2$ kinetics.* The dynamic $StO_2$ responses from a representative PAD and control subject across all exercise work rates are presented in Figure 1. This figure demonstrates that at very low work rates, both patients and controls had no change in $StO_2$, indicating a balance between oxygen supply and oxygen demand leading to no net decrease in $StO_2$. However at higher work rates, both PAD and control subjects had a progressive decrease in $StO_2$ that reflected the greater work intensities which reached a new steady-state after 20-40 seconds. The kinetic profiles of $StO_2$ dynamics were also qualitatively similar between PAD and control subjects. All subjects had a time delay from the onset of

exercise to the initial $StO_2$ decrease, following which a single exponential model was fit to the data as presented in Figure 2. In some subjects, a second phase of $StO_2$ desaturation was observed after the initial nadir that occurred approximately 80-100 seconds following the onset of exercise, was slower and heterogeneous between subjects, characterized by an increase in some, or further decrease in others. This phase was excluded from exponential modeling in order to describe the primary $StO_2$ response. Additionally, mathematical modeling could not be performed for some subjects at 1% and 5% MVC work rates as $StO_2$ did not decrease below baseline values (Table 2). Within control subjects, the $StO_2$ time constant ($\tau 1$) did not vary as a function of increasing work rate ($p>0.05$, Table 2) ranging from 6.9±1.2 seconds at 5% MVC to 7.5±1.2 seconds at 50% MVC. However, the time delay (TD1) prior to the primary fall in $StO_2$ became shorter with increasing work rates ($p<0.05$). Post-hoc analyses indicated that the time delay at 30% and 50% MVC was significantly shorter than at 5% and 10% MVC constant work rate transitions. As a consequence, the mean response time (MRT) decreased with constant work rate exercise of increasing percent MVC. The magnitude of $StO_2$ desaturation generally became greater with increasing work rates ($p<0.05$), with post-hoc analyses indicating the amplitude of the primary $StO_2$ response (A1) was significantly greater at 30% and 50% than at 5% or 10% MVC exercise transitions. Within PAD subjects, the $StO_2$ time constant ($\tau 1$) was altered as a function of increasing work rate ($p<0.05$). Post-hoc analyses revealed significantly slower $StO_2$ time constants for PAD subjects at 1% , 5% and 10% than at 30% or 50% MVC constant work rate exercise ($p<0.05$, Table 2). As in controls, the time delay (TD1) became shorter with increasing constant work rates in PAD ($p<0.05$). Post-hoc tests indicated that the time delays at 30% and 50% MVC were significantly shorter than for 1%, 5%, or 10% MVC

transitions. The MRT decreased with increasing % MVC exercise, similar to that observed in control subjects. The magnitude of the primary $StO_2$ desaturation became greater with increasing exercise work rates ($p<0.05$), with post-hoc tests revealing that hemoglobin desaturation (A1) was significantly greater at 10%, 30%, and 50% than for 5% or 1% MVC exercise transitions.

Comparing control with PAD subjects, baseline $StO_2$ was not different for any work rate. The $StO_2$ time constants at 5% and 10% MVC exercise were significantly slowed in PAD subjects compared with controls ($p<0.05$, Table 2). No differences between groups were observed for the $StO_2$ time constants at 30% or 50% MVC exercise. There were no differences in time delay between groups at any work rate. The MRT was longer in PAD subjects compared with controls at 5% and 10% MVC transitions ($p<0.05$), but no differences in MRT were observed between groups for 30%, and 50% MVC transitions. The magnitude of the primary $StO_2$ desaturation in PAD subjects was significantly greater than controls at 10% MVC exercise ($p<0.05$) and tended to be greater at 30 and 50% MVC exercises although these differences did not reach statistical significance.

*Calf blood flow responses.* Resting calf blood flow was not different between groups ($p>0.05$, Figure 3A). There were no differences in post-exercise blood flow responses between PAD and controls at 1%, 5%, or 10% MVC exercise. However, the post-exercise blood flow of PAD subjects was significantly reduced compared with controls at 30% and 50% MVC exercise ($p<0.05$). The difference in blood flow between groups was 8.6 ml/100mltissue/min at 30% and 21.8 ml/100mltissue/min at 50% MVC exercise. The slope of the leg blood flow/power output relationship was not different between PAD and control subjects compared across 1, 5, and 10% MVC work rates (PAD 2.6± 0.55 vs.

control 2.8±0.55 ml/100mltissue/min · watt$^{-1}$, p>0.05, Figure 3B). However above 4 watts (30% and 50% MVC), the slope of the leg blood flow/power output relationship decreased in PAD (2.0±0.40 ml/100mltissue/min · watt$^{-1}$), but this did not reach statistical significance (p=0.14). This contrasts with the controls in which the blood flow-work relationship was relatively constant or tended to become steeper when 30% and 50% MVC work rates were included in the regression.

*Correlations.* There was a significant positive correlation between the post exercise blood flow at the 50% MVC work rate and whole body peak oxygen uptake (ml/min) in the PAD group (R = 0.82, p<0.05). A significant inverse correlation was observed between the time constant ($\tau$1) of $StO_2$ during 10%MVC exercise and peak oxygen uptake (ml/min) in PAD subjects (R=-0.88, p<0.01).

**Discussion**

The present study demonstrated a work rate dependence of muscle hemoglobin desaturation responses ($StO_2$ kinetics) during exercise in subjects with PAD that was not observed in healthy controls. At lower work rates, and under conditions of normal increase in end-exercise calf blood flow, the PAD $StO_2$ kinetics were slowed compared with control subjects. In contrast, with higher work rate intensities where end-exercise blood flow did not increase to the same degree as control subjects, PAD $StO_2$ kinetics became faster and similar to the control $StO_2$ kinetic responses. Thus, in PAD subjects, an exercise state characterized by a high relative exercise intensity and inability to increment end-exercise blood flow to the level expected for the work demand was associated with speeded $StO_2$ kinetics.

Previous studies have demonstrated that pulmonary oxygen uptake kinetics are slowed in PAD patients following the onset of exercise (Bauer et al. 1999, Barker et al.

2004, Bauer et al. 2004b). Further, there is an inverse correlation between pulmonary oxygen uptake kinetics and peak exercise performance in PAD, suggesting that the determinants of oxygen uptake at the beginning of exercise in PAD may have functional significance (Barker et al. 2004). The slowed pulmonary oxygen uptake kinetics in PAD could not be explained by the severity of the arterial disease as defined by resting ankle-brachial index, number of limbs involved (Bauer et al. 1999), reactive hyperemic limb blood flow, or by systemic cardiovascular abnormalities (Bauer et al. 2004b). These observations raised the possibility that slowed pulmonary oxygen uptake kinetics could reflect a limitation in skeletal muscle oxygen uptake independent of an oxygen delivery impairment due to arterial disease. Numerous examples of abnormal skeletal muscle metabolism in the affected legs of patients with PAD support this concept. For example, aerobic metabolism is abnormal in PAD-affected skeletal muscle resulting in the accumulation of short chain acylcarnitines that are inversely correlated with peak exercise performance (Hiatt et al. 1992). The skeletal muscle mitochondria of patients with PAD demonstrate numerous histological irregularities that may be associated with impaired function (Teravainen and Makitie 1977). Several key complexes in the electron transport chain have also been shown to exhibit decreased activities relative to citrate synthase activity (Brass et al. 2001). Moreover, the initial rate of oxidative ATP synthesis following the onset of exercise appears reduced in PAD skeletal muscle (Kemp et al. 2002). Thus, changes in PAD skeletal muscle metabolism may result in a reduced ability to increase oxygen utilization in response to submaximal metabolic demands. Indeed, such changes could contribute to the magnitude of metabolic inertia of oxidative metabolism in PAD-affected skeletal muscle, resulting in impaired dynamics of muscle oxygen extraction that are particularly evident when resting blood flow and initial

increments in limb blood flow during submaximal exercise are normal (Pernow et al. 1975, Bernink et al. 1982).

The present finding of slowed $StO_2$ kinetics in PAD muscles during lower intensity work rates is consistent with the notion that the initial increase in muscle oxygen utilization is impaired relative to local oxygen delivery in PAD following the onset of exercise. These observations in PAD muscles during lower intensity calf work rates are consistent with our previous findings of slowed $StO_2$ kinetics during submaximal treadmill exercise (Bauer et al. 2004a). Moreover, the similarity between end-exercise calf blood flows in PAD and control subjects at the same relative and absolute lower intensity work rates strengthens the possibility of abnormal oxygen utilization in PAD muscle when exercise blood flow responses may not be impaired, although the kinetics of muscle blood flow immediately following the onset of exercise in PAD limbs remains unresolved. If true, this would imply altered $QO_2$ to $VO_2$ matching in PAD muscle. Nevertheless, as $StO_2$ measurements provide an indirect assessment of the dynamic balance between oxygen delivery and oxygen utilization in muscle, the presence of a time delay and prolonged desaturation kinetics of $StO_2$ in PAD support the concept of an intrinsic impairment to increase oxygen utilization relative to oxygen delivery following the onset of exercise in PAD skeletal muscle, at least during low levels of calf exercise. These observations are also consistent with direct measurements of intramuscular $PO_2$ during exercise contractions in PAD, which indicated slower kinetics in PAD-affected calf muscles compared with controls during exercise of similar absolute decreases in muscle $PO_2$ (Holm and Bylund-Fellenius 1981).

An interesting finding was that $StO_2$ kinetics became faster in PAD subjects at higher calf exercise intensities. The work rates at which $StO_2$ kinetics accelerate in PAD

subjects were the same at which the absolute post-exercise blood flows were less than those observed in control subjects. Thus, these work rates appear to be associated with an oxygen utilization/oxygen delivery mismatch at the level of the skeletal muscle secondary to the obstructive arterial lesions associated with PAD. This contrasts with the apparent work rate independence of muscle hemoglobin desaturation kinetics observed during submaximal treadmill exercise (Bauer et al. 2004a) or across cycling work intensities in healthy subjects (Grassi et al. 2003, Shibuya et al. 2004), a factor that may be explained by the different exercise conditions of the current investigation. Indeed, the present study employed a different exercise from treadmill walking or cycling, consisting of isolated calf muscle contractions in the supine position. At the highest work rates, the force of calf muscle contraction (%MVC) likely exceeded that experienced even during peak treadmill exercise (Cavagna 1975). Accordingly, calf blood flow responses may have become more limited in PAD subjects given the high contractile forces, lower hydrostatic forces, and reduced arterial pressures distal to the arterial stenoses (Richardson 1981, Leyk et al. 1994). Thus, the effects of exercise type and position on PAD blood flow responses may have exacerbated any oxygen utilization/oxygen delivery mismatch, resulting in the speeded PAD $StO_2$ kinetics observed (Ferreira et al. 2005). Kemp and colleagues found similar results in PAD muscles during similarly-intense, claudication-producing calf exercise (Kemp et al. 2001). In that study, the calculated rate constant of muscle hemoglobin desaturation was faster in PAD than control subjects despite a lower oxidative generation of ATP in legs of individuals with PAD (Kemp et al. 2001). The authors reasoned that the faster rate of muscle hemoglobin desaturation was reflective of local oxygen utilization that greatly outpaced oxygen delivery, consistent with the kinetic models of muscle venous oxygen content predicted by Ferreira et al.

(Ferreira et al. 2005). Kemp et al. suggested the apparent slowing of oxidative ATP resynthesis was likely due to a limitation in oxygen delivery; however no measures of blood flow were assessed (Kemp et al. 2001). An interesting alternative possibility could relate to the altered regulation of oxidative metabolism in PAD, and potentially the increased magnitude of metabolic inertia in PAD muscle that may be modified under the conditions of high work intensity and extreme mismatch of oxygen supply to metabolic demand. Walsh et al., using saponin-skinned skeletal muscle fibers, have demonstrated that free ADP control of mitochondrial respiration may be sensitized by the decreasing phosphocreatine-to-creatine ratio during high intensity exercise (Walsh et al. 2001). As changes in this ratio as well as accumulation of free [ADP] and inorganic phosphate may occur rapidly following the onset of heavy exercise in both healthy subjects and patients with PAD, these changes could easily affect the kinetics of oxygen utilization, particularly if mitochondrial sensitivity (a potential contributor to metabolic inertia) to these metabolites may be abnormal (e.g. PAD). Indeed, increased changes in free [ADP], [CrP], [creatine], and [Pi] for a given rate of oxidative ATP synthesis are characteristic of exercising PAD skeletal muscle (Kemp et al. 1993). In this context, an increase in mitochondrial sensitivity to potential metabolic controllers could reduce the magnitude of PAD metabolic inertia, allowing an early acceleration of oxidative phosphorylation and oxygen utilization that may exacerbate any developing oxygen utilization/oxygen delivery mismatch, speeding the muscle $StO_2$ kinetics as observed. Clearly, further study will be necessary to directly assess muscle oxygen uptake, intracellular energetics, and blood flow kinetics following the onset of exercise at low compared with high intensity exercise in patients with PAD in order to clarify the muscle $StO_2$ and local oxygen uptake kinetic responses.

*Implications of altered $StO_2$ kinetics in PAD.*

Peripheral arterial disease is associated with a profound impairment in peak exercise capacity as well as limitations in the ability to sustain even low levels of walking exercise. The importance of the primary hemodynamic limitation of leg blood flow during peak exercise is supported by the observation of a positive correlation between end-exercise blood flow at 50% MVC and peak pulmonary oxygen uptake. However, the PAD $StO_2$ time constants during 10% MVC exercise were inversely correlated with peak oxygen uptake during graded treadmill testing, consistent with previous work describing an inverse relationship between peak oxygen uptake and the accumulation of metabolic intermediates in PAD skeletal muscle (Hiatt et al. 1992). The metabolic abnormalities in PAD-affected skeletal muscle are likely reflected in the altered dynamic increase and regulation of muscle oxygen utilization throughout the range of tolerable work rates rather than simply reflecting a reduced maximal oxygen consumption per gram of muscle, a factor that is often overlooked during study of isolated mitochondria. For example, in addition to specific abnormalities in electron transport chain component activities in PAD muscle, an increased dynamic change in intracellular metabolite concentrations required for a given level of oxidative phosphorylation could contribute to the level of metabolic inertia in PAD during low levels of exercise, predisposing the muscle to early fatigue and symptom development. This could, in part, explain how factors related to the slowed kinetics of oxygen uptake during the early period of exercise may be related to the functional limitation of patients with PAD. Thus, in addition to a primary hemodynamic impairment, metabolic abnormalities of the affected skeletal muscle may contribute to the profound exercise intolerance in patients with PAD.

*Limitations.*

A limitation of this study was that the kinetics of limb blood flow following the onset of exercise could not be measured with the plethysmographic technique. Thus, the effects of the early and rapid changes in limb blood flow at the onset of exercise could not be directly assessed and compared with the work load dependent changes in muscle $StO_2$ in the PAD patients. In addition, the assessment of muscle $StO_2$ by NIRS may be influenced by factors other than the balance between oxygen delivery and oxygen utilization within the muscle microvasculature. For example, the absorption spectra of myoglobin and hemoglobin are nearly identical and have differing kinetics of desaturation (Tran et al. 1999, Marcinek et al. 2003). However the actual quantitative contributions of myoglobin to the overall $StO_2$ signal is not known, but is estimated to be relatively small (Seiyama et al. 1988, Mancini et al. 1994).

The dynamic $StO_2$ measurements may also be influenced by the redistribution of oxyhemoglobin and deoxyhemoglobin within the NIRS sample window (McCully and Hamaoka 2000, DeLorey et al. 2003). In the present study, following the onset of contractions, there was a rapid decrease in the total absorbance of hemoglobin, representing a decrease in the total admixture of arteriolar (pre-capillary), capillary and venular (post-capillary) blood. Thus, shifts in the relative distribution of oxy- and deoxy-hemoglobin could also explain a portion of the transient increase or time delay immediately following the onset of contractions. Moreover, skin thickness and muscle depth can alter measures of muscle oxygenation (van Beekvelt et al. 2001). In order to minimize this latter effect, we excluded subjects with a skin and subcutaneous adipose thickness equal to or greater than 15mm. However, because near infrared light must pass through these superficial layers, it is possible that the $StO_2$ measurements also reflected a portion of hemoglobin oxygen saturation in the non-active (fat and skin) tissue. Thus,

while the kinetic response of $StO_2$ desaturation primarily reflects the changes in muscle microvascular hemoglobin oxygen saturation due to the exercise contractions, the inclusion of non-active tissue could alter the magnitude of $StO_2$ decrease (e.g. A1).

*Conclusions*

Muscle $StO_2$ kinetics demonstrate a work rate dependence following the onset of calf exercise in PAD compared with healthy older subjects. The muscle $StO_2$ time constants were prolonged in PAD as compared with controls during lower intensity work rates, consistent with previous observations during submaximal treadmill exercise. However, the accelerated muscle $StO_2$ responses in PAD patients during higher levels of exercise intensity and blood flow limitation indicates an extreme oxygen utilization/oxygen delivery mismatch and suggests the possibility that a greater degree of metabolic inertia associated with PAD might be overcome at high intensity work rates. Studies measuring dynamic muscle oxygen consumption, muscle bioenergetics, and blood flow following the onset of exercise will be critical in further understanding the pathophysiology of impaired blood flow and muscle metabolism in the exercise limitation observed in PAD.

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**Table 1: Subject Characteristics**

| | Control | PAD |
|---|---|---|
| Age (years) | 63±2 | 64±3 |
| ABI, Index Leg | 1.25±0.02 | 0.68±0.05 ** |
| Peak $\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$) | 26.2±1.2 | 17.3±1.40 ** |
| MVC, Index Leg (N) | 591±67 | 528±68 |
| Medications | (n) | (n) |
| Aspirin | 0 | 8 |
| Statin | 2 | 8 |
| $Ca^{2+}$ blockers | 0 | 4 |
| Diuretic | 0 | 2 |

Values are mean± S.E.M. ABI, Ankle-Brachial Index. Peak $\dot{V}O_2$, peak oxygen uptake during graded treadmill exercise testing. MVC, maximal voluntary contraction. ** $p < 0.01$ for PAD vs. Control.

**Table 2: $StO_2$ dynamics during constant work rate calf muscle exercise in peripheral arterial disease patients and healthy controls.**

| MVC | | Power output (w) | $StO_2$(b) (%) | A1 (%) | τ1 (s) | TD1 (s) | MRT (s) |
|---|---|---|---|---|---|---|---|
| **1%** | | | | | | | |
| **Control** | n=0 | 0.8 ± 0.1 | 83 ± 4 | No $StO_2$ desaturation | | | |
| **PAD** | n=4 | 1.4 ± 0.3 | 82 ± 4 | -12 ± 4 | 15.7 ± 4.3 | 22.3 ± 2.9 | 38.0±5.3 |
| **5%** | | | | | | | |
| **Control** | n=6 | 1.7 ±0.2 | 81 ±5 | -12± 3 | 6.9 ±1.2 | 32.1 ± 3.2 | 30.8±1.5 |
| **PAD** | n=7 | 2.3 ±0.5 | 82 ± 3 | -21 ± 4 | 13.5 ± 1.7* | 20.3 ± 2.5 * | 36.1±3.0* |
| **10%** | | | | | | | |
| **Control** | n=8 | 3.2 ±0.3 | 79 ±5 | -18 ± 5 | 6.8 ±1.1 | 18.6 ±1.9 | 25.4±1.6 |
| **PAD** | n=8 | 3.5 ±0.5 | 80 ± 3 | -43 ± 8*, ‡ | 14.5 ± 2.7* | 15.4 ± 2.6 | 34.8±2.7* |
| **30%** | | | | | | | |
| **Control** | n=8 | 6.9 ±0.6 | 78 ± 5 | -45 ± 7† | 7.5 ± 1.1 | 11.8 ± 1.5 † | 19.3±1.7† |
| **PAD** | n=8 | 6.2 ±0.8 | 81 ± 3 | -58 ± 7‡ | 7.9 ± 0.7† | 9.1 ± 1.3† | 17.1±1.4† |
| **50%** | | | | | | | |
| **Control** | n=8 | 8.6 ±0.8 | 83 ± 4 | -56 ± 6† | 7.5 ± 1.2 | 10.5 ± 1.3† | 10.4±1.9† |
| **PAD** | n=8 | 7.9 ±1.1 | 82 ± 4 | -62 ± 7‡ | 6.8 ± 0.8† | 7.6 ± 1.3† | 10.5±0.7† |

Table 2. Values are mean±S.E.M. $StO_2$(b), muscle saturation (%) at baseline. τ1, $StO_2$ time constant. TD1, time delay. A1, Amplitude of $StO_2$ response. No $StO_2$ desaturation was observed during 1% MVC exercise for the controls. * $p < 0.05$ for PAD vs. Control. † $p < 0.05$ versus 1, 5 and 10% MVC. ‡, $P<0.05$ versus 1 and 5% MVC.

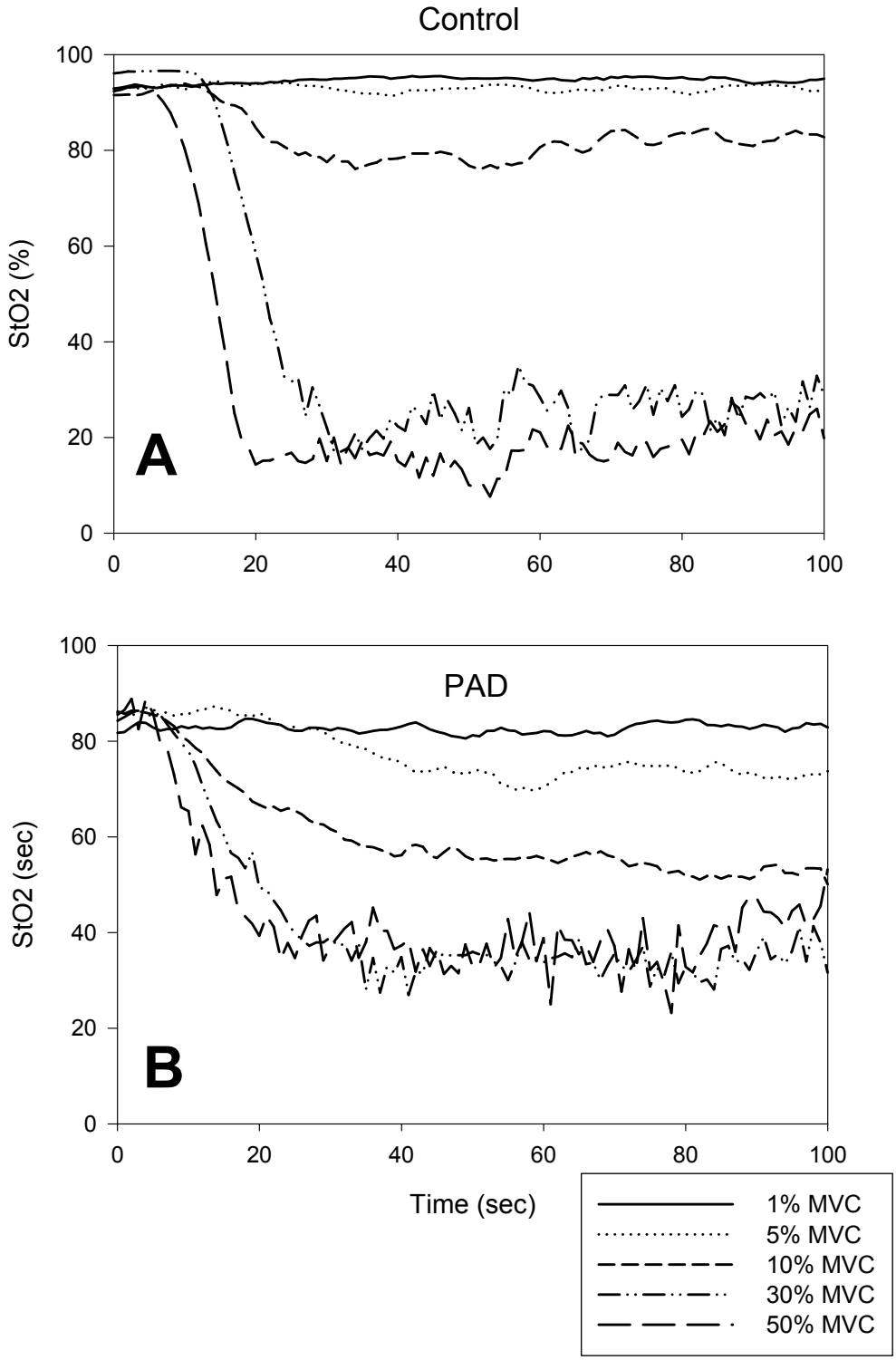


**Figure 1.** Representative $StO_2$ responses from a control subject (Panel A) and a patient with peripheral arterial disease (PAD, Panel B) in the transition from rest to exercise across all constant work rates. Exercise time begins at time=0.

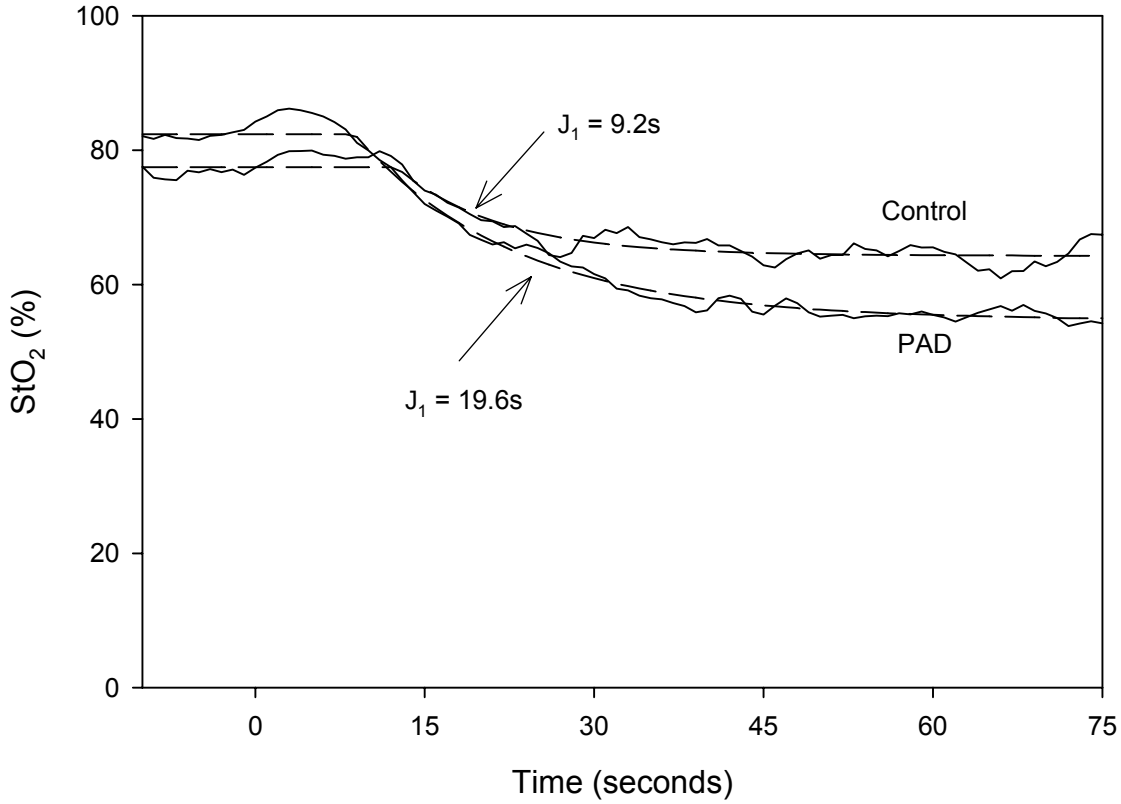


**Figure 2.** Representative $StO_2$ kinetics during 10% MVC calf plantar flexion exercise in a control subject (Panel A) and patient with peripheral arterial disease (Panel B). $StO_2b$ is resting $StO_2$ prior to exercise onset. Exercise begins at time=0. Following a time delay, $StO_2$ falls to a nadir with a time constant ($\tau_1$). Dashed lines denote curve fit.

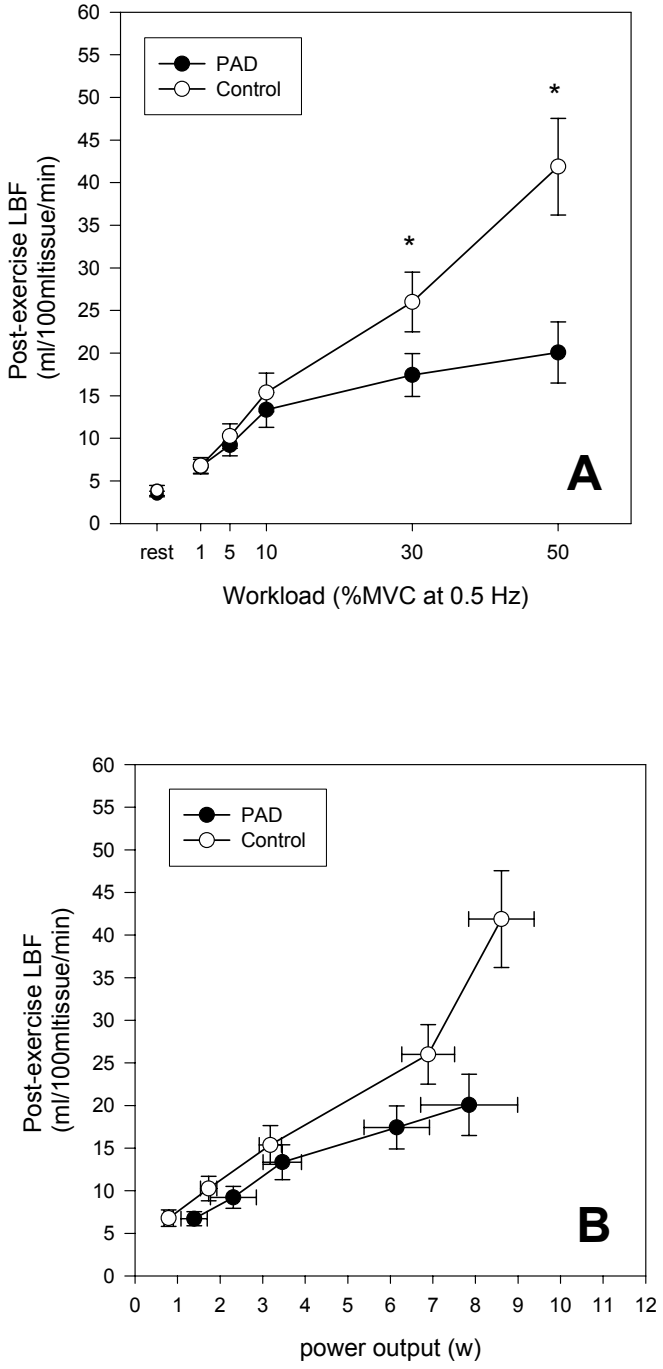


**Figure 3.** Comparison of calf blood flow (LBF) in peripheral arterial disease (PAD) patients and control subjects measured at rest and immediately following the last contraction at each work rate (Panel A) and as a function of power output (Panel B). Note reduced LBF/power output relationship at highest power outputs in PAD patients compared with controls. *, $p<0.05$ PAD vs. control.

## Chapter 5

## Effect of Revascularization on Pulmonary Oxygen Uptake Kinetics in Peripheral Arterial Disease


### Abstract

Patients with peripheral arterial disease (PAD) have a profound reduction in exercise tolerance and impaired oxygen uptake ($\dot{V}O_2$) kinetics during the rest to exercise transition. Previous studies have suggested that abnormalities of limb blood flow, impaired skeletal muscle metabolism, or both may contribute to the impaired exercise responses. The present study was designed to investigate the relative contribution of impaired blood flow on the slowed $\dot{V}O_2$ kinetics in patients with PAD by evaluating patients before and after revascularization. We hypothesized that if blood flow limitation was the primary determinant of the slowed pulmonary $\dot{V}O_2$ kinetics in PAD, revascularization would accelerate the pulmonary $\dot{V}O_2$ kinetic response. Eight patients with PAD (age 55.9±9.4 yrs.) were studied before and following revascularization procedures. Peak $\dot{V}O_2$ was assessed by graded treadmill exercise testing. Limb hemodynamics were evaluated by ankle-brachial index (ABI), and limb blood flow at rest and during reactive hyperemia using venous plethysmography. Pulmonary $\dot{V}O_2$ kinetics were evaluated from the average of three bouts of constant work rate treadmill exercise at 2.0 mph and 4% grade. Prior to revascularization, all PAD patients experienced claudication pain during exercise testing, but no patient experienced claudication during follow-up testing following revascularization. Revascularization improved peak reactive hyperemia in patients (11.5±3.5 increased to 19.3±6.2 ml/100ml tissue/min, $p<0.01$) and the ABI of the index legs of PAD patients (from 0.66±0.12 to 0.99±0.10, $p<0.01$). The phase 2 $\dot{V}O_2$ kinetic time constant was reduced in 6 of 8 patients after revascularization but the magnitude of improvement was not statistically significant (from 52.0 ± 13.7 to

44.2 ± 11.2 sec. after revascularization, n.s.). In contrast, the magnitude of the estimated $\dot{V}O_2$ slow component (6-3 $\dot{V}O_2$) in PAD patients was reduced (from 92 ±59 to 52 ±35 ml/min post-revascularization, $p<0.05$). Peak $\dot{V}O_2$ of PAD patients improved following revascularization (from 14.2±3.0 to 18.5±3.6 ml/kg/min, $p<0.01$). The increase in reactive hyperemia after revascularization was correlated with the improvement in peak $\dot{V}O_2$ ($r = 0.94$, $p < 0.05$), but no correlation was observed between RH or ABI and the phase 2 kinetic time constant after revascularization. In summary, revascularization resolved claudication symptoms, increased peak $\dot{V}O_2$ and tended to improve phase 2 pulmonary $\dot{V}O_2$ kinetics. However, despite the significant improvement in calf blood flow responses, pulmonary $\dot{V}O_2$ kinetics may remain abnormal in PAD patients. These findings are consistent with a persistent abnormality in muscle oxygen utilization in PAD post-revascularization and suggest that local abnormalities of the PAD-affected skeletal muscle may contribute to the slowed pulmonary $\dot{V}O_2$ kinetics and exercise intolerance of patients with PAD.

**Introduction**

Patients with peripheral arterial disease (PAD) are characterized by a low exercise tolerance and reduced peak oxygen uptake ($\dot{V}O_2$) (Eldridge and Hossack 1987, Hiatt et al. 1992). Moreover, pulmonary oxygen uptake responses during submaximal exercise are abnormal in patients, and the dynamic increase of pulmonary $\dot{V}O_2$ following the onset of exercise ($\dot{V}O_2$ kinetics) is slowed in PAD (Auchincloss et al. 1980, Haouzi et al. 1997, Womack et al. 1997b, Bauer et al. 1999, Barker et al. 2003, Barker et al. 2004, Bauer et al. 2004b). The slowing of $\dot{V}O_2$ kinetics in PAD is significant and is inversely related to peak exercise performance, consistent with the notion that slowed $\dot{V}O_2$ kinetics in PAD may have functional consequences (Barker et al. 2003). Previous studies of $\dot{V}O_2$ kinetics in PAD have attributed the abnormally slowed responses to the primary impairment in

lower extremity blood flow due to the arterial stenoses (Auchincloss et al. 1980, Haouzi et al. 1997), or alternatively in combination with the spectrum of metabolic abnormalities in PAD-affected skeletal muscle (Bauer et al. 1999, Barker et al. 2003). However, the relative contributions of the potential muscle metabolic abnormality and primary blood flow impairment to the slowed pulmonary $\dot{V}O_2$ kinetics and reduced exercise performance in PAD remains to be more fully elucidated.

The present study was designed to investigate the relative contribution of impaired blood flow on the slowed $\dot{V}O_2$ kinetics in patients with PAD by evaluating patients before and after revascularization. We hypothesized that if blood flow limitation was the primary determinant of the slowed pulmonary $\dot{V}O_2$ kinetics in PAD, then revascularization would accelerate the pulmonary $\dot{V}O_2$ kinetic response. A finding of faster $\dot{V}O_2$ kinetics in PAD would reflect the contribution of revascularization, and presumably improvements in leg blood flow, on the PAD $\dot{V}O_2$ kinetic response. Additionally, we anticipated that revascularization would result in improvements in the symptoms of claudication and increase the peak exercise capacity of patients with PAD.

**Methods**

*Subjects.* Eight subjects with intermittent claudication secondary to PAD were recruited. Subjects with PAD were enrolled that were scheduled for lower extremity angiogram as part of the standard care for the PAD condition. The study was approved by the University of Colorado Multiple Institutional Review Board, and subjects provided informed consent prior to study participation.

Peripheral arterial disease was confirmed by a resting ankle-brachial index (ABI) value < 0.90, that fell at least 0.10 following peak treadmill exercise. All PAD subjects exhibited typical symptoms of intermittent claudication during walking, defined as localized discomfort or cramping in the calf muscles of the affected legs that occurred

only with exercise and that was completely relieved within ten minutes of rest. Subjects with PAD included four subjects with unilateral occlusive disease (defined as a reduced ABI and claudication symptoms in one leg, but no symptoms and a resting ABI greater than 0.90 in the other leg) and four subjects with bilateral occlusive disease (leg claudication symptoms and ABI < 0.90 in both legs). In all PAD subjects, an index leg was identified that was most symptomatic and for which revascularization was planned. Participants were excluded from study if they had: 1) a history of stroke, congestive heart failure, or diabetes mellitus or 2) evidence of cardiac ischemia (by Electrocardiogram) during baseline graded exercise testing. Additionally, subjects with ischemic rest pain or ulceration of the affected lower extremities were excluded from study.

*Protocol Design*. Each participant visited the Vascular Research Laboratory at the University of Colorado-Health Sciences Center for evaluation a total of four times: two visits within one-week prior to- and two visits following revascularization. Subjects were instructed to avoid the consumption of alcohol, caffeine, and smoking within the 12 hours prior to each visit and to avoid food consumption within 4 hours before each visit. The first visit was used to obtain initial screening measurements, lower extremity blood flow measurements, and establish baseline peak exercise capacity. The second visit consisted of three constant work rate (CWR) exercise tests for the analysis of pulmonary oxygen uptake ($\dot{V}O_2$) kinetics as described previously (Bauer et al. 1999, Bauer et al. 2004b). Each participant repeated these procedures following revascularization (Table 1).

*Graded Exercise Testing*. Peak treadmill (Q-stress, Quinton Instruments, Seattle, WA, USA) exercise performance (peak $\dot{V}O_2$) was assessed before and after revascularization as previously described (Bauer et al. 1999, Bauer et al. 2004b). For assessment of baseline peak $\dot{V}O_2$, PAD subjects performed a Gardner Protocol (speed constant at 2.0 mph with a 2% increase in grade every 2 minutes) to maximal claudication pain that prevented any

further walking (Gardner et al. 1992). In order to accommodate an expected increase in exercise performance following revascularization, PAD participants performed an incremental treadmill protocol with greater work rate increments (Astrand protocol) for determination of peak $\dot{V}O_2$ (Astrand and Rodahl 1977). Heart rate (HR) was measured continuously using 12-lead ECG monitoring during exercise testing.

*Constant Work Rate Exercise Testing.* On a separate day, subjects performed three treadmill exercise transitions from rest to constant work rate (CWR) walking (2.0 mph, 4% grade) as previously described (Bauer et al. 1999). Each CWR exercise test consisted of a 3-minute resting period to obtain baseline gas exchange data, followed by 6-minutes of CWR walking exercise, and a 30-minute period of seated rest. Pulmonary gas exchange measurements and heart rate data were recorded continuously throughout the resting and exercise periods of each CWR test.

*Ankle Brachial Index.* The ABI was calculated in all subjects at rest as previously described (Bauer et al. 1999). Prior to revascularization, a post-exercise ABI was also obtained within one-minute following the graded treadmill exercise. The ratio of ankle-to-brachial systolic pressure was determined by taking the highest brachial artery pressure divided into the higher of the dorsalis pedis or posterior tibialis artery pressure in each ankle.

*Reactive Hyperemia (RH) Blood Flow Measurements*. Calf blood flow was assessed using venous occlusion strain gauge plethysmography (D.E. Hokanson Inc. Issaquah, WA) at rest and during reactive hyperemia (RH) following release of supra-systolic cuff occlusion as previously described (Bauer et al. 2004b). Blood flow was expressed as ml of flow/100ml of tissue/minute. Resting blood flow was calculated as the average of 6 separate measurements. Peak RH was determined following a 5-minute period of limb ischemia induced by a proximal thigh cuff inflated to 50 mmHg above systolic blood

pressure. Post-occlusion blood flow measurements were followed for 5 minutes following cuff release. The highest value achieved was taken as the peak RH value. The sum of peak RH values for both legs were recorded.

*Measurement of Pulmonary Gas Exchange.* For all exercise tests, oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}E$), and other respiratory variables were measured and recorded breath-by-breath using a metabolic measurement system (MedGraphics CPX/D, Medical Graphics Corp., St. Paul, MN, USA). The $O_2$ and $CO_2$ analyzers were calibrated prior to each test, and pneumotach volumes were calibrated using a syringe of known volume (3.0 L). Breath-by-breath data were stored to computer disk for off-line analysis. During graded exercise, the highest $\dot{V}O_2$ attained from 20-second averages was defined as peak $\dot{V}O_2$. The respiratory exchange ratio (RER) was calculated as the ratio of $\dot{V}CO_2/\dot{V}O_2$.

*$\dot{V}O_2$ Kinetic Analysis.* Breath-by-breath gas exchange data for each CWR exercise transition were processed using a software program as previously described (Bauer et al. 1999). Three constant work rate tests were time-aligned and averaged to provide a single, average $\dot{V}O_2$ kinetic response for each patient before and after revascularization. The averaged responses were evaluated by non-linear regression techniques (Sigmaplot 2001, SPSS, Inc., Chicago, IL, USA) using 2- or 3-component exponential mathematical models that describe the dynamic changes in pulmonary$\dot{V}O_2$ as described previously (Bauer et al. 1999, Bauer et al. 2004b). The two component mathematical model is given as: $\dot{V}O_2(t) = \dot{V}O_2(b) + A_1(1 - e^{-(t-TD1)/\tau1}) + A_2[1- e^{-(t-TD2)/\tau2}]$ and the three component exponential model by: $\dot{V}O_2(t) = \dot{V}O_2(b) + A_1(1 - e^{-(t-TD1)/\tau1}) + A_2[1- e^{-(t-TD2)/\tau2}] + A_3[1- e^{-(t-TD3)/\tau3}]$. For each subject, best-fit modeling of the $\dot{V}O_2$ kinetic response was determined by F-test. Estimates of the total exercise $\dot{V}O_2$ increase above resting baseline (Atot) and the magnitude of the $\dot{V}O_2$ slow component ($A_3'$) were calculated from the individual model parameters as

described (Bauer et al. 2004b): Atot = $A_2 + A_3'$ and $A_3' = A_3(1-e^{(-(ED-TD3)/\tau 3)})$, where ED is the time at end-exercise (6 minutes).

*Statistical Analysis.* Two-tailed paired Student t-tests were used for comparison of exercise parameters before and after revascularization within PAD subjects. Statistical significance was declared at $p < 0.05$. A Pearson's R product was employed to evaluate significant correlations using a Bonferroni correction of alpha level, $p<0.016$ based on three comparisons.

**Results**

Patient demographics and chronic medications are listed in Table 1. Lower extremity arterial stenoses were localized to the iliac arteries in four subjects, three subjects had involvement of both iliac and femoral arteries, and one subject had an abdominal aortic stenosis (Table 2). Percutaneous transluminal angioplasty of the iliac arteries was performed in seven subjects. A common femoral artery bypass was also performed in two subjects. Seven PAD patients returned to the laboratory for evaluation within 33 days post-revascularization (mean 14±10 days). However, one patient was unable complete follow-up evaluations until 113 days post-revascularization.

*Limb Hemodynamics.* Prior to revascularization, the resting ABI of the index and contralateral leg were significantly lower in PAD subjects ($p<0.01$, Table 3). Revascularization significantly improved the ABI of the index leg ($p<0.01$) and the contralateral leg ($p <0.05$) given that revascularization procedures treated bilateral symptoms in four subjects. Resting blood flows of the index legs were not significantly different between pre and post-revascularization (2.9±1.2 versus 3.7±2.5 ml/100ml tissue/min, Figure 1). However, following revascularization peak reactive hyperemia increased 69% in the index legs of PAD subjects (11.5±3.5 at baseline versus 19.4±6.2 ml/100ml tissue/min after revascularization, $p<0.01$).

*Peak Exercise Performance.* Prior to revascularization, all PAD patients experienced claudication symptoms during incremental exercise testing, and the total claudication-limited walking time during graded treadmill exercise was approximately 344 ± 122 seconds. Following revascularization, peak $\dot{V}O_2$ and peak HR were significantly increased compared with pre-revascularization findings ($p<0.05$, Table 4). Peak RER tended to be increased following revascularization but did not reach statistical significance ($p=0.11$). No subject experienced claudication symptoms during graded treadmill exercise following revascularization, and incremental tests post-revascularization continued until patient volitional fatigue.

*Constant work rate exercise*. While all PAD subjects experienced claudication symptoms during CWR exercise prior to revascularization, no claudication symptoms were observed in PAD subjects at the same absolute treadmill work rate post-revascularization (Table 5). The increase in HR ($\Delta$HR) from rest to the end of CWR exercise was reduced in PAD subjects following revascularization ($p<0.01$). End-exercise oxygen consumption and the RER during the same absolute treadmill CWR exercise work rate were reduced post-revascularization ($p<0.05$). Consistent with an increase in peak $\dot{V}O_2$ following revascularization, the PAD relative intensity of the CWR treadmill exercise decreased by approximately 27% following revascularization ($p<0.01$), and the increase in $\dot{V}O_2$ from minute 3 to minute 6 of exercise prior to revascularization (e.g. an indicator of the $\dot{V}O_2$ slow component) was reduced in PAD subjects after revascularization ($p<0.05$).

*$O_2$ uptake kinetics.* Prior to revascularization, pulmonary $\dot{V}O_2$ kinetic data from four PAD subjects were best fit using a 3-component model (to include the $\dot{V}O_2$ slow component), and four PAD subjects were best fit by 2-component modeling (no slow component) (Table 6). Following revascularization, two PAD subjects' $\dot{V}O_2$ kinetics remained best-fit by 3-component modeling (Figure 2), and the remaining 6 PAD subjects were best-fit

using the 2-component model. No differences in phase 1 $\dot{V}O_2$ kinetic responses were noted following revascularization. The phase 2 time constant ($\tau_2$) (Figure 3) and time delay (TD2) tended to become faster following revascularization procedures, but the changes in these parameters did not reach statistical significance (p=0.09 and p=0.05, respectively). Moreover, the amplitude of the phase 2 response were similar between pre- and post-revascularization. The amplitude of the $\dot{V}O_2$ slow component as modeled by a third exponential term comprised approximately 16% of the total $\dot{V}O_2$ response pre-revascularization (n=4) and was reduced by 6% post- revascularization (n=2). Assessed from model parameters, a significant reduction of the total amplitude of $\dot{V}O_2$ response during CWR treadmill walking was observed following revascularization within PAD subjects ($p<0.05$).

*Relationships between exercise and hemodynamic parameters.* The improvement in peak $\dot{V}O_2$ post-revascularization in PAD subjects was significantly correlated with the increase in total peak reactive hyperemia (e.g. sum of both legs) ($R = 0.94$, $p<0.01$). However, the improvement in hemodynamic parameters in PAD subjects was not correlated with a change in the phase 2 time constants ($\tau_2$) following revascularization (ABI: $R = -0.06$ or peak RH: $R = -0.26$, $p>0.5$).

**Discussion**

The primary finding of the present investigation is that revascularization of the lower limbs tended to speed the phase 2 time constant of pulmonary $\dot{V}O_2$ kinetics in PAD (6 of 8 subjects), but that the faster $\dot{V}O_2$ time constants were not correlated with the improvement in limb blood flow responses following revascularization. In contrast, revascularization improved peak $\dot{V}O_2$, and the magnitude of improvement was significantly correlated with the increase in reactive hyperemia responses following revascularization. These data indicate that a primary benefit of revascularization may

relate to an increased peak exercise capacity in PAD. However, revascularization may not normalize pulmonary $\dot{V}O_2$ kinetics in PAD, suggesting that an acute improvement of leg blood flow may not fully address the pathophysiology of PAD.

The dynamic increase of pulmonary $\dot{V}O_2$ during the rest to exercise transition is dictated by the interaction between the rates of skeletal muscle mitochondrial oxygen consumption, oxygen delivery, circulatory transit delays, and pulmonary gas exchange (Whipp and Mahler M. 1980). In healthy subjects, the time course of phase 2 pulmonary $\dot{V}O_2$ kinetics closely reflect the dynamic increase in $\dot{V}O_2$ of the exercising muscles (Grassi et al. 1996) and during moderate exercise is generally considered to be limited by the 'metabolic inertia' intrinsic to the working skeletal muscle (Whipp and Mahler M. 1980, Grassi et al. 1996, Grassi et al. 1998a, Grassi et al. 1998b). In contrast, cardiovascular diseases have been shown to slow pulmonary $\dot{V}O_2$ kinetics presumably through impairment of blood flow dynamics (and thus oxygen delivery) (Sietsema et al. 1986, Koike et al. 1995) or in combination with peripheral impairments in peripheral skeletal muscle oxygen utilization (Sietsema et al. 1994, Grassi et al. 1997) . In patients with PAD, lower extremity blood flow responses during exercise are limited due to the primary arterial stenoses. However, PAD is also associated with skeletal muscle pathology that may contribute to impaired muscle oxygen utilization. For example, there is fiber degeneration (Teravainen and Makitie 1977, Regensteiner et al. 1993), pathologic changes in mitochondrial structure (Teravainen and Makitie 1977, Sjostrom et al. 1980, Farinon et al. 1984), decreased electron transport chain activity (Brass et al. 2001) as well as derangements in capillary ultra-structure (Makitie 1977) in the affected skeletal muscle of PAD patients. Thus, a fundamental question arises as to whether the slowed pulmonary $\dot{V}O_2$ kinetics in PAD are attributable solely to the primary blood flow impairment or rather reflect also the metabolic dysfunction or other pathologic factors local to the

affected skeletal muscle. To date, the contribution of impaired muscle blood flow to the slowed pulmonary $\dot{V}O_2$ kinetics in PAD has not been previously addressed.

In the present study, the patients demonstrated pulmonary $\dot{V}O_2$ kinetic responses prior to revascularization that were quantitatively similar to the abnormally slowed pulmonary $\dot{V}O_2$ kinetics previously reported for patients with PAD (Bauer et al. 1999, Barker et al. 2003, Bauer et al. 2004b). After revascularization, the phase 2 time delay tended to be shorter, and the phase 2 time constant tended to be faster compared with the pre-procedure baseline. Although not statistically significant, the shorter time delay of the primary component of pulmonary $\dot{V}O_2$ kinetics following revascularization is consistent with the expected speeding of circulatory transit of venous blood from the exercising leg to the lung for gas exchange (Barstow and Mole 1987, Barstow et al. 1990) and may provide an indirect indication that arterial inflow dynamics were improved. Moreover, the tendency to speed the phase 2 time constant after revascularization could suggest that a primary impairment in oxygen delivery to the exercising legs was improved, consistent with the early conclusions by others (Auchincloss et al. 1980, Haouzi et al. 1997). However, the improvement in phase 2 time constants among patients following revascularization was modest, accelerating the time constant by approximately 7.9 seconds. Thus, despite a significant improvement in blood flow responses following revascularization (~69%), by comparison the improvement in the phase 2 time constant was relatively small (~17%). Indeed, the lack of correlation between improvements in limb blood flow and the phase 2 time constants with revascularization supports the notion that the slowed pulmonary $\dot{V}O_2$ kinetics following the onset of exercise in patients with PAD may be predominantly determined by factors independent of the potential limb blood flow limitation during exercise.

Revascularization of the affected lower extremities in patients improved the resting ABI and blood flow responses during reactive hyperemia, consistent with previous observations of hemodynamic improvements with revascularization at rest and during exercise (Pernow et al. 1975). However, the phase 2 time constants in patients remained slowed, consistent with the notion that accumulated changes and metabolic injury associated with PAD-affected skeletal muscle may persist following revascularization procedures. In the present study, the phase 2 time constants improved in 6 of 8 patients, but on average did not normalize as compared with healthy control data collected under similar testing conditions (Figure 3). This could reflect the presence of gross residual atherosclerotic disease in the legs in patients after revascularization. However, residual stenoses were less than 5% or not apparent by post-revascularization angiography. An alternative explanation may be related to the skeletal muscle pathology distal to the primary arterial stenoses. Using $^{31}$P-NMR, Zatina et al. observed abnormally slowed phosphocreatine (PCr) recovery following exercise in PAD patients that persisted for several months despite the hemodynamic improvement with revascularization (Zatina et al. 1986). An intrinsic impairment in oxidative metabolism in PAD muscle is also consistent with the slowed kinetics of muscle deoxygenation (e.g. oxygen extraction) following the onset of exercise (Bauer et al. 2004a) (Bauer unpublished results 2005) and abnormal $\dot{V}O_2$ kinetics previously observed in PAD patients (Auchincloss et al. 1980, Haouzi et al. 1997, Bauer et al. 1999, Bauer et al. 2004b). Thus, the slowed pulmonary $\dot{V}O_2$ kinetics after revascularization may reflect a persistent abnormality in the control of oxidative metabolism or other local factors limiting oxygen utilization of the affected lower limbs in PAD.

An interesting finding was that revascularization appeared to reduce end-exercise $\dot{V}O_2$ and parameters associated with the $\dot{V}O_2$ slow component. In healthy subjects, the

$\dot{V}O_2$ slow component occurs during exercise in the heavy and severe exercise domains (e.g. above the lactate threshold) and describes an additional increase in oxygen consumption above that predicted from moderate exercise (Poole et al. 1991). Thus, the $\dot{V}O_2$ slow component may be considered an exercise intensity dependent phenomenon. The reduction in the $\dot{V}O_2$ slow component in PAD patients may be clinically significant as less oxygen consumption may be required to perform a given submaximal exercise task, particularly given that low work rates can represent a large proportion of the limited peak $\dot{V}O_2$ of PAD patients (Womack et al. 1997a, Womack et al. 1997b). Similar changes in exercise $\dot{V}O_2$ have been observed in PAD during treadmill walking following aerobic exercise training (Hiatt et al. 1994, Womack et al. 1997a). Thus, an improvement in exercise blood flow resulting from either revascularization or from potential hemodynamic and/or metabolic changes that occur with exercise training could reflect a common mechanism of clinical benefit between these different treatments. Further study will be necessary to explore the mechanisms of the $\dot{V}O_2$ slow component and functional consequences in the treatment of claudication in PAD.

*Study limitations.*

In the present study, plethysmography could not be used to measure exercise blood flow or blood flow dynamics during treadmill walking. Thus, although revascularization may have improved exercise blood flow we could not determine the effect of revascularization on this important measurement. However, the increase in peak reactive hyperemia responses following revascularization was correlated with an improvement in peak $\dot{V}O_2$, which supports the notion that reactive hyperemia provided a useful marker of bulk limb blood flow improvement with revascularization. Another limitation of the study was in the inclusion of two PAD subjects chronically taking selective beta-1 adrenergic blocking agents. Patients' medical therapy was maintained prior to and following

revascularization such that the only change pre-to-post revascularization was the change in lower limb blood flow resulting from the revascularization procedure. Beta-blocking drugs lower peak $\dot{V}O_2$ by limiting cardiac output, and may slow pulmonary $\dot{V}O_2$ kinetics in healthy subjects (Petersen et al. 1983, Hughson 1984). Moreover, reduced reactive hyperemia responses in healthy and PAD subjects have also been described (Lepantalo 1985), although lesser reductions in RH have been observed with selective beta-1 blocking agents (Lepantalo 1985). Despite this potential confounding influence, prior to revascularization the phase 2 time constants were similar between PAD patients taking beta-blocking medications to those tested without these agents (56.4 vs. 50.8s, respectively) and were similarly affected by revascularization (48.9 vs. 42.3s, respectively). Thus, in this small study of PAD patients, the changes in $\dot{V}O_2$ kinetic responses following revascularization appear to be of similar magnitude and consistent among subjects.

In conclusion, the present study demonstrated that revascularization of the lower limbs may speed pulmonary $\dot{V}O_2$ kinetics in patients with PAD. However, the improvement in the phase 2 time constant was modest consistent with a limited role for impaired blood flow on the abnormal pulmonary $\dot{V}O_2$ kinetics in PAD patients. Moreover, that pulmonary $\dot{V}O_2$ kinetics may not normalize following revascularization suggests a persistent impairment in PAD oxygen uptake dynamics following exercise onset. In addition to the limitation of lower limb blood flow, factors related to the abnormal oxidative metabolism of PAD skeletal muscle may offer a putative mechanisms for the slowed pulmonary $\dot{V}O_2$ kinetics of this patient population.

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**Table 1: Patient Characteristics**

| | |
|---|---|
| Age (years) | 55.9 ±9.3 |
| Weight (kg) | 81.9 ±20.4 |
| BMI ($kg/m^2$) | 27.4 ±6.92 |
| Pack-years | 38.3 ±5.8 |
| Current Smokers | n=5 |

| Medications | |
|---|---|
| Aspirin | n=7 |
| $Ca^{2+}$ blocking agents | n=5 |
| Beta Blocking agents | n=2 |
| Statins | n=3 |

**Table 2: PAD Angiographic Characteristics**

| Subject | Lateralization | Leg | Artery | PRE % Stenosis | POST % Stenosis | procedure |
|---|---|---|---|---|---|---|
| 01 | Uni | L | CIA | 100 | 0 | S |
| 02 | Uni | L | CIA | 61 | 0 | S |
| 03 | Uni | L | EIA | 100 | 0 | S |
| | | L | CFA | 100 | 0 | E |
| | | L | PFA | 100 | 0 | E |
| 04 | Bi | L | CIA | 100 | <5 | S |
| | | R | CIA | 40 | 0 | A |
| 05 | Bi | L | CIA | 29 | <5 | A |
| | | R | SFA | 100 | 0 | E |
| 06 | Uni | L | CIA | 100 | 0 | B |
| | | L | EIA | 100 | 0 | B |
| 07 | Bi | L | EIA | 59 | 0 | S |
| | | R | SFA | 75 | <5 | A |
| 08 | Bi | L,R | Ao | 77 | <5 | S |

Angiographic characteristics of PAD patients before and after revascularization. Uni, unilateral peripheral arterial disease; Bi, bilateral peripheral arterial disease. Ao, distal Aorta, CIA, Common Iliac Artery; EIA, External Iliac Artery; CFA, Common femoral Artery; PFA, Profunda Femoral Artery; SFA, Superficial Femoral Artery. S, stent; A, angioplasty, E, endarterectomy, B, surgical bypass.

**Table 3: Ankle-Brachial Index**

| | Pre | Post |
|---|---|---|
| ABI | | |
| Index leg resting | 0.66 ± 0.12 | 0.99 ± 0.10** |
| - post-exercise | 0.29 ± 0.25 | - |
| Contra-lateral leg resting | 0.86 ± 0.25 | 1.03 ± 0.15* |
| - post-exercise | 0.71± 0.30 | - |

Values are mean ±SD. ABI, Ankle-Brachial Index measured at rest and immediately following peak treadmill exericse in patients with PAD. Contra-lateral data include the revascularization of less affected legs of 3 bilateral PAD subjects. * $p<0.05$, ** $p<0.01$ Pre- vs. Post-revascularization.

**Table 4: Peak Exercise Characteristics Pre- and Post-Revascularization**

| | Pre | Post |
|---|---|---|
| $\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$) | 14.2 ± 3.0 | 18.5 ± 3.6** |
| RER | 1.00 ± 0.09 | 1.12 ± 0.12 |
| HR ($beats \cdot min^{-1}$) | 110 ± 15 | 128 ± 21* |
| ICT (seconds) | 66 ± 34 | No claudication |
| ACT (seconds) | 344 ± 122 | No claudication |

Values are mean ±SD. ICT, Time to onset of claudication symptoms; ACT, Claudication limited total walking time on Gardner Protocol. No subject experienced claudication pain during graded exercise post-revascularization (Astrand Protocol). *$p<0.05$, ** $p<0.01$ Pre- vs. Post-revascularization.

**Table 5: Constant Work Rate (CWR) Exercise Characteristics Pre- and Post-Revascularization**

| | Pre | Post |
|---|---|---|
| ICT (seconds) | 75 ±34 | No claudication |
| Δ HR (bpm) | 27 ±10 | 22 ±10** |
| End Exercise $\dot{V}O_2$ ($ml \cdot min^{-1}$) | 1055 ±205 | 970 ±199* |
| End Exercise $\dot{V}O_2$ (% of peak) | 94 ±13 | 67 ±10** |
| End Exercise RER | 0.96±0.06 | 0.90 ±0.06* |
| 6-3 minute $\dot{V}O_2$ ($ml \cdot min^{-1}$) | 92 ±59 | 52 ±35* |

Values are mean ±SD. PAD patients exercised for 6-minutes at 2.0 mph, 4% grade. All PAD subjects experienced typical claudication symptoms during CWR treadmill testing pre-revascularization. No PAD subject experienced claudication symptoms post-revascularization. ICT, Time to onset of claudication symptoms during CWR treadmill walking. ΔHR, 6-minute heart rate minus resting baseline. * $p<0.05$, ** $p<0.01$ Pre- vs. Post-revascularization.

**Table 6: $\dot{V}O_2$ Kinetic Parameters for Constant Work Rate Exercise Pre- and Post-Revascularization**

| | Pre | Post |
|---|---|---|
| Resting $\dot{V}O_2$ (ml/min) | 329 ± 66 | 326 ±52 |
| Phase 1 | N=8 | N=8 |
| $\tau_1$ (s) | 4.9 ± 5.0 | 2.7 ± 2.2 |
| $TD_1$ (s) | 1.9 ± 1.9 | 2.0 ± 2.0 |
| $A_1$ (ml/min) | 297 ± 96 | 270 ± 103 |
| Phase 2 | N=8 | N=8 |
| $\tau_2$ (sec) | 52.0 ± 13.7 | 44.2 ± 11.2 |
| $TD_2$ (s) | 42.8 ± 12.7 | 36.3 ± 9.1 |
| $A_2$ (ml/min) | 381 ± 104 | 362 ± 81 |
| Phase 3 | n=4 | n=2 |
| $A_3$' (ml/min) | 100 ± 26 | 60 ± 12 |
| SC (%) | 16 ± 6 | 10 ± 3 |
| Atot (ml/min) | 724 ± 186 | 646 ± 155* |

Values are mean ± SD. $\tau$, Exponential time constant. TD, time delay. A, Amplitude of $\dot{V}O_2$ response. Atot, total amplitude of $\dot{V}O_2$ response. Phase 3 data are presented for subjects that demonstrated a slow component of $\dot{V}O_2$ increase. *$p<0.05$ Pre- vs. Post-revascularization.

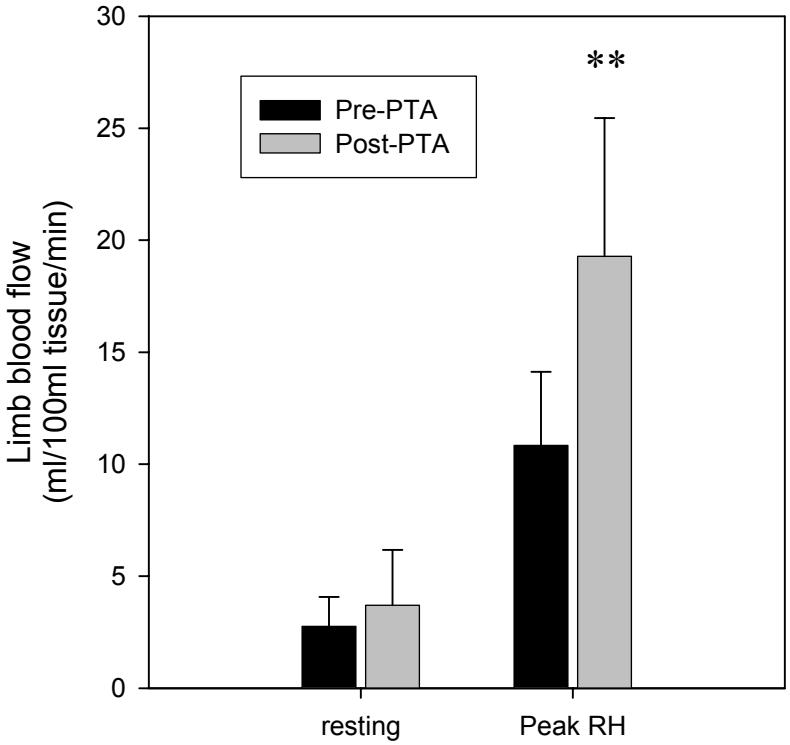


**Figure 1**. Mean ± SD. Resting blood flow and peak reactive hyperemia (RH) pre- and post-revascularization of the index legs in PAD patients. ** $p<0.01$ Pre- vs. Post-revascularization.

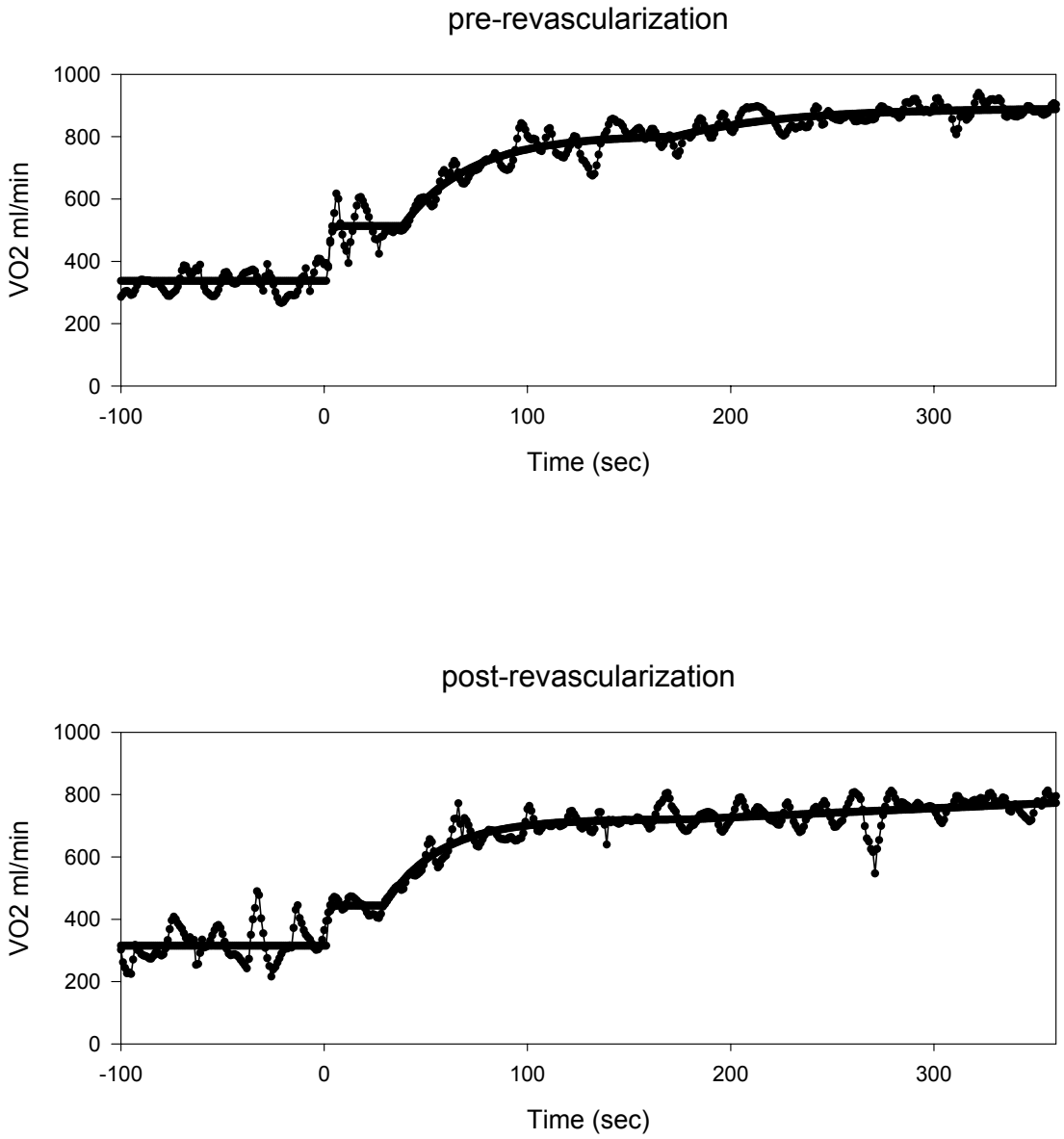


**Figure 2.** Pulmonary $\dot{V}O_2$ kinetic responses and model fit from a representative PAD patient before (top) and after revascularization (bottom) during treadmill constant work rate (CWR) exercise. Treadmill exercise was performed at 2.0 mph, 4% grade. Exercise was initiated at time 0. Note presence of $\dot{V}O_2$ slow component and modeling of third component during PAD CWR treadmill exercise and which remains present, but reduced post-revascularization.

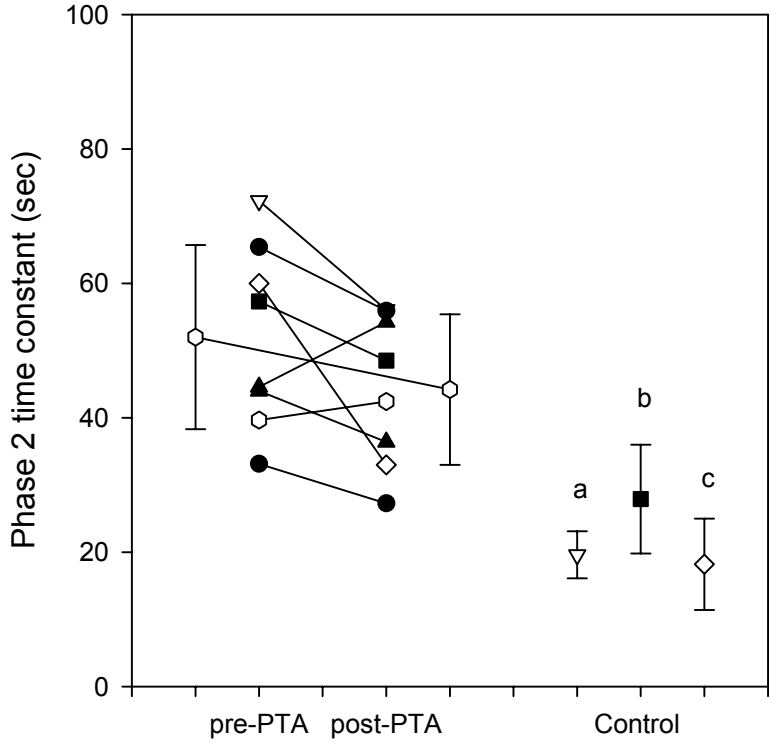


**Figure 3.** Pulmonary $\dot{V}O_2$ kinetic phase 2 time constants of the individual PAD patients before and after revascularization. Open cirlces with error bars represent group mean (±S.D.). Phase 2 time constants from healthy control subjects during treadmill walking provided for reference (mean ± S.D.) ([a] Bauer et al. 2004 (Bauer et al. 2004b); [b] Bauer et al. 1999 (Bauer et al. 1999), [c] Barker et al. 2002 (Barker et al. 2003)).

# Chapter 6.

## Synthesis and Future Directions

## Synthesis

The transport of oxygen from the atmosphere to the skeletal muscles during exercise requires the tightly coupled integration of pulmonary gas exchange and cardiovascular function to meet the demands of oxygen utilization at the level of the mitochondria. In cardiovascular diseases, the integration of these physiological systems becomes altered leading to slowed kinetics of pulmonary oxygen uptake following the onset of exercise and reduced exercise tolerance. In peripheral arterial disease (PAD), arterial stenoses of the lower limbs limit blood flow increase (and oxygen delivery) during exercise. However, it has been previously shown that the reduced arterial pressure and impaired leg blood flow do not entirely explain the abnormal oxygen uptake responses during exercise in PAD, consistent with the notion that alterations in metabolism and the microcirculation at the level of the affected skeletal muscle as well as systemic abnormalities in cardiovascular function may contribute to the abnormal oxygen uptake kinetics of this patient population.

The present series of investigations systematically addressed key questions regarding the abnormal oxygen uptake responses during exercise in patients with peripheral arterial disease. First, we demonstrated abnormal pulmonary oxygen uptake kinetics in patients with PAD during exercise of the affected lower limbs but not during exercise of the unaffected upper extremities, suggesting that the abnormal oxygen uptake responses in PAD were localized to the affected lower limbs. Thus, the slowed pulmonary $VO_2$ kinetics in peripheral arterial disease likely reflects an impairment of

oxygen utilization of the affected skeletal muscle and appears not to be related to the systemic sequelae secondary to the disease process of atherosclerosis. In the second experiment, using non-invasive near infrared spectroscopy of local muscle deoxygenation responses during treadmill exercise, we demonstrated slowed calf muscle $StO_2$ kinetics in the transition from rest to submaximal walking in patients with peripheral arterial disease compared with control subjects. The slowed kinetics of the fall in calf muscle $StO_2$ are consistent with the concept that the dynamic increase in muscle oxygen utilization may be impaired relative to local oxygen delivery during the transition from rest to exercise in peripheral arterial disease, forming a basis for the hypothesis that local muscle metabolic abnormalities in PAD may influence the adjustment of oxidative metabolism in the rest-to-exercise transition. In the third experiment using isolated calf exercise, we confirmed that the fall of muscle $StO_2$ kinetics were abnormally slowed in the working muscle of patients during lower intensity exercise and extended these observations to show that the slowed PAD $StO_2$ kinetic responses occurred under conditions where total increases in exercise blood flow were normal compared with controls. However, the kinetic fall of $StO_2$ became faster in PAD calf muscle during higher intensity exercise where the total increase in blood flow with exercise was significantly compromised. This dichotomous response is consistent with a work rate and potentially blood flow dependence of $StO_2$ kinetics in PAD patients, as might be predicted from the Fick principle under conditions where leg blood flow kinetics may be impaired. Whether these latter data simply reflected a shortfall of oxygen delivery relative to oxygen utilization during the transition from rest-to-high intensity exercise or were the result of improved mitochondrial respiration in PAD muscle remains unclear. However, even in the context of slowed

activation of mitochondrial oxygen utilization, the significant impairment in muscle oxygen delivery relative to oxygen utilization during high intensity exercise is the most likely explanation for the faster fall of $StO_2$ in PAD-affected muscle. In the final study, we showed that an acute improvement of leg blood flow following revascularization tended to speed the abnormal pulmonary oxygen uptake kinetic responses in patients with peripheral arterial disease. However, an acute increase in leg blood flow capacity did not fully normalize the pulmonary oxygen uptake kinetic responses, consistent with a limited role for impaired blood flow in the abnormal oxygen uptake responses in peripheral arterial disease. These findings suggest there may remain a persistent abnormality in the control of oxidative metabolism and/or other local factors limiting oxygen utilization at the level of the affected skeletal muscle even after the restoration of peripheral hemodynamics. Thus, the current series of investigations present a body of work that has clarified and advanced our understanding of the impaired oxygen uptake responses in peripheral arterial disease. Moreover, these studies have identified several key issues regarding the mechanisms of slowed oxygen uptake kinetics at the level of the affected skeletal muscle in peripheral arterial disease that may direct future research.

**Future Directions**

A key element to understanding the limitations of oxygen uptake and exercise intolerance in PAD is the investigation of blood flow and oxygen uptake made at the level of the microcirculation of the affected skeletal muscle. The characterization of lower limb blood flow kinetics following the onset of exercise is paramount to further our understanding of the hemodynamic compromise that occurs during exercise in these patients. Not to be understated, this poses a significant technical challenge as large vessel

arterial occlusions and collateral vessel formation complicate direct measures of conduit artery inflow. Furthermore, separating the potentially normal conduit artery blood flow kinetics from those in the affected microcirculation in PAD will be important. In this area, the measurement of blood flow using magnetic resonance imaging may prove a useful tool in the description of this important parameter and offer insight into the exercise hemodynamics of both the macro and microcirculation in PAD.

Secondary to the blood flow impairment in PAD are many physiological changes in the affected skeletal muscle that may alter the transport and utilization of oxygen during the rest-to-exercise transition. For example, changes in capillary ultra structure and basement membrane alterations may impair oxygen transport from the microcirculation to the myocyte. Moreover, mitochondrial irregularities and abnormalities in the activities of mitochondrial electron transport complexes may impair mitochondrial oxidative metabolism following the onset of exercise. Understanding how these changes may affect oxygen utilization, particularly oxygen transport from capillary to muscle cell as well as the control of mitochondrial respiration at low intracellular $PO_2$, are crucial to understanding the limiting factors of oxygen uptake at the level of the muscle and whole body in PAD. Direct studies of skeletal muscle oxygen uptake and intramuscular energetics in PAD skeletal muscle will provide useful insight into the determinants of muscle oxygen uptake in PAD. More broadly, these data will advance our understanding of the interaction between muscle metabolism and blood flow limitation on the local and systemic exercise responses in diseases where impaired oxygen delivery and muscle ischemia are critical factors.