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Exercise and peripheral vascular function in health and disease

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EXERCISE AND PERIPHERAL VASCULAR FUNCTION
IN HEALTH AND DISEASE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the
requirement for the degree of
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By

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Abstract

“Chronic disease will never reach its clinical horizon to compromise health if it is attacked at its origin” [1]. Knowledge that one’s risk for cardiovascular events is related to the severity of endothelial dysfunction, and evidence that exercise training can improve endothelial function, has prompted speculation that measures of vascular function may serve as a “barometer” for cardiovascular health. This dissertation consists of three experiments intended to study vascular function and the manner in which it may influence or may be influenced by physical function.

Project one examined the influence of high and low volume circuit weight training on forearm vascular function. Thirty-five individuals participated in a program consisting of 3 sessions/week for 5 weeks. Results indicated significant but similar strength gains in both groups (Hand Grip: Δ15.55%, Knee Extension: Δ21.00%, Bench Press: Δ35.31%; p<0.05). Reactive hyperemic responses, a measure of vascular function, only changed in those individuals with the lowest pre-training vascular measures, independent of group assignment.

Project two examined the link between vascular and physical function in peripheral neuropathy patients. Reactive hyperemic responses were significantly related to the time up and go test (r=−0.31, p=0.02) and the 6-minute walk distance (r=0.37, p=0.007). These data suggest a link between measures of vascular and physical function in these patients, indicating that those with better vascular function have greater physical function.

Project three examined the effects of an acute bout of exercise on nitric oxide, oxidative stress and anti-oxidants, and brachial vasoreactivity, before, at peak exercise, and in recovery, in trained individuals. The results indicated a significant increase in reactive oxygen species and peroxynitrite, and a decrease in the anti-oxidant glutathione peroxidase at peak exercise. Brachial
vasoreactivity was significantly lower immediately after exercise, but returned to pre-exercise levels at 20 minutes into recovery. These findings suggest an acute bout of exercise contributes to a significant rise in oxidative stress, which can in part be buffered by anti-oxidants systems, but may cause temporary blunting of arterial reactivity. Collectively, these findings indicate the importance of examining vascular function, and its controllers, and may extend the current understanding of preserving and/or maintaining vascular health.
Chapter 1

Introduction

The purpose of this introductory chapter is to provide a foundation upon which the subsequent doctoral work is based. Chapter 2 is an extensive review of the scientific literature pertinent to this dissertation. Chapters 3 to 5 are original investigations conducted by this investigator. Finally, chapter 6 is a conclusion chapter designed to give the reader a summary and connect the findings of the projects outlined.

The endothelium regulates vascular homeostasis by elaborating a variety of paracrine factors that act locally in the blood vessel wall and lumen [2]. Under normal conditions, the sum total effect of these endothelial factors is to maintain normal vascular tone, blood fluidity, and limit vascular inflammation and smooth muscle cell proliferation [2]. Endothelial function may be measured invasively or non-invasively. For example, invasive measures involve the intra-arterial administration of substances (e.g. acetycholine and bradykinin) that enhance the release of endothelial nitric oxide and lead to measurable vasodilatation in normal subjects but vasoconstriction in states of endothelial dysfunction [3]. However, the obvious disadvantage with such a method is that its invasive nature precludes its widespread use in the population. Therefore, the development of non-invasive techniques could easily be used to evaluate endothelial function in conditions where the invasive techniques could not be implemented properly.

Vascular function assessments in this laboratory are done using non-invasive techniques in order to assess vascular reactivity and structure in a variety of populations. The non-invasive techniques commonly used in this laboratory are: (a) mercury strain gauge plethysmography, it is used to determine upper and/or lower regional blood flow, and (b) the brachial artery flow
mediated dilation model using ultrasonography, is a tool used to evaluate functional and/or structural changes and alterations of one single conduit artery. Generally it has been observed that individuals who are less fit, are older, or have chronic diseases, have lower vascular reactivity than fitter, younger and healthier individuals [4].

The main focus of this dissertation was to determine how the vasculature may be influenced or may influence physical function. The first step in the research process started with a follow-up study of previous work from this laboratory. Previous data from this laboratory, has reported regional vascular adaptations when a localized forearm training stimulus was used [5,6]. These studies were used as a foundation to keep exploring the effects of training on the vasculature, specifically the effects of resistance training on blood flow responses. Consequently the goal of the first study was to examine the influence of two volumes (high and low) of whole-body circuit weight training on reactive hyperemia blood flow, venous capacitance and outflow in younger healthy college individuals. The findings revealed significant and similar increases in strength for the high and low volume groups. Although vascular function did not change following circuit weight training, those individuals with the lowest pre-training vascular measures did have significant increases in forearm reactive hyperemia blood flow and venous outflow, independent of group assignment.

The second project in this dissertation focused on individuals who may have lower vascular function due to some sort of pathologic condition. The prevalence of peripheral neuropathy increases significantly in the older adult population and nearly one-third of all diabetics suffer from this condition [7]. In addition, irrespective of etiology, pathological changes associated with peripheral neuropathy are in many cases consistent with ischemia, secondary to vascular insufficiencies [8]. Thus the second study was intended to examine the
relationship between measures of vascular (both arterial and venous) and physical function (mobility and lower extremity strength) in individuals with peripheral neuropathy. These data indicated a relationship between measures of vascular and physical function in peripheral neuropathy. Indeed, individuals with greater physical function scores exhibited more favorable measures of arterial inflow and venous outflow.

Although the previous studies were able to provide important information on how the vasculature may be influenced or may influence physical function, these findings also demonstrated how complex the study of vascular function could be. Finally, last step in this process was to begin the exploration in some putative factors thought to control vascular function. Clearly, vascular homeostasis is important to maintain vascular function. In that regard, Deanfield et al., [9] have speculated the vascular function is very much influenced by the balance of pro- and anti-oxidants. Thus the last study investigated the effects of an acute bout of exercise on nitric oxide bioavailability, oxidative stress markers, and antioxidants markers at three time points, before, at peak exercise, and at 20 minutes into the recovery phase in aerobically trained individuals. These data suggest that an acute bout of exercise contributes to a significant rise in reactive oxygen stress on the brachial artery, which can in part be buffered by anti-oxidants systems, but may cause temporary blunting of the reactivity of the brachial artery. In summary the findings of this dissertation further add to the current understanding of the role of the vasculature in physical function.
Chapter 2
Review of Literature

2.1 Introduction

Cardiovascular diseases remain the leading cause of death in the United States and most western countries. Mortality data show that cardiovascular disease total mentioned in 2002 constituted about 58% of all deaths that year [10]. Furthermore, cardiovascular disease is the underlying cause of death accounted for 36.3% of all deaths in 2004, or 1 of every 2.8 deaths in the United States. In fact, an estimated 79 million American adults (1 in 3) have 1 or more types of cardiovascular disease. Of these, 37 million are estimated to be age 65 or older [11]. Finally, the economic burden of cardiovascular disease is enormous [12]. In fact, in 2004 the costs of cardiovascular disease totaled an estimated $352 billion dollars in the United States [12].

Proper treatment of cardiovascular disease includes risk factor modification, pharmacotherapy, and surgery. Among the modifiable behaviors that increase risk of cardiovascular disease are cigarette smoking, high blood pressure, obesity, and physical inactivity. In regard to this last factor, there is no doubt that physical activity is beneficial for improving health. Therefore, the role of physical activity will be addressed in this review, in part because of the interest by the author, and second because evidence suggests that regular physical activity, as a therapeutic intervention, not only beneficially effects symptoms and enhances myocardial perfusion but also reduces mortality in patients with coronary artery disease/myocardial infarction [13,14].

Over the last decade, exercise training has emerged as a very important intervention for primary and secondary prevention of coronary artery disease [15]. Position statements developed by the Surgeon General [16], the National Institutes of Health Consensus Development Panel on
Physical Activity and Cardiovascular Health [17], and the Centers for Disease Control and Prevention and the American College of Sports Medicine [18] call for every adult to accumulate at least 30 minutes of moderate-intensity physical activity on most, preferably all, days of the week to prevent cardiovascular heart disease and other chronic diseases. Furthermore, exercise should promote all aspects of physical conditioning, including aerobic capacity and muscular endurance, range of motion and flexibility, and muscular strength.

Importantly, despite the evidence of the benefits of regular physical activity in the management of individuals with cardiovascular disease, the manner in which exercise is prescribed is often “generic” rather than tailored to an individual’s state, severity and type of cardiovascular disease. This is particularly important given the evidence that individuals’ responses, adaptations, and compensations to a disease are often highly variable. In fact, the problems with cardiovascular disease are not just limited to the cardiovascular system. In most cases cardiovascular disease is the “tip” of the iceberg, as many other physiologic systems attempt to compensate for the problems incurred by the cardiovascular system. These “compensatory changes” are critical to understand as they to a large degree contribute to the clinical course of patients afflicted by cardiovascular diseases.

This is particularly relevant in terms of choosing an appropriate treatment path. More specifically, a “generic” recommendation to accumulate 30 minutes of exercise over the course of a day may be insufficient to truly alter the disease progression or could in fact be harmful. For example, clinicians may need to recognize that in many cases compensatory changes to a particular form of cardiovascular disease is dramatically different from other manifestations of the same disease. This is critical as we need to move from generic exercise programs to individualized targeted treatment plans that may be more effective in its delivery. It is with this
concern in mind that has led me to examine the literature more closely. In doing so, two interesting distinctive outcomes of cardiovascular disease are discussed: (a) Heart Failure, and (b) Peripheral Arterial Disease.

The first one, heart failure is a condition that results from myocardial dysfunction that impairs the heart's ability to pump blood at a rate sufficient to maintain the metabolic needs of peripheral tissues and various organs. As a result of the cardiac problems, skeletal muscle blood flow decreases contributing to the cardinal symptoms of heart failure including breathlessness and fatigue on exertion. Although the primary defective organ in heart failure is the heart, the periphery, including skeletal muscle becomes a secondary defective organ of major clinical significance in the manifestation of the disease. Of major concern is preferential skeletal muscle atrophy, in that more oxidative muscle fibers (Type I) are lost, and non-oxidative muscle fibers (Type IIa and IIb) are preferentially maintained.

The second condition included is peripheral arterial disease, a manifestation of systemic atherosclerosis in the lower limb distal to the aortic bifurcation. As with heart failure, peripheral arterial disease is associated with significant morbidity and mortality. Anatomically peripheral arterial disease is defined as atherosclerotic arterial disease, while functionally it is defined as arterial narrowing, causing a decrease in muscle blood flow, and consequently a mismatch between the oxygen supply and metabolic demand resulting in symptoms of intermittent claudication, exercise limitations, or tissue loss. Patients with peripheral arterial disease also undergo significant compensatory changes in skeletal muscle. However, in contrast to heart failure, peripheral arterial disease patients show significant atrophy in non-oxidative (Type IIa and IIb) skeletal muscle fibers, and preferentially retain the oxidative fibers (Type I). Thus, two conditions, which essentially have a common underlying problem of reduced muscle blood flow
and subsequent exercise intolerance, have vastly different physiological and morphological compensatory changes. It is this differential compensatory adaptation that serves as the major stimulus for this review. The reason being that if patients with such a different compensation to essentially the same problem, that is reduced blood flow, a generic recommendation for exercise cannot be effective. Thus ultimately the quest is to determine the most effective exercise strategies for individuals with cardiovascular disease. Those strategies can only evolve from a detailed understanding of the underlying pathophysiological changes. Therefore, it is with that in mind that this review is written. Consequently, the main focus of this review is to discuss the central and peripheral mechanisms of exercise intolerance in patients with heart failure and peripheral arterial disease.

2.2 Heart Failure

A major physiologic problem for patients with heart failure is a reduction in muscle blood flow. As a consequence the cardinal symptoms of this disease are exercise intolerance, shortness of breath, and chronic fatigue. In this section the following issues will be addressed: (a) the definition, (b) the incidence and prevalence, (c) the etiology and pathophysiology, (d) the cardinal symptoms and clinical outcomes, (e) the diagnostic tests and the prognostic value of exercise testing, and finally (f) the classification of heart failure related to the degree of symptom severity. Next, this section will present information regarding the principal circulatory and muscular compensatory adaptations in heart failure patients. Finally, this section will discuss the acute exercise responses, and the response to exercise training in heart failure patients. The purpose of this section is to increase the understanding of the manner in which patients with heart failure compensate to their reduction in blood flow, and the potential ramifications for treatment strategies.
2.2.1 Definition

Heart failure is defined as "the pathophysiological state in which an abnormality of cardiac function is responsible for failure of the heart to pump blood at a rate commensurate with the requirements of the metabolizing tissues and/or to be able to do so only from an elevated filling pressure" [19]. This definition implies that there is an alteration in the normal physiological pumping capacity of the heart.

Heart failure is a complex clinical syndrome that can result from any structural or functional cardiac disorder (e.g. myocardial dysfunction) that impairs the heart's ability to circulate blood at a rate sufficient to maintain the metabolic needs of peripheral tissues and various organs [20]. The cardinal manifestations of heart failure are fatigue or dyspnea on exertion progressing to dyspnea at rest [20].

Patients with heart failure suffer from decreased exercise capacity, impaired quality of life, and poor prognosis [21]. Furthermore, the inability to perform exercise without discomfort may be one of the first symptoms experienced by patients with heart failure and is often the principal reason for seeking medical care. Therefore, exercise intolerance is inextricably linked to the diagnosis of heart failure [22].

2.2.2 Incidence and Prevalence

Heart failure is a major and growing public health problem in the United States. Approximately 5 million patients in this country have heart failure, and over 550 000 patients are diagnosed with heart failure for the first time each year [23]. The disorder is the primary reason for 12 to 15 million office visits and 6.5 million hospital days each year [24]. Indeed, heart failure is the most common Medicare diagnosis-related group (i.e. hospital discharge diagnosis), and more Medicare dollars are spent for the diagnosis and treatment of heart failure than for any
other diagnosis [25]. In addition, the estimated direct and indirect cost of heart failure in the United States for 2007 is $33.2 billion [11].

The incidence of heart failure approaches 10 per 1000 population after age 65 [23]. Heart failure is primarily a condition of the elderly [26], and thus the widely recognized “aging of the population” also contributes to the increasing incidence of heart failure. Furthermore, a community-based cohort study using the resources of the Rochester Epidemiology Project conducted in Olmsted County, Minnesota [27], showed that the incidence of heart failure has not declined during 2 decades, but survival after onset has increased overall, with less improvement among women and elderly persons. Finally, the Framingham Heart Study [28] have revealed that at age 40, the lifetime risk of developing heart failure for both men and women is 1 in 5, and the lifetime risk of heart failure occurring without antecedent of myocardial infarction is 1 in 9 for men and 1 in 6 for women.

The Framingham Heart Study and the Framingham Offspring Study [29] indicated that hypertension is a very common risk factor for heart failure. Truly, seventy-five percent of heart failure cases have antecedent of hypertension [28], and the lifetime risk doubles for people with blood pressure greater than 160/90 mm Hg versus those with blood pressure less than 140/90 mm Hg [28]. In addition, the Heart and Estrogen/progestin Replacement Study (HERS) of the predictors of heart failure among women with coronary heart disease found that diabetes was the strongest risk factor [30]. Furthermore, it is relevant to mention that heart failure is a common manifestation of the later stages of various cardiovascular diseases, including coronary artery disease, hypertension, valvular disease, and primary myocardial disease [11].

Heart failure total-mentioned mortality in 2002 was 286 700 [11]. On the basis of the 44-year follow-up among Framingham Heart Study participants, 80% of men and 70% of women
under age 65 who have heart failure will die within 8 years [28]. After heart failure is diagnosed, survival is poorer in men than in women, but fewer than 15% of women survive more than 8 to 12 years. In people diagnosed with heart failure, sudden cardiac death occurs at 6 to 9 times the rate of the general population [28]. The 2004 overall death rate for heart failure was 19.1%. Death rates were 20.3% for white males, 22.9% for black males, 18.3% for white females, and 19.0% for black females [31].

2.2.3 Etiology and Pathophysiology

The etiology of heart failure is displayed in table 2.1. The clinical syndrome of heart failure may result from disorders of the pericardium, myocardium, endocardium, or great vessels, but the majority of patients with heart failure has symptoms due to an impairment of left ventricular myocardial function [32]. Heart failure may be associated with a wide spectrum of left ventricular functional abnormalities, which may range from patients with normal left ventricular size and preserved ejection fraction to those with severe dilatation and/or markedly reduced ejection fraction [32].

In most patients, abnormalities of systolic and diastolic dysfunction coexist, regardless of ejection fraction. Patients with normal ejection fraction may have a different natural history and may require different treatment strategies than patients with reduced ejection fraction, although such differences remain controversial [32]. The most common causes of heart failure in the United States are coronary artery disease, systemic hypertension, non-ischemic dilated cardiomyopathy, and valvular heart disease [23].

Other frequent causes are myocarditis and diabetes mellitus, but there are a great many less common causes [20]. As a result, when an excessive workload is imposed on the heart by increased systolic blood pressure (pressure overload), increased diastolic volume (volume
overload), or loss of myocardium, normal myocardial cells hypertrophy in an effort to enhance contractile force of the normal areas [33]. The subsequent alterations in biochemistry, electrophysiology, and contractile function lead to mechanical alterations of myocardial function.

**Table 2.1 Etiology of heart failure**

<table>
<thead>
<tr>
<th>Etiology</th>
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<tr>
<td>Coronary artery disease</td>
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<tr>
<td>Hypertension</td>
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<tr>
<td>Valvular heart disease</td>
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<tr>
<td>Familial/genetic cardiomyopathy</td>
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<tr>
<td>Metabolic disorders</td>
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<td>Myocarditis</td>
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<td>Collagen vascular disease</td>
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<td>Endocrine disorders</td>
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<td>Electrolyte disorders</td>
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<td>Acidosis</td>
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<td>Sepsis</td>
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<tr>
<td>Hypoxia</td>
</tr>
<tr>
<td>Alcohol</td>
</tr>
<tr>
<td>Toxins (chemotherapy, cocaine)</td>
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<tr>
<td>Peripartum</td>
</tr>
</tbody>
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The rate of contraction slows, the time to develop peak tension increases, and myocardial relaxation is delayed. Peak force development may be well preserved with enough viable myocardium and adequate time for the development of force. Thickening of the ventricular wall limits the rate of ventricular filling (diastolic dysfunction), which is worsened by increased heart rate because it shortens the duration of ventricular filling [34].

The force of myocardial contraction is eventually reduced as cell loss and hypertrophy continues, leading to significant geometric ventricular alterations and increased volumes [20]. After the initial compensatory phase, the increase in intracavitary volume is usually associated with further reductions in ventricular ejection fraction (progressive systolic dysfunction) and
eventually abnormalities in the peripheral circulation from activation of various neurohumoral compensatory mechanisms [33].

Finally, the abnormal neurohumoral responses lead to increased systemic sympathetic tone and activation of the renin-angiotensin system [35]. Production of angiotensin increases, causing peripheral vasoconstriction. The increase in peripheral arterial resistance limits cardiac output during exercise. The increased levels of angiotensin II also stimulate release of aldosterone by the adrenal glands, enhancing sodium retention and thus leading to fluid retention and peripheral edema [33].

2.2.4 Symptoms

The symptoms of heart failure can be caused by either an abnormality in systolic function that leads to a defect in the ejection of blood from the heart (systolic heart failure) or an abnormality in myocardial diastolic function that leads to a defect in ventricular filling (diastolic heart failure) [32]. Reduced left ventricular filling caused by diastolic dysfunction leads to decreased stroke volume and associated symptoms of low cardiac output, whereas increased filling pressures lead to symptoms of pulmonary congestion [20]. Thus, some characteristics of heart failure (e.g. the inability of the left ventricle to provide adequate forward output to meet the demands of the skeletal muscles during exercise while maintaining normal filling pressures) may result primarily from diastolic dysfunction, which, in some patients, can occur with normal left ventricular systolic function [32]. There are no exact data on the prevalence of diastolic dysfunction leading to heart failure in the presence of normal systolic function.

Several studies have shown, however, that as many as 40% of all patients with a clinical diagnosis of heart failure may have preserved LV systolic function, and many of these patients have evidence of diastolic dysfunction [32]. The following section will present a brief overview
of the clinical findings in heart failure patients. First, the major symptoms that affect heart failure patients will be outlined.

2.2.4.1 Shortness of Breath

Dyspnea or breathlessness (a subjective feeling of air hunger), is the earliest and most frequent symptom in heart failure. Initially, dyspnea on exertion will be noted by a change in the extent of physical activity that causes shortness of breath. As heart failure worsens, the intensity of exertion required will decrease. The patient with heart failure will progressively develop paroxysmal nocturnal dyspnea, orthopnea, and eventually dyspnea at rest [36].

2.2.4.1.1 Paroxysmal Nocturnal Dyspnea

Paroxysmal nocturnal dyspnea normally occurs after the patient has been asleep and in the supine position for some time. Suddenly, the patient wakes up with a sensation of choking and air hunger; assuming an upright posture usually relieves the symptoms. Frequently the patient will feel better after opening a window or going outside to catch a breath of fresh air. Paroxysmal nocturnal dyspnea usually precedes orthopnea; it may be associated with bronchospasm and wheezing (cardiac asthma) and can be confused with an attack of bronchial asthma, especially in patients with prior known chronic obstructive pulmonary disease [33]. Many patients with heart failure also have chronic obstructive pulmonary disease; it may be difficult to distinguish between the dyspneic symptoms secondary to heart failure and those that are due to chronic obstructive pulmonary disease.

Most severe episodes of paroxysmal nocturnal dyspnea can cause a feeling of intense suffocation and may leave the patient gasping for breath. The uncomfortable sensation may persist for 30 minutes or more after the patient has assumed an upright posture [37]. Paroxysmal nocturnal dyspnea is primarily caused by mobilization of interstitial fluid (especially in patients
with edema). The result is increased circulating blood volume and increased pulmonary venous pressure, especially in patients with left ventricular diastolic dysfunction [38].

2.2.4.1.2 Orthopnea

Orthopnea is defined as dyspnea that occurs often within a few minutes when the patient assumes a supine position; sitting up or standing usually improves the symptoms. The most severely affected patients usually sleep sitting upright in a chair. Orthopnea has the same cause as paroxysmal nocturnal dyspnea, but it represents more severe cardiac impairment [33].

2.2.4.1.3 Nocturnal Dyspnea or Exertional Dyspnea

Nocturnal dyspnea is a dry or nonproductive cough. This is due to pulmonary congestion and in patients with heart failure is usually relieved by successful treatment of the heart failure [36].

2.2.4.2 Fatigue and Weakness

Fatigue and generalized weakness are present in heart failure patients particularly in the limbs. These symptoms are secondary to low cardiac output with decreased perfusion of skeletal muscles and can occur with exertion or at rest; they may be worsened after eating because of the increased splanchnic demand for blood flow, which may stress the limited reserve. Low-output syndromes can be present, without evidence of pulmonary congestion, and limit performance during exercise testing [37]. It is important to emphasize that breathlessness and fatigue on exertion are the dominant symptoms of heart failure [39]. Indeed, these symptoms are the primary determinants of their health-related quality of life [22].

A traditional view of the pathophysiology of the symptoms runs as follows: first, the failing heart has an impaired response to exercise. In turn, this leads to failure to perfuse the exercising muscle adequately; the unhappy skeletal muscle signals the brain, and this sensation is
interpreted as fatigue. Second, the failing heart requires an ever higher left ventricular filling pressure to maintain output, particularly during exertion; the filling pressure can rise high enough to cause either stiff lungs or even transudation of fluid into the alveoli, which causes breathlessness [39].

2.2.4.3 Nocturia and Oliguria

Nocturia is a common and early symptom in heart failure. Renal filtration of sodium and water is decreased in patients with compromised left ventricular function, in part, because of the redistribution of blood flow away from the kidneys in the upright position and during physical activity. Urine formation is enhanced in the recumbent position when renal stimulus for vasoconstriction decreases and venous return to the heart increases. Oliguria is associated with a markedly reduced cardiac output and is usually a sign of terminal heart failure; it indicates a poor prognosis [36].

2.2.4.4 Cerebral Symptoms

Elderly patients with advanced heart failure may develop confusion, memory impairment, anxiety, headaches, insomnia, nightmares, and, occasionally, disorientation, delirium, and hallucinations. These cerebral symptoms are predominantly related to reduce cardiac output and poor perfusion of brain and other neurologic tissues [33].

2.2.4.5 Abdominal Symptoms

Patients with heart failure may also develop gastrointestinal complaints because of hepatic congestion and edema of the abdominal wall and intraabdominal organs [36]. Congestion of abdominal organs may be present with ascites, abdominal fullness and enlargement, easy satiety, bloating, anorexia, nausea, vomiting, constipation, and upper abdominal discomfort. The abdominal discomfort is usually described as a dull ache or heaviness that can be enhanced or
reproduced by upper abdominal or hepatic palpation. This is consistent with the likely cause, the stretching of the hepatic capsule [40]. Patients can frequently detect early re-accumulation of fluid by the recurrence of abdominal fullness before the signs become obvious. This symptom can be easily overlooked, however. Asking the patient about a recent change in waist size or atightening of the clothing at the waist is often helpful [40].

After describing the major symptoms among heart failure patients, it is necessary to review the clinical outcomes obtained in a medical examination. Normally, during a physical examination, patients with heart failure of recent onset appear acutely ill but well nourished. On the other hand, patients with advanced stages of heart failure frequently appear malnourished, and occasionally cachectic. Thus, some of the clinical outcomes or signs revealed throughout a physical examination will be outlined in the next section.

2.2.5 Clinical Outcomes

2.2.5.1 Cardiomegaly

Cardiomegaly with a laterally displaced, enlarged, and sustained ventricular impulse may be found on physical examination, but this is a nonspecific finding and can be absent, particularly in patients with acute heart failure. The decrease in ventricular compliance may initially become apparent by the presence of a late diastolic atrial sound (S4 gallop). A protodiastolic sound (S3 gallop) occurs in patients with more advanced heart failure and is caused by acute deceleration of ventricular inflow after the early filling phase [41]. An S3 gallop, however, can also be detected in other conditions such as mitral and tricuspid regurgitation and a left-to-right shunt.

Gallop sounds are more readily audible in the presence of a rapid heart rate. The presence of a third heart sounds appears to be associated with an increased risk of death, death from pump
failure, and hospitalization for heart failure [41]. Systolic murmurs are common in heart failure and are largely secondary to mitral or tricuspid regurgitation that can result from ventricular dilatation. These murmurs frequently diminish or disappear after adequate treatment and reduction of ventricular size [40].

2.2.5.2 Systemic Venous Hypertension

Can be detected by abnormal distension of the jugular veins (the upper limit of the jugular venous pressure is about 4 cm above the sternal angle with the patient recumbent at a 45° angle) [41]. Although the jugular venous pressure normally declines on inspiration, it can rise in patients with right heart failure (Kussmaul's sign). Persistent elevation of the jugular venous pressure is one of the earliest and most reliable signs of right-heart failure [41]. The inability of the right ventricle to accept transient increases in venous return (hepatojugular reflux) is observed during transient compression (≥30 s) of the upper abdomen [40].

Systolic pulsations of the liver may be felt in patients with tricuspid regurgitation. Liver enlargement and tenderness on palpation are marked by epigastric fullness and dullness to percussion in the right upper quadrant. These findings may persist after other signs of heart failure have disappeared because it takes longer for hepatic congestion to disappear. In some cases, the liver enlargement does not disappear because of structural changes in patients with long-standing heart failure. The presence of an elevated jugular venous pressure appears to be associated with an increased risk of death, death from pump failure, and hospitalization for heart failure [36,41].

2.2.5.3 Pulsus Alternans

Pulsus alternans is common in patients with heart failure; when severe, it can be detected by sphygmomanometry or by palpation of peripheral pulses, particularly the femoral pulse. This
sign is characterized by a regular rhythm of alternating strong and weak pulsations [42]. Sometimes the weak beat may be so small that the aortic valve does not open and no aortic or arterial pulse is produced, resulting in total alternans and a pulse that is only half as fast as the apical beats [40]. With total alternans, a first heart sound will occur, but no second heart sound if both the aortic and pulmonic valves fail to open.

Pulsus alternans appears to be due to an alternation in the stroke volume of the left ventricle, possibly because of an incomplete recovery of myocardial cells and thus a decrease in the responsiveness of contracting cells on alternate beats [43]. It can be persistent or paroxysmal, or it may occur only after a premature beat or with the Valsalva maneuver. The hemodynamic response to the Valsalva maneuver has been found useful in the clinical evaluation of patients with heart failure [43].

In this maneuver the blood pressure cuff is inflated to just about 15 mm Hg above the systolic blood pressure before the maneuver. In a normal response, the Korotkoff sounds disappear during sustained maneuver and reappear after completion of the maneuver. An abnormal response, in which blood pressure sounds either do not disappear or are maintained throughout the maneuver, has been found in patients with heart failure [42]. In normal patients, the decrease in blood pressure during the maneuver is associated with tachycardia and the increase in blood pressure after release with bradycardia. No changes in heart rate are observed in heart failure patients.

Although heart failure is a clinical diagnosis based on a careful history and physical examination, there are diagnostic tests that have shown being reliable in the evaluation of patients with heart failure [40]. Thus, the following section will present information regarding these diagnostic tests.
2.2.6 Diagnostic Tests

2.2.6.1 Chest Radiograph

Cardiomegaly (cardiothoracic ratio > 50%) can be found on chest film in 87% of patients when first diagnosed with primary dilated cardiomyopathy [40]. The lower lobes of the lung are normally better perfused than are the upper lobes; with heart failure, there is progressive vasoconstriction of vessels in the lower lobes and redistribution of the pulmonary flow to the upper lobes. Interstitial and perivascular edema develop with acute increases in pulmonary capillary wedge pressure above 20-25 mm Hg; bronchovascular markings at the bases are prominent.

Interstitial edema can present as perivascular or peribronchial edema (initially in perihilar and then in peripheral zones) [36]. Kerley's lines, spindle-shaped linear opacities at the periphery of the lung bases, occur in the later stages of heart failure; pleural fluid can produce discrete interlobular-type linear opacities and subpleural fluid accumulation between the lung and adjoining pleura [40].

The accumulation of fluid in major and minor lung fissures can be of considerable size and may be incorrectly diagnosed as a tumor mass in the lung [37]. These "phantom tumor" shadows, however, have smooth margins and disappear with resolution of heart failure. With acute increases in pulmonary capillary wedge pressures above 25 mm Hg, alveolar edema, pleural effusions, or both may occur. Heart failure patients may show elevated pulmonary capillary pressures in the range of 25-35 mm Hg or more without interstitial or alveolar edema, reflecting associated increased lymphatic flow [44]. After therapy that lowers pulmonary capillary pressure, there may be a delay of 24-48 h before improvement and clearing of pulmonary infiltrates can be seen on chest radiograph [40].
2.2.6.2 Electrocardiography

Changes in the 12-lead electrocardiography are generally nonspecific. Sinus tachycardia is usually present in uncompensated heart failure or in end-stage disease with a low stroke volume that requires tachycardia to maintain the cardiac output. Isolated premature ventricular beats are common, and complex ventricular arrhythmias can be detected in most patients during prolonged (24-48 h) Holter monitoring. Electrocardiographic findings suggestive of atrial and ventricular chamber enlargement may be evident. Intraventricular conduction delays are also common and include left bundle branch block as well as other, nonspecific repolarization changes [40].

2.2.6.3 Exercise Stress Testing

There are three major types of exercise tests used to obtain an objective measurement of exercise performance in heart failure patients [45]; (a) the symptom-limited progressive exercise test carried to the point of exhaustion (cardiopulmonary exercise stress testing); (b) the test of endurance at a predetermined level of exercise (which is submaximal); and (c) tests of submaximal exercise performance, such as the time taken to complete a typical walking task or the distance covered at a comfortable walking speed along a corridor (the corridor walking test) or a self-propelled treadmill [46]. The first two tests are done using a bicycle ergometer or treadmill [47]. For the last one, submaximal exercise performance, the 6-min walk test has been promoted as a safe, well-tolerated, and a simple measure of functional capacity in heart failure patients [48].

In addition, the most common measure of fatigue in practice is the Borg scale [49], which can be graded separately for fatigue and dyspnea. Although fatigue remains a major complaint of patients, it is rare in practice to monitor this symptom or its progression [50]. It is important to
emphasize the prognostic value of some of the above exercise tests used to evaluate patients with heart failure. Therefore, two specific modalities will be addressed in the next section. The first one, an incremental test with metabolic gas exchange measurement, which is the most useful [39]; the second, the 6-minute walk test, which is commonly used and simple to administer [51]. The prognostic value of exercise testing in heart failure is as follow:

2.2.6.3.1 Cardiopulmonary Exercise Stress Testing

A cardiopulmonary exercise stress testing properly performed provides an objective measurement of peak functional capacity, and has become an important clinical tool with which to define disease severity and to predict outcome in patients with heart failure due to left ventricular systolic dysfunction [52]. Indeed, cardiopulmonary exercise stress testing plays a pertinent role in decision-making about the workup of the heart failure patient [47].

During a cardiopulmonary exercise stress testing, the subject exercises to exhaustion, breathing through a tight fitting mask or mouthpiece, and the expired air is collected and analysed [53]. Contrary to what is observed in athletes or fit subjects, what is measured at the end of exercise in a heart failure patient is not a plateau of maximum oxygen consumption ($V_{O2_{max}}$) [54]. Heart failure patients generally stop exercising early because of fatigue or dyspnea. Other reasons for stopping a test include chest pain, ischemia, decrease in blood pressure, arrhythmias [53].

The cardiopulmonary exercise stress testing provides information concerning three important variables [39]: (a) peak oxygen consumption ($V_{O2}$), (b) the slope of the relation between ventilation and carbon dioxide production ($V_e/V_{CO2}$), and (c) the respiratory exchange ratio (RER). Peak oxygen consumption is defined as the maximum rate of oxygen consumption achieved and is used as an index of exercise performance. The slope of the relation between
ventilation and carbon dioxide production is used as an index of the ventilatory response to exercise. Finally, the respiratory exchange ratio is defined as the ratio of carbon dioxide production to peak oxygen consumption.

There is overwhelming evidence of the role played by peak oxygen consumption in stratifying risk in heart failure [39,55,56]. In fact, a study by Mancini et al., [57] is considered to be the cornerstone of the documentation of the prognostic power of peak oxygen consumption. In that regard, the study by Mancini et al., [57] divided heart failure patients into the following three groups (see table 2.2).

**Table 2.2** Peak oxygen consumption (VO\(_2\)) in stratifying risk in heart failure patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Peak VO(_2)</th>
<th>1-Year Survival Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Patients with peak VO(_2) of &lt; 14 mL/kg/min who had been accepted for heart transplantation</td>
<td>48%</td>
</tr>
<tr>
<td>2</td>
<td>Patients with peak VO(_2) of ≥ 14 mL/kg/min who had transplant deferred</td>
<td>94%</td>
</tr>
<tr>
<td>3</td>
<td>Patients with peak VO(_2) of &lt; 14 mL/kg/min but with significant comorbidity that precluded heart transplantation</td>
<td>47%</td>
</tr>
</tbody>
</table>

Furthermore, a peak VO\(_2\) of < 10 mL/kg/min was associated with significantly poorer predicted survival [45]. Lastly, the results of the study by Mancini et al., [57] were adopted by the American Heart Association/American College of Cardiology consensus statement on the selection and treatment of candidates for heart transplantation [58].

In addition, the anaerobic threshold has been proposed as a submaximal index of exercise capacity, independent of the patient’s motivation, and has been classically defined as the point where lactate increases in plasma during exercise, as a consequence of the transition from total aerobic to aerobic plus anaerobic metabolism [45]. A failure to reach the ventilatory anaerobic
threshold strongly suggests poor motivation or non-cardiovascular limitation of exercise tolerance. Indeed, Opasich et al., [59] evaluated the predictive role of peak VO₂ in 505 heart failure patients when the ventilatory anaerobic threshold (VAT) was or was not detected (see table 2.3).

These findings were also reported by Corra et al., [45]. Indeed, the study by Corra et al., [45] concluded that when ventilatory anaerobic threshold is detected, patients with a peak VO₂ of < 10 mL/kg/min have a high event rate, whereas those with a peak VO₂ of > 18 mL/kg/min have a good prognosis. However, it is important to recognize that a multiplicity of factors influence any given peak VO₂ value [45]. In fact, it is well-known that peak VO₂ is affected by age, gender, body weight, muscle mass, and conditioning status [60,61].

Table 2.3 Predictive role of peak VO₂ and VAT in heart failure patients

<table>
<thead>
<tr>
<th>Peak VO₂</th>
<th>VAT was identified cardiac event rates</th>
<th>VAT was not detected cardiac event rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 10 mL/kg/min</td>
<td>59%</td>
<td>46%</td>
</tr>
<tr>
<td>&gt; 10 to ≤ 14 mL/kg/min</td>
<td>32%</td>
<td>29%</td>
</tr>
<tr>
<td>&gt; 14 to ≤ 18 mL/kg/min</td>
<td>32%</td>
<td>23%</td>
</tr>
<tr>
<td>&gt; 18 mL/kg/min</td>
<td>15%</td>
<td>22%</td>
</tr>
</tbody>
</table>

In addition, many studies have revealed that ventilatory expired gas parameters obtained from symptom-limited cardiopulmonary exercise stress testing represent prognostic value in heart failure patients [62,63]. In that regard, figure 2.1 displays the relationship between ventilation and carbon dioxide production (VE/ VCO₂). The VE/ VCO₂ slope is highly linear, and the slope is increased with increasing severity of heart failure that is, for any given level of carbon dioxide production, the ventilator response is increased with worsening heart failure symptoms [39].
Finally, Corra et al., [45] after reviewing some of those findings have suggested that the risk stratification procedure with symptom-limited cardiopulmonary exercise stress testing in heart failure patients, traditionally based on peak oxygen consumption and ventilator anaerobic threshold, should be replaced by a new prognosticating algorithm that is structured on a multiparametric decoding scrutiny employing the stepwise introduction of peak VO$_2$, VE/VCO$_2$ slope, and peak respiratory exchange ratio [45].

![Graph](image)

**Figure 2.1.** Ventilation/carbon dioxide production (VE/ VCO$_2$) slope in heart failure. Note that the relation between VE and VCO$_2$ remains linear but that the slope increases with worsening heart failure. Thus, for a VCO$_2$ of 1 L/min, a normal subject has to ventilate at 22 L/min and the patient with moderate heart failure ventilates at 42 L/min [39].

2.2.6.3.2 The 6- Minute Walk Test

The 6-minute walk test is considered to be realistically related to daily physical activity [64]. Furthermore, the 6-minute walk test is used to estimate functional capacity in patients with a variety of medical conditions, and can be performed by those who are unable or unwilling to
undertake formal treadmill or bicycle exercise tests [65]. Thus, among heart failure patients, it has been incorporated into studies of assessment of exercise capacity [66], of quality of life [67], of efficacy of new therapeutic agents [68] including beta-blockers [69], of physical training programs [70], and of prognostic stratification [71,72].

According to the American Thoracic Society (ATS) Statement for Guidelines for the Six-Minute Walk Test [73], the 6-minute walk test should be performed indoors, along a long, flat, straight, enclosed corridor with a hard surface that is seldom traveled. If the weather is comfortable, the test may be performed outdoors. The walking course must be 30 m in length. A 100-ft hallway is, therefore, required. The length of the corridor should be marked every 3 m. The turnaround points should be marked with a cone (such as an orange traffic cone). A starting line, which marks the beginning and end of each 60-m lap, should be marked on the floor using brightly colored tape.

In order to study the potential usefulness of the 6-minute walk test, the Studies of Left Ventricular Dysfunction (SOLVD) Registry Substudy followed 833 heart failure patients for an average of 242 days [71]. This cohort reported that the 6-minute walk test is a safe and simple clinical tool that strongly and independently predicts morbidity and mortality in patients with left ventricular dysfunction. Indeed, table 2.4 displays the significance of the 6-minute walk test in estimating a patient’s prognosis in heart failure [71]. Furthermore, previous studies have identified that walking <300 m during the 6-minute walk test is a simple prognostic marker of subsequent cardiac death in patients with mild-to-moderate heart failure [74].

However, studies that have evaluated the relationship between the 6-minute walk test to peak oxygen consumption (VO₂) in heart failure patients have reported that the 6-minute walk test cannot be used in the individual heart failure patient as an alternative to, or as a surrogate of,
peak VO$_2$ [48,64,75]. In that regard, Opascih et al., [64] found no significant correlation between the distance walked during the 6-minute walk test and hemodynamic indices (left ventricular ejection, cardiac index, right and left filling pressures) and found only a moderate correlation with functional capacity indicators (peak VO$_2$).

Therefore, these investigators concluded that the walking performance does not provide prognostic information which can either complement or substitute that provided by peak VO$_2$ or the New York Heart Association (NYHA) classification for heart failure [64].

**Table 2.4** Relationship between 6-minute walk test distance and heart failure readmission rates and mortality

<table>
<thead>
<tr>
<th>6-minute walk test distance</th>
<th>Heart failure readmissions</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;300 m</td>
<td>22.16%</td>
<td>10.23%</td>
</tr>
<tr>
<td>300–374.9 m</td>
<td>11.20%</td>
<td>7.88%</td>
</tr>
<tr>
<td>375–449.9 m</td>
<td>3.72%</td>
<td>4.19%</td>
</tr>
<tr>
<td>≥450 m</td>
<td>1.99%</td>
<td>2.99%</td>
</tr>
</tbody>
</table>

Furthermore, the prediction of peak VO$_2$ based on regression equations derived from the relationship between peak VO$_2$ and 6-minute walk test results in too much variability to be clinically useful [48]. While 6-minute walk test may be appealing based on its ease of application and potential prognostic value in a subset of heart failure patients, this variable does not correlate well with peak and submaximal exercise parameters that have proven validity and reliability in heart failure patients [48]. This investigator concluded that when an accurate determination of functional capacity is required, direct measurements of peak VO$_2$ and ventilatory anaerobic threshold are still the preferred outcome measures in heart failure patients.

In summary, peak VO$_2$ is a convincing, strong, and independent predictor of outcome, supporting the value of cardiopulmonary exercise stress testing as a clinical tool in heart failure.
However, in the last few years recent research have discovered that a single arbitrary cut point value for peak VO$_2$ is unlikely to describe the true risk of events, and that a multiparametric stepwise approach may be more successful. Finally, the 6-minute walk test, a submaximal exercise test, may serve as a more straightforward clinical tool to assess functional capacity when the cardiopulmonary exercise stress testing could not be evaluated in heart failure patients.

The above section have outlined the significant prognostic value of two exercise tests modalities, the cardiopulmonary exercise stress testing and the 6-minute walk test. The next section will continue addressing the additional diagnostic tools used in patients with heart failure.

2.2.6.4 Echocardiography

The single most useful diagnostic test in the evaluation of patients with heart failure is the comprehensive 2-dimensional echocardiogram coupled with Doppler flow studies to determine whether abnormalities of myocardium, particularly cardiomyopathy (dilated, restrictive, hypertrophic), heart valves, or pericardium are present and which chambers are involved [40].

2.2.6.5 Laboratory Blood Tests

In severe heart failure, neurohumoral compensatory mechanisms frequently lead to hyponatremia and other significant electrolyte abnormalities, even without the use of diuretics, which (especially the thiazide type) may contribute to hyponatremia [76]. This effect is less prominent with furosemide, the only diuretic that increases free water clearance through a mechanism that is resistant to the action of vasopressin [43]. In fact, furosemide may be necessary to correct hyponatremia in patients with heart failure [43]. Hypokalemia is also very common with use of diuretics, especially the long-acting thiazides. Hyperkalemia may occur in patients with very low cardiac output or renal insufficiency. It can also occur in patients who are ingesting additional potassium through salt substitutes, especially when given in addition to a potassium-sparing
diuretic or angiotensin-converting-enzyme inhibitor, such as captopril, which also blocks the release of aldosterone. Reduced renal blood flow can lead to moderate increases in the blood urea nitrogen and mild increases in sodium creatinine [40].

2.2.6.6 Cardiac Catheterization

Left-heart catheterization and angiography are necessary when the presence and extent of coronary artery disease need to be determined, particularly if cardiac surgery (valve replacement, aneurysmectomy, coronary artery bypass surgery) can possibly improve ventricular dysfunction. Right-heart catheterization may be useful in evaluating and selecting patients with refractory heart failure who require customized treatment [40].

2.2.6.7 Radionuclide Ventriculography

This method is helpful in documenting the severity of left ventricular systolic dysfunction and indicating whether the wall motion abnormalities are global or regional. This test is especially helpful in patients who present technical difficulties for echocardiography; it can, for example, be obtained easily even in obese patients. Comparison of right and left ventricular stroke volumes is also helpful in establishing the severity of regurgitant lesions [76].

So far, this review has presented the major symptoms, signs and diagnostic tools in heart failure patients that help physicians to determine the current clinical status of these patients. In addition, the information presented until now is needed to properly quantify the degree of functional limitation in heart failure patients.

2.2.7 Classification According to the Symptom Severity

The approach that is most commonly used to quantify the degree of functional limitation imposed by heart failure is one first developed by the New York Heart Association (NYHA) [77]. The NYHA classification has 4 classes. This system assigns patients to 1 of 4 functional
classes, depending on the degree of effort needed to elicit symptoms (see table 2.5).

In addition, The American College of Cardiology (ACC) and the American Heart Association (AHA) have jointly engaged in the production of guidelines in the area of cardiovascular disease since 1980. This effort is directed by the ACC/AHA Task Force on Practice Guidelines. The 2001 report by the ACC/AHA Guidelines for the Evaluation and Management of Chronic Heart Failure in the Adult [78] have identified 4 stages involved in the development of the heart failure syndrome (see table 2.6). Stages A and B patients are best defined as those with risk factors that clearly predispose toward the development of heart failure.

Table 2.5 The NYHA classification for heart failure

<table>
<thead>
<tr>
<th>Class</th>
<th>The New York Heart Association Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea</td>
</tr>
<tr>
<td>Class II</td>
<td>Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, or dyspnea</td>
</tr>
<tr>
<td>Class III</td>
<td>Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnea</td>
</tr>
<tr>
<td>Class IV</td>
<td>Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms are present even at rest or minimal exertion</td>
</tr>
</tbody>
</table>

This classification recognizes that there are established risk factors and structural prerequisites for the development of heart failure and that therapeutic interventions introduced even before the appearance of left ventricular dysfunction or symptoms can reduce the population morbidity and mortality of heart failure. Furthermore, this classification system is intended to complement but in no way to replace the New York Heart Association functional classification [77], which primarily gauges the severity of symptoms in patients who are in Stage C or Stage D.
Table 2.6 The ACC/AHA classification for heart failure

<table>
<thead>
<tr>
<th>Stage</th>
<th>The American College of Cardiology and the American Heart Association Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage A</td>
<td>Patients with coronary artery disease, hypertension, or diabetes mellitus who do not yet demonstrate impaired left ventricular (LV) function, hypertrophy, or geometric chamber distortion</td>
</tr>
<tr>
<td>Stage B</td>
<td>Patients who are asymptomatic but demonstrate LV hypertrophy (LVH) and/or impaired LV function</td>
</tr>
<tr>
<td>Stage C</td>
<td>Patients with current or past symptoms of heart failure associated with underlying structural heart disease (the bulk of patients with heart failure)</td>
</tr>
<tr>
<td>Stage D</td>
<td>Patients with truly refractory heart failure who might be eligible for specialized, advanced treatment strategies, such as mechanical circulatory support, procedures to facilitate fluid removal, continuous inotropic infusions, or cardiac transplantation or other innovative or experimental surgical procedures, or for end-of-life care, such as hospice</td>
</tr>
</tbody>
</table>

In summary, heart failure is a major and growing public health problem in the United States. Heart failure is a clinical syndrome that impairs the heart's ability to circulate blood at a rate sufficient to maintain the metabolic needs of peripheral tissues and various organs. The cardinal manifestations of heart failure are fatigue or dyspnea on exertion. In addition, exercise intolerance is inextricably linked to the diagnosis of heart failure. Finally, the New York Heart Association classification is used to quantify the degree of functional limitation imposed by heart failure. The next section will present information regarding the principal circulatory and muscular compensatory adaptations in heart failure patients.

2.2.8 Compensatory Adaptations to Heart Failure

Heart failure develops in response to an insult to the cardiovascular system [79]. This alteration is principally due to three distinct etiologies [80]; (a) intrinsic myocardial disease secondary to coronary heart disease, cardiomyopathy, and infiltrative disease; (b) excess work load due to increased resistance to ejection (pressure load) secondary to hypertension and hypertrophic cardiomyopathy or increased stroke volume (volume load) secondary to aortic
insufficiency and valvular insufficiency; and (c) iatrogenic myocardial damage from drugs (doxorubicin and disopyramide) or radiation therapy for mediastinal tumors or Hodgkin's disease.

The insult to the cardiovascular system is frequently met with a variety of compensatory adaptations with short-to long-time constants aimed to maintain cardiac output and arterial pressure to adequately perfuse the brain and the heart. These compensatory adaptations include: (a) an increase in ventricular end-diastolic volume and pressure (ventricular dilatation); (b) sympathetic nervous system activation; (c) neurohumoral vasoconstriction; (d) renal sodium and water retention; (e) myocardial hypertrophy; (f) impaired vasodilatory capacity; and (g) intrinsic changes in skeletal muscle. Although, these compensatory adaptations may be remarkably effective under resting conditions, the capacity to sustain cardiac performance in the face of hemodynamic overload relative to myocardial contractility is finite and exacts a price [81]. Indeed, it is those compensatory mechanisms that ultimately contribute significantly to the clinical severity of the disease.

Therefore, the focus of this section of this review is on the morphological, biochemical, cellular, and neurohumoral changes characteristic of heart failure and how these relate to the exercise tolerance, a marker of the clinical severity of the disease. A particular focus of the review is the manner in which these changes alter skeletal muscle blood flow, thereby significantly affecting the patient’s functional abilities.

2.2.8.1 Cardiovascular Compensatory Adaptations

It is important to reinforce that regardless of the etiology, heart failure results from myocardial dysfunction that impairs the heart's ability to circulate blood at a rate sufficient to maintain the metabolic needs of peripheral tissues and various organs [82]. This applies to both
the resting and active states and results in significant vasoconstriction and the shunting of blood toward vital organs and away from peripheral structures [83].

In addition, the manifestations of heart failure depend on the rate at which the syndrome develops [82]. Generally, if the underlying cardiac abnormality develops slowly, compensatory mechanisms have time to become activated, and the patient will be able to adjust to the altered cardiac output [40]. In contrast, if the underlying condition develops rapidly or an acute precipitating factor is present, the result may be inadequate organ perfusion or acute congestion of the venous bed draining into the affected ventricle, causing sudden cardiac decompensation, with a concomitant reduction in cardiac output and an acute onset of symptoms [40,82].

Thus, the development and progression of heart failure depend on the etiology and severity of the underlying disease. Furthermore, there are basically four mechanisms that initiate the sequence leading to heart failure: (a) pressure overload, (b) volume overload, (c) primary myocardial disease (myocarditis, cardiomyopathy), and (d) myocardial infarction [40,84]. In each case, through different mechanisms, the myocardium mechanically compensates for the primary defect before the stage of overt myocardial failure develops. In addition, complex neuroendocrine changes [84,85] attempt to maintain the circulation and normal organ perfusion in the face of decreasing myocardial function. These changes engage activation of the renin-angiotensin and other systems that cause peripheral vasoconstriction, thereby maintaining the blood pressure while punishing the heart by increasing the afterload [83,84]. Although the central hemodynamic abnormalities are the primary pathophysiological features of heart failure, the neurohumoral responses play an important role in its progression and prognosis. Therefore, this section will review first the major hemodynamic adaptations, and then the neurohumoral reactions.
2.2.8.1.1 Hemodynamic Adaptations

2.2.8.1.1 The Heart

In healthy circumstances, the anatomy and physiology of the heart are carefully construed and maintained so as to perform compressive mechanical work at near-maximal efficiency to deliver an adequate cardiac output over the normal range of rest and exertional pressure-volume conditions, commensurate with the demands of the body’s metabolizing tissues [83]. On the other hand, chronic heart failure is the pathophysiological state in which either the delivery of cardiac output is inadequate to meet the metabolic demands of tissues or an adequate cardiac output is delivered only under conditions of abnormally elevated intracardiac pressures, which prematurely precipitate systemic or pulmonary venous congestion [86].

Although still debated, the primary ‘‘locus’’ of dysfunction in most instances of heart failure appears to reside largely within the myocyte compartment of the heart [83]. Indeed, studies support a marked reduction in ‘‘contractility reserve’’ in response to increasing heart rate of sympathetic stimulation [87]. The normal integrated function of the heart depends upon a host of factors that include: (a) normal ultrastructural and gross architecture of the heart, (b) an adequate number of myocytes, (c) normal myocyte contractile and relaxant function, (d) normal structure and function of cardiac valves, (e) normal structure, composition, and metabolism of the myocardial extracellular matrix, (f) adequate myocardial perfusion, and (g) normal myocardial metabolism [40,83].

However, heart failure patients exhibit abnormalities in any of these major anatomic and functional components. Indeed, heart failure patients can present: (a) loss of myocytes (ischemic, inflammatory or toxic necrosis, apoptosis), (b) acute or chronic contractile dysfunction of myocytes (inflammation, alcohol, chemotherapeutic agents, sepsis, hypoxia), (c) excessive
myofiber architectural disorganization or disarray (hypertrophic cardiomyopathy), (d) infiltrative cardiomyopathy, (e) extracellular matrix structural or functional abnormalities (excessive fibrosis leading to abnormal force transduction, myocyte linkage, and inadequate compressive force efficiency), (f) distortion of the three-dimensional shape of the heart itself (aneurysm, infarction, chronic valvular disease), and (g) intractable pressure or volume overload (hypertension, valvular heart disease) [83,87]. As a result, an initial injury causes dysfunction of the left ventricle that leads to decrease in cardiac output and blood pressure [83].

2.2.8.1.1.2 Cardiac Hypertrophy

Left ventricular dysfunction is generally a progressive process, even in the absence of a new identifiable insult to the heart [40]. The principal manifestation of such progression is a change in the geometry and structure of the left ventricle, so the chamber dilates and/or hypertrophies and becomes more spherical, a process referred to as cardiac remodeling [88]. Left ventricular remodeling is defined as the alterations in ventricular mass, chamber size, and shape that result from myocardial injury, or pressure or volume overload [88]. This change in chamber size and structure not only increases the hemodynamic stresses on the walls of the failing heart and depresses its mechanical performance but may also increase regurgitant flow through the mitral valve. These effects, sequentially, serve to sustain and exacerbate the remodeling process.

Cardiac remodeling generally precedes the development of symptoms (occasionally by months or even years), continues after the appearance of symptoms, and contributes substantially to worsening of symptoms despite treatment [88]. The increase in pressure as a result of aortic stenosis or sustained severe hypertension requires that the left ventricular pressure must increases in order to overcome the resistance to the ejection of blood [40,88]. The initial mechanism for the increase at a cellular level is probably a stretch-induced increase in the inotropic state,
involving mechanoreceptors and increased cytosolic calcium. The result is that the left ventricular systolic pressure rises, the obstruction to the outflow of blood from the left ventricle is overcome, and the cardiac output is maintained [40,84]. However, the disadvantage of this form of adaptations is that the left ventricle wall stress is greatly increased.

As a result cardiac concentric hypertrophy develops, it means that the left ventricle becomes thicker without increasing in radius, and the wall stress normalizes. However, the compensatory hypertrophy in chronic heart failure patients has a greater susceptibility to ischaemia and abnormal diastolic function [84]. In addition, left ventricular hypertrophy is characterised by an increased left ventricular wall thickness and mass, with abnormal diastolic properties [37]. There is a triad of loss of (a) distensibility (so that the ventricle is stiffer), (b) impaired relaxation and (c) decreased early diastolic filling. This pattern is responsible for the exertional shortness of breath that chronic heart failure experience. [84,89].

In contrast, the increase in volume due to valvular regurgitation (incompetence) of either the mitral valve or the aortic valve requires that the heart respond in a different way. First, the preload increases, so that the heart functions at the length limit of the Frank-Starling curve [38]. Second, the sustained volume load causes longitudinal hypertrophy, thereby increasing the size of the left ventricle cavity, which, in turn, could increase the chamber size without increasing the wall thickness [90]. The end-result of the volume overload is enhanced early diastolic filling and decreased left ventricular stiffness [91]. As a result, the diastolic function improves rather than deteriorates, as in a pressure overload. Nonetheless, as the chamber size increases, wall tension must rise. The consequence will be modest and proportional hypertrophy, to allow the left ventricle cavity to regain a normal wall stress [40,84]. At the cellular level, myocardial pathologic changes accompany left ventricular remodeling and involve myocyte hypertrophy and
fibroblast hyperplasia accompanied by an increase in collagen deposition within the interstitium. These processes, which occur in the noninfarcted myocardium, contribute to progressive left ventricular remodeling and left ventricular dysfunction [50].

Progression of coronary artery disease, diabetes mellitus, hypertension, or the onset of atrial fibrillation may also contribute to the progression of heart failure. The development of structural abnormalities can have 1 of 3 outcomes: (a) patients die before developing symptoms (in Stage A or B of the New York Heart Association Classification for Heart Failure), (b) patients develop symptoms controlled by treatment, or (c) patients die of progressive heart failure. Sudden death can interrupt this course at any time. Although several factors can accelerate the process of left ventricular remodeling, there is substantial evidence that the activation of endogenous neurohormonal systems plays an important role in cardiac remodeling and thereby in the progression of heart failure. Indeed, substantial amounts of experimental and clinical data now exist that support the pivotal role of the Renin-Angiotensin-Aldosterone-System in contributing to these cellular processes [32,84,89].

Patients with heart failure have elevated circulating or tissue levels of norepinephrine, angiotensin II, aldosterone, endothelin, vasopressin, and cytokines, which can act (alone or in concert) to adversely affect the structure and function of the heart. These neurohormonal factors not only increase the hemodynamic stresses on the ventricle by causing sodium retention and peripheral vasoconstriction but may also exert direct toxic effects on cardiac cells and stimulate myocardial fibrosis, which can further alter the architecture and impair the performance of the failing heart. Neurohormonal activation also has direct deleterious effects on the myocytes and interstitium, altering the performance and phenotype of these cells [78]. Finally, it is important to mention that in response to a sustained pressure or volume load, the myocardium passes from a
compensated phase to a dilated failing phase. It is speculated that the hypertrophied myocardium is susceptible to an imbalance in the ratio of oxygen supply and demand [84,92].

2.2.8.1.1.3 Cardiac Output

Cardiac output is defined as the amount of blood ejected from the ventricles per minute, and it represents a measure of the pumping ability of the heart. It is determined by the heart rate and the stroke volume [83]. Among healthy individuals the average resting cardiac output is around 5.00 L/min [93]. Early in the process of heart failure, cardiac output may be normal at rest [20]. During stressful conditions, such as physical exercise and other periods of increased metabolic demand, however, cardiac output fails to increase normally.

As a result the glomerular filtration rate declines and the renal mechanism for salt and water retention becomes activated [20,83]. Furthermore, ventricular filling pressure as well as pressures in the atrium and venous system behind the affected ventricle may rise abnormally during periods of stress. This may cause transudation of fluid and symptoms of tissue congestion during exercise [20,40]. In such early stages, physical rest may induce diuresis and relieve symptoms in many patients with mild heart failure; however, excessive and repeated strenuous physical activities will worsen the compromised hemodynamic state and cause progression of heart failure [37,38]. The impaired left ventricular function causes inadequate blood flow to active skeletal muscle secondary to reduced cardiac output and consequently low maximal oxygen uptake in these patients [83].

2.2.8.1.2 Neuroendocrine (neurohumoral) Reaction

The impaired contraction and/or relaxation of the heart, which is the major component in the pathophysiology of heart failure patients, result in the inability to fulfill its pumping function normally. In addition, left ventricular dysfunction leads to reduction in cardiac output and blood
pressure. As a result, a complex series of events leading to a neuroendocrine reaction, also called the neurohumoral response [84], which creates the self-perpetuating vicious circle of heart failure (see figure 2.2).

![Diagram of the neuroendocrine vicious circle in CHF](image)

**Figure 2.2.** The neuroendocrine vicious circle in chronic heart failure [84].

The initial activation of the neuroendocrine system would restore the hemodynamic balance through vasoconstriction and retention of salt and water. However, at the same time this also contributes to progression of heart failure due to the hemodynamic effects and the direct harmful hormonal effects on the cardiomyocytes [89]. Several neurohumoral systems are activated in heart failure. Furthermore, extensive reviews discussing the impact the neuroendocrine system and heart failure can be found elsewhere [85,94-96].

Although several neurohormonal systems are activated in heart failure patients, the next section will outline the more important of those systems with emphasis on prognostic and
diagnostic of heart failure. The topics presented will include; (a) alterations in cardiovascular reflexes, (b) catecholamines and the sympathetic nervous system, (c) humoral activation, (d) renin-angiotensin-aldosterone system, (e) vasopressin, (f) atrial natriuretic peptide, (g) endothelin-1, (h) cytokines, and (i) the ergoreflex.

2.2.8.1.2.1 Alterations in Cardiovascular Reflexes

The arterial baroreflexes include an afferent limb, a central neural component, and an autonomic neuroeffector component. A rise in pressure stretches the baroreceptors located in the heart, lungs, and great vessels and causes them to transmit signals to the central nervous system [97]. These signals normally inhibit sympathetic outflow as well as suppress renin and vasopressin release while increasing parasympathetic outflow.

Conversely, a sudden reduction in arterial pressure results in a strong sympathetic discharge throughout the body. The short-term effect of the sympathetic discharge is to enhance stroke volume, cardiac output and increase vasoconstriction in an effort to compensate for a loss in arterial pressure [98]. However, the long-term consequences are thought to be deleterious [99]. In the acute phase of low-output heart failure, arterial and cardiopulmonary baroreflexes are activated to help maintain systolic blood pressure. However, both arterial and cardiopulmonary baroreceptors become desensitized in heart failure [100]. The absence of baroreflex inhibitory input to medullary centers results in excessive sympathetic excitation [39,84]. It is thought that the marked desensitization of this reflex contributes to the neurohumoral abnormalities [89].

2.2.8.1.2.2 Catecholamines and the Sympathetic Nervous System

A rise in systemic vascular resistance is accomplished initially, by activation of the sympathetic nervous system. Activation of the sympathetic nervous system is very effective over the short term to maintain blood pressure [101]. Plasma norepinephrine concentration [102,103],
an index of the activity of this system, is generally two or three times higher in patients with heart failure in comparison to healthy controls, and it is usually correlated to the degree of left ventricular dysfunction and hemodynamic abnormalities [104], and it carries an ominous prognosis [102,103]. In addition, it has been shown that plasma norepinephrine reflects sympathetic activity in heart failure patients fairly well [105].

The increase in sympathetic activation is to maintain cardiac output and arterial pressure through stimulation of myocardial contractility and \( \alpha \)-1 adrenoreceptors in the vascular wall. Stimulation of the \( \alpha \)-1 adrenoreceptors causes an increase in vasomotor tone and systemic vascular resistance. However, any supportive effect of the increased sympathetic nervous system activity on arterial blood pressure is, in time, superseded by a number of organ-specific consequences. The potential adverse effects of sympathetic activation on cardiac muscle, the kidneys, and the vasculature are numerous and contribute to the clinical manifestations of heart failure [106].

2.2.8.1.2.3 **Humoral Activation**

Humoral activation is one of the biochemical hallmarks in patients with heart failure [107,108]. The main function of the humoral activation is to maintain arterial pressure despite a loss in myocardial function. However, as a result of chronic humoral activation two vicious cycles develop: (a) vasoconstriction and (b) sodium and water retention. Arterial vasoconstriction causes an increase in systemic vascular resistance and inevitably an increase in afterload and systolic wall stress.

The sodium and water retention causes an increase in circulating volume, which may be beneficial at first, but ultimately results in an increased ventricular filling pressure and diastolic wall stress. Together, these vicious cycles may eventually lead to progressive myocardial and
vascular dysfunction, peripheral tissue abnormalities, fluid accumulation, and finally the clinical picture of heart failure [109].

2.2.8.1.2.4 Renin-Angiotensin-Aldosterone System

Renin is a proteolytic enzyme synthesized and secreted into the blood by the juxtaglomerular cells located in the macula densa in the afferent arterioles of the glomeruli [110]. Renin, itself, is not a vasoactive substance. Instead it acts on another plasma protein called angiotensinogen, produced by the liver, to release a 10-amino acid peptide, angiotensin I [110]. Although angiotensin I has mild vasoconstrictor properties, within a few seconds following its formation two additional amino acids are split to form an 8-amino acid peptide, angiotensin II. The formation of angiotensin II is catalyzed by angiotensin converting enzyme which is located in the endothelium of the vascular tree [110].

The effects of angiotensin II are multiple and include: (a) raising peripheral resistance through vasoconstriction, (b) enhancing renal sodium reabsorption, (c) facilitating catecholamine release from sympathetic nerve endings, and (d) stimulating mineralcorticoid production in the adrenal gland [89,111]. More recently angiotensin II has also been implicated as a growth factor or growth modulator in the cardiovascular system [112-114]. In fact, Dzau et al., [112] have reported that the renin-angiotensin-aldosterone system is markedly activated in patients with decompensated heart failure. In addition, data from the Study of Left Ventricular Dysfunction (SOLVD) Registry report resting values for plasma renin activity in healthy adults ranging from 0.3 to 0.8 (mean 0.6) ng/ml•h⁻¹ [115]. In the same study, plasma renin activity in patients with heart failure ranged from 0.5 to 8.7 (mean 2.5) ng/ml•h⁻¹ [115]. The mechanism of the increased plasma renin activity in heart failure is not entirely known but is thought to be secondary to (a) a reduction in renal perfusion, (b) elevated efferent renal sympathetic nerve
activity, (c) increased circulating catecholamines, (d) alterations of sodium load presented to the macula densa, or (e) a combination of all the above [108,116].

As a result of the increased renin-angiotensin-aldosterone system activation systemic vascular resistance increases [89]. In addition, the formation of angiotensin II causes sodium and fluid retention secondary to production of the mineralcorticoid, aldosterone. Aldosterone causes a marked increase in sodium reabsorption from the renal tubules, thereby raising extracellular sodium, followed by fluid expansion [110]. The function of the increased fluid is to increase preload in patients with heart failure secondary to myocardial dysfunction. It is thought that the increase in preload subsequently stretches the sarcomeres in the myocardium to a more optimal length during the intermediate period until myocardial hypertrophy develops.

Angiotensin II is potentially a stimulus to fibrosis, as is aldosterone. Indeed, in animal experiments it has been shown that angiotensin II has a direct effect on the myocardium which eventually leads to hypertrophy, remodeling and fibrosis [117,118] and thereby to loss of function. In physiological amounts, collagen may help to limit ventricular dilation when it is increased in proportion to the degree of myocardial hypertrophy. On the contrary, when there is an excessive collagen response to ischaemia or endocrine signals such as angiotensin II, then compliance decreases with an increase in chamber stiffness, or a decrease in distensibility [119].

These modifications promote poor diastolic relaxation and, hence, diastolic failure. The wall tension therefore rises more than expected, with a corresponding increase in the oxygen demand. Therefore, it contributes to relative ischaemia of the hypertrophied myocardium and further promotes interstitial fibrosis. With the discovery that the renin-angiotensin-aldosterone system is markedly activated in heart failure, it is not surprising that a significant number of investigators have focused on the role of angiotensin-converting-enzyme (ACE) inhibitors
as a strategy to treat patients with heart failure. Results from clinical trials, The SOLVD Investigators [120] and The CONSENSUS Trial Study Group [121], have shown that cardiovascular morbidity and mortality can be reduced in patients with left ventricular dysfunction (ejection fraction < 40%) secondary to ischemic heart disease following long-term use of ACE-inhibitors.

Interestingly, the precise mechanism(s) of action of ACE-inhibitors are still not fully understood. It is thought that the mechanism of action of ACE-inhibition is in part cardioprotective and vasculoprotective. A reduction in systemic vascular resistance secondary to inhibition of angiotensin II generation may reduce afterload and myocardial work. Mechanisms for the vasculoprotective properties include in addition to a reduction in angiotensin II production, decreased bradykinin degradation, antagonism of macrophage function and migration as well as inhibition of sympathetic nervous system and thrombotic activity [122]. All of these could result in plaque stabilization, protection against plaque rupture, and prevention of acute coronary occlusion [122].

2.2.8.1.2.5 Vasopressin

Vasopressin is synthesized as a larger pre-hormone in nerve cells located in the hypothalamus. Then, it is transported through the axons of the nerve cells to their end located in the neurohypophysis [110]. The physiological actions of vasopressin are vasoconstriction mediated via V₁-receptors and decrease in free-water clearance mediated via renal V₂-receptors. In heart failure patients the vasopressinergic system is activated, contributing to vasoconstriction and water retention. It has been shown that the sensitivity of the vasopressin system for changes in osmolality and volume is decreased in heart failure [123,124]. In addition, some studies have reported that vasopressin is increased in heart failure [125,126] and that the increase could not be
explained by changes in osmolality. Vasopressin is especially elevated in heart failure with hyponatraemia. Hyponatremia is a common manifestation of severe heart failure and occurs when water is retained in excess of sodium. It has been shown to be a powerful independent predictor of cardiovascular mortality [127].

2.2.8.1.2.6 Atrial Natriuretic Peptide

Atrial natriuretic peptide is primarily produced in the atria and released by atrial distention. Atrial natriuretic peptide dilates arterioles and increases sodium excretion from the kidneys and is an antagonist to both the sympathetic and the renin-angiotensin-aldosterone system. Three known natriuretic receptors exist, A, B and C. Atrial natriuretic peptide has highest affinity for the A receptor [110]. Thus, the action of atrial natriuretic peptide is to decrease plasma volume. It have been reported that atrial natriuretic peptide caused a net transfer of fluid from the vascular to the interstitial space. This raises the possibility that atrial natriuretic peptide may have a vascular effect which may contribute to edema formation in heart failure [128].

The plasma concentration of atrial natriuretic peptide is elevated in heart failure and in general correlates with the degree of heart failure [103,126,129,130]. In addition, the activation of the atrial natriuretic peptide in heart failure patients initially counteracts the effect of the vasoconstrictor systems, but the effect is not lasting. Hence, it has been reported that the natriuretic response to atrial natriuretic peptide is blunted in heart failure [131].

2.2.8.1.2.7 Endothelin-1

Endothelin-1 is synthesized from a larger pre-propeptide which after enzymatic cleavage becomes the propeptide “big endothelin-1”. Endothelin-1 levels are increased in heart failure patients [132,133], and it has been reported that correlates strongly with the pulmonary pressure
Endothelin-1 acting via selective receptors has strong vasoconstrictor effects and possibly also a trophic effect on cardiac myocytes, found in in vitro studies [135]. In addition, endothelin-1 was found to contain prognostic information, in a multivariate analysis including other hormones (atrial natriuretic peptide and noradrenaline) and hemodynamic measures [136].

2.2.8.1.2.8 Cytokines

Cytokines act locally in one of several manners: (a) autocrine; active on the cells of origin, (b) paracrine; acting on neighboring cells, or (c) justacrine; acting on adjacent cells [84]. Among the cytokines that appear to be involved in heart failure, tumor necrosis factor-alpha has drawn special attention. Indeed, Bozkurt et al., [137] have reported that tumor necrosis factor-alpha causes dilatation and remodeling of the left ventricle in rats. In addition, tumor necrosis factor-alpha is increased in heart failure and seems to reflect the severity of the disease [138,139].

2.2.8.1.2.9 The Ergoreflex

Ergoreceptors are intramuscular receptors sensitive to work performed. They are stimulated by metabolites formed during skeletal muscular activity. Their stimulation increase ventilation and sympathetic activation (the ergoreflex). The stimulus is in part sensitive to work performed by each unit of muscle mass. This effect can be seen in normal subjects, where the ventilation and carbon dioxide production slope is considerably greater when arms are used than when legs are used to perform the same external workload [140].

In addition Piepoli et al., [141] showed how these receptors were more active in patients with heart failure and that the increased ventilatory response to exercise was proportional to the ergoreflex activity. The signal is due to metabolic stimulation of receptors rather than being merely a response to movement. Possible stimuli for initiating the ergoreflex are local release of
prostaglandins, potassium, and hydrogen ion. Furthermore, in heart failure there is enhanced activity of these receptors (“Coats hypothesis”) [142], which contributes to the excessive neuroendocrine response.

2.2.8.2 Skeletal Muscle Compensatory Adaptations

The result of impaired cardiac pumping capacity and limited vasodilatory capacity is an inadequate blood supply to metabolizing tissues [143]. Therefore, a reduction in skeletal muscle perfusion may be an important contributor to the decreased exercise capacity and tolerance in heart failure patients. However, even when oxygen availability to skeletal muscle is improved oxygen uptake remains unchanged [144,145]. This observation suggests the presence of intrinsic alterations of skeletal muscle. Recently, considerable attention has been given to identify those intrinsic alterations in skeletal muscle which may contribute to the clinical severity of patients with heart failure [146-152].

These studies strongly suggest the presence of skeletal muscle abnormalities including (a) ultrastructural abnormalities, such as significant atrophy of muscle fibers classified as Type I [144,148,150,151]; (b) a marked decline in mitochondrial enzyme concentration and activity (Succinate dehydrogenase, Citrate synthase and cytochrome oxidase); and (c) a reduction in mitochondrial volume and density [144,146].

2.2.8.2.1 Skeletal Muscle Atrophy

Muscle atrophy is common in patients with heart failure [144,148,150,153,154]. Indeed, Minotti et al., [154] found that maximal cross-sectional area of the thigh, measured by magnetic resonance imaging, was markedly reduced in patients with end-stage heart failure compared to age-matched controls. These data have been confirmed by Magnusson et al., [155] who reported a 13% smaller muscle cross-sectional area of the quadriceps in heart failure patients. In addition,
Mancini et al., [156] noted a 15% reduction in muscle volume in the lower legs of patients with mild-to-moderate heart failure.

The mechanism for the muscle atrophy in heart failure is presently not clear but has been linked to malnutrition, deconditioning, an increased catabolic state due to sympathetic nervous system hyperactivation, an increase in serum cortisol, corticotropin, and/or tumor necrosis factor [126]. Consequently, it appears that skeletal muscle atrophy could be an important and potentially reversible contributor to exercise intolerance in patients with heart failure. However, it should be noted that parameters of muscle mass only show a weak correlation with peak oxygen consumption (\(\text{VO}_{2\text{peak}}\)), suggesting that muscle atrophy contributes only modestly to exercise intolerance in heart failure [156].

2.2.8.2.2 Skeletal Muscle Blood Flow

Many studies have reported reduced resting blood flow to the arm and leg in heart failure [143,144,157,158]. On the other hand, Wiener et al., [159] found no evidence of reduced blood flow at rest in ambulatory, optimally diuresed heart failure patients. Although the reason for these different findings at rest are speculative it may be related to (a) the level of circulatory dysfunction, (b) the position in which the measurements were obtained (supine or standing), (c) the amount of muscle atrophy present, and/or (d) the pharmacotherapy of the patient.

2.2.8.2.3 Histologic and Biochemical Alterations in Skeletal Muscle

It appears that skeletal muscle atrophy in heart failure is selective and more pronounced in the type I (oxidative) muscle fibers. Indeed, Lipkin et al., [160] have reported atrophy of type I and type II fibers, increased interstitial cellularity, and excess lipid accumulation, in patients with heart failure. Mancini et al., [146] and Sullivan et al., [148] also reported a relative greater decrease of highly oxidative, fatigue resistant, type I fibers in the calf muscle of patients with
heart failure secondary to coronary artery disease. In addition, Drexler et al., [149] found a shift in fiber type distribution to type II fibers. In this comprehensive study, the authors indicate that because type IIb fibers possess less oxidative capacity than type IIa or even I, a reduction in oxidative capacity could be attributed to a shift in fiber type distribution.

Furthermore, to these morphologic changes, Sullivan et al., [148] reported a decrease in the number of capillaries per fibers for type I and type IIa fibers. The apparent loss in oxidative capacity in heart failure is further evident from studies which have performed biochemical analysis of skeletal muscle. In fact, Lipkin et al., [160] was the first to demonstrate an accumulation of intracellular lipids in heart failure suggesting a possible abnormality in lipid metabolism. Sullivan et al., [148] examined patients with long-standing heart failure and found significant reductions in mitochondrial enzyme concentration for succinate dehydrogenase and citrate synthetase. Furthermore, they found a decrease in 3-hydroxacyl-coenzyme A-dehydrogenase and glycogen content.

Drexler et al., [144] reported that the ultrastructural morphometry of muscle biopsies of the vastus lateralis indicated significant abnormalities of skeletal muscle as compared to normals. The volume density of mitochondria and surface density of mitochondrial cristae, markers of structural correlates of oxidative capacity, were significantly reduced by 20% in patients with severe heart failure.

In addition, capillary length density was reduced and fiber type distribution of skeletal muscle was shifted to type II fibers. Cytochemical analysis of cytochrome oxidase activity also revealed significant decreases in heart failure. Both the volume density of mitochondria and surface density of mitochondrial cristae were significantly related to oxygen consumption (VO_{2peak}), but inversely related to the duration of heart failure.
These studies support the notion that a major component of heart failure is a reduction in oxidative capacity due to intrinsic alterations in skeletal muscle. This reduction in oxidative capacity may play an important role in the clinical syndrome of heart failure by adversely affecting exercise capacity. Although, the mechanisms for the alterations in skeletal muscle metabolism in heart failure patients are unknown, several factors (neurohumoral, chronic reductions in muscle perfusion, and deconditioning) may be involved [144].

Chronic deconditioning may be a key factor in the alterations in skeletal muscle metabolism, since recent studies have shown that physical training can improve exercise capacity in patients with heart failure by delaying the onset of anaerobic metabolism [161-163]. However, chronic muscle underperfusion (at rest or during exercise) and/or increased sympathetic stimulation could also cause many of the above mentioned abnormalities in skeletal muscle metabolism.

2.2.8.3 Summary of Compensatory Adaptations in Heart Failure

In summary the "Heart Failure Syndrome" is characterized by a series of compensatory mechanisms designed to maintain cardiac output and arterial pressure at a level compatible with life. There are compensatory mechanisms designed to cope quickly with a reduction in stroke volume, and there are mechanisms which operate over a longer period of time.

The cardiac compensatory changes include (a) ventricular dilation to stretch the sarcomeres (fast adaptation); (b) ventricular hypertrophy (slow adaptation) and aim to preserve cardiac output. The circulatory changes include (a) autonomic nervous system activation (fast adaptation); (b) humoral activation (intermediate to slow adaptation); (c) impaired vascular responsiveness (slow adaptation), and aim to maintain blood pressure. Alterations in skeletal muscle include (a) skeletal muscle atrophy (slow adaptation) and (b) impaired oxidative
metabolism (slow adaptation). Although speculative, it is thought that those mechanisms with an intermediate to long-time constant eventually contribute to the severe clinical manifestations.

2.2.9 The Role of Exercise in Heart Failure

2.2.9.1 The Acute Exercise Response in Heart Failure

Under normal resting conditions, patients with heart failure generally do not exhibit signs or symptoms of their disease. It is not until a physiologic stress, such as exercise, is introduced that the morbidity associated with heart failure becomes apparent. Therefore, the purpose of this section is to review the effects of a single bout of exercise on patients with heart failure. In particular, this review will focus on: (a) the cardiovascular responses and (b) the skeletal muscle responses.

2.2.9.1.1 Cardiovascular Responses to an Acute Bout of Exercise

2.2.9.1.1.1 Cardiac Output

Normally, the capacity for performing aerobic exercise depends on the ability of the heart to increase cardiac output to the exercising muscles and the ability of these muscles to utilize oxygen from the delivered blood. The increase in cardiac output during acute exercise can be accomplished through an increase in heart rate and/or stroke volume. In general, patients with heart failure demonstrate characteristic responses to dynamic exercise. However, as functional class declines there is a progressive decrease in peak cardiac output, stroke volume, and heart rate [164]. Cardiac output is the product of heart rate and stroke volume. It has been shown that the increase in heart rate contributes to cardiac output throughout exercise [165], whereas stroke volume is thought to be most important during the early stages of exercise [166]. Normally, during maximal upright exercise, cardiac output increases 4 to 6 fold in healthy subjects. This is accomplished by a 2- to 4-fold increase in heart rate and a 20% to 50% augmentation of stroke
volume. The stroke volume increase is accomplished both by use of the Frank-Starling mechanism to maintain left ventricular end-diastolic volume and by more complete left ventricular emptying to reduce end-systolic volume. Both enhanced left ventricular contractility and peripheral vasodilation contribute to the more complete left ventricular emptying observed during exercise. Studies have reported that cardiac output is reduced at any given peak oxygen consumption (VO₂max) in patients with heart failure [163,167]. The reduction in aerobic capacity in heart failure patients seems to be largely mediated by inadequate blood flow to active skeletal muscle secondary to impaired cardiac output [157,168]. Indeed, patients with heart failure may achieve < 50% of the maximal cardiac output attained by healthy individuals at peak exercise.

Stroke volume, which is already decreased at rest, rises only modestly up to a peak of 50 to 65 mL, compared with ≥ 100 mL in healthy subjects.

Stroke volume is determined by four factors; [97] (a) the volume of blood returned to the heart, (b) ventricular distensibility, or the capacity to enlarge the ventricle, (c) ventricular contractility, and (d) aortic and/or pulmonary artery pressure, or the pressure against which the ventricles must contract. Although there are conflicting reports about stroke volume changes during exercise, the traditional thought in healthy individuals suggests that stroke volume increases with increasing rates of work, and seems to level off at approximately 50-60% of maximal capacity [166]. The explanation for the increased stroke volume in healthy individuals is that the Frank-Starling mechanism, defined by an increase in left ventricular end-diastolic volume, operates at lower work rates, whereas an increased degree of contractility, defined by a decrease in left ventricular end-systolic volume, has its greatest effect at higher work rates.

In patients with heart failure stroke volume is consistently lower at any given work load compared to normals [169]. In fact, Weber et al., [169] have reported that the stroke volume
response to exercise was related to severity of disease as defined by functional class. Using this classification they showed that as functional class declined so did stroke volume at rest. In addition, they indicated that those patients with the most severe impairments lost the reserve capacity to raise stroke volume during exercise. Studies have shown that the mechanism to increase stroke volume during exercise in heart failure patients with diastolic dysfunction is further impaired [36]. In these patients changes in ventricular relaxation and filling may increase diastolic pressure in the absence of an increase in left ventricular end-diastolic volumes. This indicates that the exercise intolerance in patients with diastolic dysfunction may be secondary to an inability to rely on the Frank-Starling mechanism to increase left ventricular end-diastolic volumes and stroke volume. This has been confirmed in several studies indicating a close association between indices of ventricular filling and exercise tolerance. In that regard, Sullivan et al., [163] have reported increases in left ventricular filling pressures in patients with heart failure without an increase in left ventricular end-diastolic volume suggesting the presence of diastolic dysfunction. These data suggest that in patients with diastolic dysfunction ventricular filling may be attenuated and contribute to a reduction in stroke volume and exercise tolerance.

The increase in heart rate observed during exercise is linearly related to the work intensity. In patients with heart failure heart rates at rest and during submaximal exercise are increased, possibly indicating an increased demand on the heart. There is an inverse relationship between resting heart rate and mortality in patients with heart failure [170]. In contrast, peak heart rate (HR\text{peak}) responses to an incremental exercise test are generally lower when compared to age-matched healthy normals. This indicates that the heart rate reserve for patients with heart failure is also reduced. The inability to raise the heart rate during exercise is termed chronotropic incompetence [171]. The mechanism responsible for chronotropic incompetence in patients with
heart failure has been linked to abnormal reflex control and down-regulation or decreased responsiveness of beta-receptors.

2.2.9.1.1.2 Autonomic Responses

The sympathetic nervous system has an important role in mediating the response to exercise in healthy individuals [172]. Normally, during exercise plasma norepinephrine levels increase in normal subjects, which is thought to be indicative of increased participation of the sympathetic nervous system [172]. In contrast, patients with heart failure have much greater rise in plasma norepinephrine levels at submaximal exercise work-loads, although at peak exercise, healthy subjects exhibit higher levels of norepinephrine [126]. It is generally believed that the rise in sympathetic nervous system with exercise causes blood to be shunted away from non-exercising tissue to working skeletal muscle [173]. Although the release of epinephrine and norepinephrine is affected by a variety of factors, plasma levels increase gradually with increasing intensities until approximately 50-70% of peak oxygen consumption (VO$_{2peak}$). At this point plasma levels increase markedly reaching an apex at peak exercise. During exercise, patients with heart failure exhibit greater-than-normal increases in plasma norepinephrine levels at submaximal workloads than healthy controls [126].

It has been speculated that this may result in significant sympathetic vasoconstrictor activity and contributing to muscle underperfusion [173]. In addition, studies have reported that patients with heart failure had significant higher resting muscle sympathetic nervous system activity than age-matched controls [174]. Wilson et al., [175] have reported that exercise did not produce a major increase in plasma norepinephrine and sympathetic vasoconstriction in ambulatory patients with marked reduced exercise capacity. In fact, blood flow to non-exercising tissue remained unchanged during exercise.
Based on this research, Wilson et al., [175] have suggested that patients with heart failure do not develop major redistribution of blood flow from non-exercising beds to working muscle. Thus, the current contention does not necessarily support that the reduced exercise tolerance in heart failure is governed by an activated sympathetic nervous system. Nevertheless, it does appear reasonable to postulate that vasoconstriction mediated in part by the sympathetic nervous system could play a role in limiting skeletal muscle blood flow during exercise in heart failure patients [101].

2.2.9.1.3 Vascular Responsiveness

During acute exercise the ability of the peripheral vasculature to dilate plays an important role in determining blood flow to exercising muscle. The ability to dilate the vasculature is largely dependent on the integrity of the vascular wall, including the endothelial lining. The vasodilatory response to increased shear stress is markedly abnormal in patients with heart failure [153, 176, 177]. Jondeau et al., [153] have linked the impaired endothelium-dependent vasodilatation to exercise capacity, and found a strong correlation between peak hyperemic response and peak oxygen consumption ($V_{O2}$peak) in patients with heart failure. Therefore, these data suggest that patients with heart failure exhibit impairment in endothelium-dependent vasodilatation following exercise induced hyperemia, which may contribute to the exercise intolerance.

2.2.9.1.2 Skeletal Muscle Responses to an Acute Bout of Exercise

2.2.9.1.2.1 Skeletal Muscle Strength

Lipkin et al., [160] measured muscle strength in patients with severe heart failure and found a 45% reduction in quadriceps strength compared to age-matched controls. In addition, Magnusson et al., [155] have observed low muscular strength in patients with heart failure and
attributed the reduced strength to a smaller muscle cross-sectional area. In contrast, Minotti et al., [154] found no difference in isometric strength between heart failure and controls, although there was evidence of a marked decline in muscular endurance. This suggests that the specific tension (i.e. tension per unit of cross-sectional area) may not be altered as a result of the disease. Furthermore, Buller et al., [178] confirmed this finding showing no difference in maximal force produced during 3 repetitions, but a marked decline in force production during prolonged knee extension exercise in heart failure. In both studies the degree of muscle dysfunction as defined by muscle endurance, correlated with peak oxygen consumption (VO$_{2peak}$) as measured by cycle ergometry [154,178]. Thus, reduced muscle strength in patients with heart failure is probably due to skeletal muscle atrophy, rather than a change in the contractile apparatus. In contrast, the impaired muscle endurance appears to reflect a qualitative change in skeletal muscle.

2.2.9.1.2.2 Skeletal Muscle Blood Flow

As previously stated a number of studies have reported reduced limb blood flow at rest in heart failure [101,179]. In addition, there is substantial evidence that the increase in blood flow to working muscle is attenuated for each given workload in heart failure [143,157]. In fact, Wilson et al., [157] have reported reduced leg blood flow during submaximal and peak exercise in patients with heart failure using the thermodilution technique. Furthermore, Sullivan et al., [143] have confirmed these findings and found a strong relationship between the leg blood flow response to exercise, and peak oxygen consumption (VO$_{2peak}$) and functional class. The failure of skeletal muscle blood flow to increase normally during exercise appears to be primarily related to an impaired vasodilatory capacity. In patients with heart failure, arteriolar vasodilatation is impaired, as evidenced by a failure of leg vascular resistance to decrease normally with exercise [101,157,180]. The mechanisms involved in the impaired metabolic vasodilatory capacity within
skeletal muscle in heart failure include [158]; (a) excessive sympathetically mediated vasoconstriction, (b) increased levels of angiotensin II and vasopressin, (c) chronic vascular deconditioning, and (d) endothelial dysfunction.

Another explanation for the alterations in skeletal muscle perfusion observed in heart failure patients may be related to the distribution of blood during exercise. It is generally accepted that exercise is associated with an increase in sympathetic vasoconstrictor activity and angiotensin II [181]. This increase in neurohumoral activation serves to increase vascular resistance in non-working tissue and helps to increase arterial blood pressure. However, Wilson et al., [175] have reported that patients with heart failure do not develop major re-distributions of blood flow from non-exercising beds to exercising muscle. The mechanism for the failure to shunt blood to working tissue was attributed to a reduced sympathetic vasoconstrictor activity. Certainly, this hypothesis appears to go in the face of traditional views, suggesting that increased sympathetic nervous system activity enhances vasoconstriction in heart failure. Irrespective of the precise mechanism, the above studies provide convincing evidence that skeletal muscle perfusion is altered in heart failure patients during exercise. It is therefore not surprising that exertional fatigue in heart failure has traditionally been attributed to muscle underperfusion [182].

2.2.9.1.2.3 Skeletal Muscle Metabolic Responses

Anaerobic metabolism occurs early during exercise in heart failure and is likely an important cause of exercise intolerance. Studies using 31P-MRI have demonstrated that early anaerobic metabolism occurs independently of reduced muscle blood flow [147,183]. In addition, abnormalities in skeletal muscle metabolism occur in patients with heart failure when compared with healthy individuals during exercise under ischemic conditions [147]. These
skeletal muscle characteristics have important ramifications on substrate and oxygen utilization during exercise. However, it appears that these metabolic alterations are independent of skeletal muscle blood flow. In fact, studies demonstrating a greater increase in the inorganic phosphate to phosphocreatine ratio, a measure of oxidative stress, and more pronounced drop in acid-base balance (pH) during progressive forearm exercise in heart failure compared to healthy controls despite similar forearm blood flows [159,183]. In addition, Massie et al., [184] explored the metabolic responses in exercising muscle under ischemic conditions. In this study, it was demonstrated that skeletal muscle metabolic responses of the flexor digitorum were still markedly different in heart failure patients, when exercise was performed with a cuff sphygmomanometer inflated to 250 mmHg. These data indicate an increased reliance on anaerobic glycolytic metabolism and decreased oxidative phosphorylation during exercise in skeletal muscle in heart failure compared to controls. This may have implications for understanding the symptoms reported by heart failure patients after repeated submaximal efforts during their daily activities.

2.2.9.1.3 Summary of the Acute Exercise Response in Heart Failure

Exercise capacity is one of the most powerful predictors of mortality in heart failure [170,185-187]. Nearly all patients with heart failure suffer from rapid onset fatigue that results in a markedly reduced exercise capacity. Yet, the physiologic responses to an acute bout of exercise in heart failure are, for the most, characteristic to those observed in healthy normal. Close examination of cardiac and circulatory responses in heart failure do reveal a marked decreased reserve capacity from pre-exercise to maximal exercise conditions. The capacity to raise skeletal muscle blood flow during exercise is reduced, secondary to changes in neurohumoral and vascular responsiveness. It is thought that the decreased reserve capacity in cardiac output,
stroke volume, heart rate, and vascular responsiveness contribute to the reduced skeletal muscle blood flow and impaired exercise tolerance in patients with heart failure. Additional factors that contribute to the early onset of fatigue in heart failure are an increased reliance on anaerobic metabolic pathways, and impaired oxidative metabolism of skeletal muscle. Therefore, the exercise intolerance characteristic of heart failure patients appears to be related to a change in oxygen and nutrient delivery to the musculature, as well as a reduced oxidative capacity of skeletal muscle.

2.2.9.2 The Response to Exercise Training in Heart Failure

The first report indicating that heart failure patients could safely participate and benefit from a cardiac rehabilitation program dates to 1979 [188]. Since that study many randomized clinical trials have been conducted and demonstrate that exercise-based cardiac rehabilitation favorably improves exercise tolerance, disease-related symptoms, and quality of life in patients with heart failure, without negatively impacting left ventricular function. No adverse exercise training–related effects have been reported despite that these patients would appear to be at higher risk for events given their left ventricular dysfunction and heightened state of sympathetic activity. In fact, exercise training contributes to a reduction of the fatigue- and dyspnea-limiting exercise intolerance that is common in these patients [189,190].

However, despite these consistent findings, the mechanism of the training response in heart failure is not fully understood. It appears that the improvements in exercise capacity are more related to reversing the peripheral compensatory abnormalities, rather than improvements in cardiac function. Therefore, this section of this review will outline the response to exercise training in heart failure. First, this section will present the information regarding (a) the cardiovascular responses, and (b) the skeletal muscle responses.
2.2.9.2.1 Cardiovascular Responses to Exercise Training in Heart Failure

2.2.9.2.1.1 Cardiac Output

Cardiac output shows either no change [182], or a small increase [191] following a period of chronic exercise training. In the study by Sullivan et al., [182] patients exercised approximately 60 min, 3-5 days/wk at 75% of peak heart rate reserve. Although there was a trend toward an increase in maximal exercise cardiac output, indices of left ventricular function did not change [182]. On the other hand, in a randomized cross-over trial by Coats et al., [191] exercise training was associated with an increase in both submaximal and maximal cardiac output during exercise in eleven patients with mild to moderate ischemic heart failure.

The increase in peak cardiac output was in large extent due to changes in stroke volume, and was achieved at a greater absolute work load. The apparent differences between these studies are difficult to interpret, but could be a function of several factors such as (a) etiology, severity and duration of disease, (b) training modality and intensity, (c) length of the training program, (d) method of cardiac evaluation, or (e) a combination of all the above. Therefore, Coats et al., [191] is the only study to report an enhanced stroke volume response to exercise following training.

2.2.9.2.1.2 Heart Rate

Perhaps one of the most consistent response to exercise training is a reduction in resting heart rate and at any given submaximal workload [192]. The reduction in resting heart rate is thought to reflect a change in the balance between sympathetic and parasympathetic activity towards greater dominance of the vagal nerve. The reduction in heart rate during exercise is, generally, attributed to a larger stroke volume, secondary to myocardial hypertrophy [192] or an increase in blood volume [193].
In the study by Sullivan et al., [182] and Coats et al., [191] resting and submaximal exercise heart rates also decreased in patients with heart failure following training. Finally, a slight but significant increase in peak heart rate reserve was observed by Coats et al., [191].

2.2.9.2.1.3 Vascular System

Hornig et al., [194] are the first to suggest that a local exercise program can enhance vasodilatory capacity in patients with heart failure. In this cross-over trial, patients participated in 4 weeks of training which consisted of handgrip exercise at 70% of peak handgrip strength for 30 min per day. Exercise training restored flow-dependent dilation in patients with heart failure. Furthermore, a study by Hambrecht et al., [195] have reported that moderate-intensity aerobic exercise training significantly improved endothelium-mediated vasodilation of the peripheral vasculature after 24 weeks in patients with heart failure and that these changes were significantly correlated with changes in peak oxygen consumption [195]. A similar study has shown that lower-limb bicycle ergometer exercise training leads to a correction of endothelial dysfunction of the upper extremity, indicating a systemic effect of local exercise training on endothelial function [196].

Furthermore, there is a need for long-term exercise training for maintaining improvement of vascular function; improvement of endothelial function after 4 weeks of handgrip exercise is lost after the cessation of training, with a return to baseline vascular function after 6 weeks [194].

These results indicate that exercise training has the potential to reverse the deleterious effects of increasing peripheral resistance and reducing oxygen delivery to the working skeletal muscle caused by endothelial dysfunction, although the improvement in vascular resistance may be more rapid and pronounced in subjects who are healthy rather than patients with heart failure [197].
2.2.9.2.2 Skeletal Muscle Responses to Exercise Training in Heart Failure

2.2.9.2.2.1 Muscular Function

Resistance training was not routinely prescribed to patients with heart failure because this type of exercise was associated with undesirable and pathological cardiovascular responses [198,199]. However, during the last decade, increasing evidence indicated that dynamic resistance training alone [200-202] or in combination with aerobic exercise [203-205] is beneficial and counteracts the negative peripheral side effects exhibited in patients with heart failure. In addition, localized skeletal muscle training has been shown to improve symptoms of heart failure and exercise capacity; even localized calf skeletal muscle training improves oxidative capacity without changes in calf blood flow, and it significantly improved a subjective fatigue score [206].

Finally, selective respiratory muscle training in 3 weeks improved respiratory muscle endurance and strength, with an enhancement of submaximal and maximal exercise capacity in patients with heart failure. Dyspnea quantified by the Borg scale was significantly reduced during progressive isocapnic hyperpnea, and dyspnea during activities of daily living was subjectively improved in most patients who underwent training [207].

2.2.9.2.2.2 Skeletal Muscle Blood Flow

There is evidence from both human and animal studies that exercise training may improve skeletal muscle blood flow [182,208]. In fact, Sullivan et al., [182] have reported significant improvements in peak leg blood flow and arterio-venous oxygen difference in heart failure patients with significantly reduced left ventricular ejection fraction (24%), following 4-6 months of exercise training. The exercise prescription during this training protocol consisted of approximately 60 min of cardiovascular activity performed 3-5 days/wk at 75% of peak heart
rate reserve. The improvement in skeletal muscle blood flow is not related to improved central hemodynamics and appears to be directly related to exercise capacity [209,210].

2.2.9.2.2.3 Skeletal Muscle Metabolism

Studies have reported on the ability of skeletal muscle to significantly improve metabolic capacity following exercise training in patients with heart failure [211,212]. For example, Minotti et al., [212] examined the ability of skeletal muscle to adapt in patients with a mean left ventricular ejection fraction of 27%. Patients participated in a 28-day localized unilateral forearm training program. Training consisted of multiple sets (8 min in duration) of wrist flexion exercise performed 6 days/week. Following training, muscle bioenergetics, as assessed by $^{31}$P-NMR spectroscopy, improved in the trained forearm, whereas muscle mass, limb blood flow and cardiac output remained unchanged. In addition, the authors reported an impressive 260% increase in muscle endurance measured as the number of minutes that a submaximal load could be lifted until exhaustion [212].

In addition, Stratton et al., [213] have reported that after training there were significant improvements in skeletal muscle responses to exercise with lesser phosphocreatine utilization, higher muscle pH at submaximal workloads, and improvement in phosphocreatine reserve, which is also an indicator of mitochondrial adenosine triphosphate synthesis rate. These findings suggest that skeletal muscle abnormalities in heart failure are, in part, due to a deconditioning process, and can be partially reversed through exercise training.

2.2.9.2.3 Summary of the Response to Exercise Training in Heart Failure

Therefore, there is growing evidence that exercise training may reverse many of the peripheral abnormalities present in the heart failure patient. Exercise training may improve autonomic function, skeletal muscle blood flow and localized oxidative capacity. Together, these
changes may translate in an increased exercise tolerance, reduction in activity-related symptoms, and improved quality of life. These findings assume added importance in light of previous reports that exercise capacity is the most powerful predictor of survival of heart failure patients. Whether exercise training can improve long-term functional and prognostic outcomes in heart failure patients is presently uncertain. Furthermore, the improvement in exercise tolerance in heart failure patients following training equals that achieved in controlled trials of ACE-inhibitors [214]. Finally, since exercise tolerance is known to be an independent marker of prognosis, an increased exercise capacity following training may itself indicate a prognostic benefit.

2.3 Peripheral Arterial Disease

Just as with heart failure patients a major physiologic problem for patients with peripheral arterial disease is a reduction in muscle blood flow. However in peripheral arterial disease patients the cardinal symptom is pain on exertion that is normally relieved by rest. As the disease progresses the pain may also occur at rest. In this section the following issues will be addressed: (a) the definition, (b) the incidence and prevalence, (c) the etiology and pathophysiology, (d) the cardinal symptoms, (e) the diagnostic tests and the prognostic value of exercise testing, and lastly (f) the classification of peripheral arterial disease related to the degree of symptom severity. This section will present information regarding the principal circulatory and muscular compensatory adaptations in peripheral arterial disease patients. Finally, this section will discuss the acute exercise responses, and the response to exercise training in peripheral arterial disease patients. The purpose of this section is to increase the understanding of the manner in which patients with peripheral arterial disease compensate to their reduction in blood flow, and the potential ramifications for treatment strategies.
2.3.1 Definition

Peripheral arterial disease is defined as obstruction of blood flow through a section of the arterial tree excluding the intracranial or coronary circulations [215]. In fact, peripheral arterial disease is a chronic manifestation of systemic atherosclerosis in the lower limb distal to the aortic bifurcation in which the arterial lumen of the lower extremities becomes progressively occluded by atherosclerotic plaque [216]. As a result, the obstruction impedes blood flow to the lower extremities that leads to a mismatch of oxygen delivery and metabolic demand that becomes more evident during physical activity [215,217].

Peripheral arterial disease is mostly silent in its early stages but may manifest as intermittent claudication when the luminal obstruction is greater than 50% [215] (see figure 2.3). As the disease progresses, the symptoms typically intensify to pain at rest, with the potential for frank tissue loss [218]. As a result, peripheral arterial disease patients have impaired function and quality of life [219]. This is true even for persons who do not report leg symptoms. Furthermore, peripheral arterial disease patients, including those who are asymptomatic, experience significant decline in lower-extremity functioning over time [220].

2.3.2 Incidence and Prevalence

Peripheral arterial disease is a major cause of disability, loss of work, and lifestyle changes in the United States [221]. In addition, peripheral arterial disease affects approximately 12 million persons in this country [222]. It is a prevalent but underdiagnosed and undertreated disorder associated with significant morbidity and mortality [223].

The prevalence of peripheral arterial disease increases with advancing age, as approximately 12 to 20% of people older than 70 years suffer this condition [224,225]. Furthermore, one half of all peripheral arterial disease patients older than 55 years are
asymptomatic. Among the symptomatic, 40% experience intermittent claudication, and 10% have critical limb ischemia [226].

**Figure 2.3.** Arterial obstructions exceeding 50% of the internal lumen diameter of a peripheral artery, such as the superficial femoral artery (A), may result in IC. The most common cause of such symptomatic obstructions is atherosclerosis, resulting from a buildup of fatty, cholesterol-laden plaque within the arterial wall (B) [215].

The Rotterdam Study [227] assessed the age- and sex-specific prevalence of peripheral arterial disease and intermittent claudication in a group of 7715 old individuals. The Rotterdam Study reported that the prevalence of peripheral arterial disease in the elderly is high whereas the prevalence of intermittent claudication is rather low, although both occurrences clearly increase with advancing age. In fact, the same study showed a frequency of claudication ranging from 1% in those 55 to 60 years of age to > 5% in those over age 80 [227].

65
Finally, in the Cardiovascular Health Study [228], the ankle-brachial index, a frequently used marker of peripheral arterial disease, was measured in patients older than 65 years of age at a baseline examination. Those with an ankle-brachial index < 0.8 were more than twice as likely to have had a myocardial infarction, angina, heart failure, stroke, or transient ischemic attack as patients with normal values. Furthermore, cumulative data after 6 years of follow-up on 5888 patients showed that an ankle-brachial index < 0.9 was an independent risk factor for occult cardiovascular disease, recurrent cardiovascular events, and mortality [229].

Despite its prevalence and cardiovascular risk implications, only 25% of patients with peripheral arterial disease are undergoing treatment [230]. The risk factors associated with peripheral arterial disease are similar to those for coronary heart disease and include increasing age, diabetes mellitus, cigarette smoking, hypertension, elevated lipid levels, and elevated homocysteine levels [217,231]. In addition, persons with peripheral arterial disease, compared with those without, have 4 to 5 times the risk of dying of a cardiovascular vascular disease event, resulting in a 2- to 3-times higher total mortality risk [217,229].

2.3.3 Etiology and Pathophysiology

Atherosclerosis (arteriosclerosis obliterans), a primarily systemic inflammatory process, is the leading cause, more than 90%, of occlusive arterial disease of the extremities in patients over 40 years of age [232,204, 205]. Peripheral arterial disease is the result of atherosclerotic disease of the lower extremities, and it is one of the major causes of disability in middle age and the elderly [233]. The segmental lesions responsible for the stenosis or occlusion are usually localized in large and medium-sized blood vessels [234]. It is important to emphasize that inflammation is currently considered to be a key factor in all stages of atherogenesis in non-diabetic as well as diabetic patients [235]. The histopathological basis of disease is identical to
that seen in the coronary vasculature and other vascular beds [236]. In general, atherosclerosis results from an excessive inflammatory and fibroproliferative response to numerous vascular insults, which leads to alteration of the normal homeostatic properties of the endothelium [237].

The initial changes that precede the formation of atherosclerotic lesions occur in the endothelium, and they include: (a) increased endothelial permeability to lipoproteins and other plasma constituents, (b) up-regulation of leukocyte adhesion molecules, (c) upregulation of endothelial adhesion molecules, and (d) migration of leukocytes into the artery wall [234,238]. These processes are mediated by many vasoactive molecules, cytokines, and growth factors [237]. Afterward, if the inflammatory response cannot counteract or remove the insulting factors, the inflammatory response will stimulate migration and proliferation of smooth muscle cells that participate in the formation of fatty streaks in atherosclerosis along with lipid-laden monocytes, macrophages (foam cells), and T lymphocytes [237,239].

The resulting inflammatory process leads to increased amounts of macrophages and lymphocytes, and it promotes cholesterol and calcium deposition and, ultimately, the buildup of plaque [232]. These macrophages and lymphocytes can emigrate from the blood and multiply in the lesion and result in further damage and eventually lead to focal necrosis [237,239]. These changes causes further enlargement and restructuring of the lesion, so it becomes covered by a fibrous cap that overlies a core of lipid, a mixture of leukocytes, and necrotic tissue. The necrotic core is the result of (a) apoptosis and necrosis, (b) increased proteolytic activity, and (c) lipid accumulation [237].

The lesions may be stable, or they may develop a thin, unstable fibrous cap, that is caused by continuing influx and activation of macrophages. As a result, the lesion is vulnerable to ulceration [240]. Once the lesion ulcerates, the underlying plaque is exposed to the bloodstream,
and the potential for thrombotic occlusion or embolization increases [241]. Therefore, atherosclerosis is a multistage, multigenic, and very complicated inflammatory disease [239]. Indeed, during atherogenesis, growth factors, cytokines, lipids, and enzymes modulate critical cell functions by inducing (a) lipid accumulation and oxidation, (b) cell-mediated inflammatory response, (c) smooth muscle proliferation, (d) vasoconstriction, and (e) a prothrombotic environment within the artery wall [237,240].

The primary sites where the lesions are located include the abdominal aorta and iliac arteries (30% of symptomatic patients), the femoral and popliteal arteries (80 to 90% of patients), and the more distal vessels, including the tibial and peroneal arteries (40 to 50% of patients) [242]. In addition, the atherosclerotic lesions have a predilection for arterial branch points where increased turbulence, altered shear stress, and intimal injury occur [232]. Typically, the narrowing of a proximal artery was considered the only factor responsible for the reduced oxygenation in the skeletal muscle. However, the hemodynamics limitations are poor predictors of exercise performance among individuals with peripheral arterial disease [243].

In addition, the natural history of peripheral arterial disease is slow progression of symptoms over time [244]; however, there is considerable variation within this overall pattern. In most patients, the disease progression is benign [215]. A great majority of patients will remain asymptomatic or with fairly stable symptoms; some may even show improvement. In that regard, a large population study found that 5 years after diagnosis of peripheral arterial disease, 63% of patients showed angiographic progression, but 66% still had no limiting intermittent claudication [245]. Many patients will simply adjust or adapt their lifestyles to accommodate their symptomatic limbs in the same way many patients with heart failure adjust their lifestyles to their heart function [215].
2.3.4 Symptoms

The symptoms and signs of peripheral arterial disease are displayed in table 2.7 [246]. The decreased blood flow to the legs caused by peripheral arterial disease may be mild or severe, resulting in a broad range of symptoms. The symptoms may range from pain on exertion that is normally relieved by rest, also known as intermittent claudication, which is the primary manifestation of peripheral arterial disease [218,247], to pain at rest also known as critical limb ischemia [216,248]. In the general population, only about 10% of persons with peripheral arterial disease have the classic symptoms of intermittent claudication. About 40% do not complain of leg pain, whereas the remaining 50% have a variety of leg symptoms different from classic claudication [223,249].

2.3.4.1 Intermittent Claudication

Intermittent claudication is described as a cramp, ache, fatigue, tightening discomfort, or frank pain located in the muscles of the buttocks, thighs, or calves [250,251]. Intermittent claudication is the result of poor oxygenation of the muscles of the lower extremities, induced by walking or exercise and relieved by rest [252]. Classic intermittent claudication, as defined by the World Health Organization (WHO)/Rose Claudication Questionnaire, is calf pain that occurs during exercise and ceases within 10 minutes of rest [253].

Intermittent claudication is usually reproducible, occurring at approximately the same distance, pace, and grade from day to day, and resolves within 2 to 5 minutes with rest. The location of the pain often corresponds to the level of stenosis [254]. For instance, patients with an iliac lesion often experience claudication in the thigh or buttock, whereas popliteal lesions are more often associated with calf claudication [216]. In addition, peripheral arterial disease patients may develop Leriche syndrome, which includes (a) claudication, (b) impotency, and (c)
global atrophy of the lower extremities due to aortoiliac obstructive disease [255]. Intermittent claudication is present in less than 1% of individuals under age 50 and approximately 5% or more of those older than age 80 [217].

**Table 2.7 Symptoms and signs of Peripheral Arterial Disease**

<table>
<thead>
<tr>
<th>Symptoms and signs</th>
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<tbody>
<tr>
<td>Exertional leg pain and relief with rest</td>
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<tr>
<td>Cool or cold feet to palpation</td>
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<tr>
<td>Nocturnal and rest pain relieved with dependency</td>
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<tr>
<td>Absent pulses</td>
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<tr>
<td>Blanching or pallor on elevation</td>
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<tr>
<td>Delayed venous filling after elevation</td>
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<td>Dependent rubor</td>
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<tr>
<td>Atrophy of subcutaneous fatty tissue</td>
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<tr>
<td>Shiny skin</td>
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<tr>
<td>Loss of hair on foot and toes</td>
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<tr>
<td>Thickened nails, often with fungal infections</td>
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<tr>
<td>Gangrene or non-healing ulcer</td>
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Intermittent claudication prevalence rates vary, but in a vascular laboratory population, only 1/3 of peripheral arterial disease subjects reported classic WHO/Rose claudication [256] [247]. This indicates that the remaining 2/3 of individuals with peripheral arterial disease are reportedly asymptomatic or have atypical symptoms. Daily activity is reduced up to 45% in patients with intermittent claudication, as compared with healthy subjects of similar age [251]. Although intermittent claudication incidence has declined since 1950, the mortality has remained high and unchanged [11].

2.3.4.2 Critical Limb Ischemia

Critical limb ischemia is defined as limb ischemia that could occur during rest [216,217]. Clinically, ischemia in the limb at rest is manifested first in the cutaneous tissues of the foot, where factors regulating perfusion differ from those governing calf muscle circulation, and
reflexive sympathetically mediated vasoconstrictor activity may reduce foot blood flow even under conditions of ischemia [255]. Critical limb ischemia is typically described as worse at bedtime with horizontal positioning (and relieved by dependent positioning), or evidence of distal tissue necrosis (non-healing ulcers, gangrene) [257]. It is a severe manifestation of peripheral arterial disease, which threatens the viability of the extremity because arterial perfusion is insufficient to meet even the basal metabolic demands of the tissue [258]. Other symptoms of ischemia at rest include hypesthesia, cold sensitivity, muscular weakness, joint stiffness, and contracture [255].

Normally, pain at rest indicates severe, often multilevel arterial occlusive disease. It characteristically occurs as a dull, aching sensation in the toes or fore foot, or as a severe, nagging burning at night with the patient lying supine [246,255]. The patient may hang the foot or leg over the side of the bed or get up to walk around to obtain relief. Some patients with severe rest pain sleep sitting in a chair with the legs dependent, resulting in edema [258]. Therefore, it has a profound effect on functional capacity and quality of life among the patients, and it requires an aggressive management, which usually includes angiographic examination and therapeutic intervention by percutaneous angioplasty or surgical revascularization. When these are not feasible, gangrene commonly ensues leading to amputation [255]. Critical limb ischemia results in some 150,000 amputations annually in the US, with perioperative mortality rates of 5–10% for below-knee amputation and up to 50% for above-knee amputation because of comorbid conditions [217]. Finally, the annual incidence of critical limb ischemia approximates 500 per one million population [221]. Among patients with intermittent claudication, approximately 10% progress to limb-threatening ischemia in 5 years, and 25% of those with critical limb ischemia require amputation [244].
2.3.5 Diagnostic Tests

This particular section will describe various diagnostic tools that are currently available in the diagnosis and management of patients with peripheral arterial disease. However, the main focus of this section is to present information regarding two of the most practical and frequently used tests in the diagnosis of patients with peripheral arterial disease, the ankle-brachial index test and exercise testing with the ankle-brachial index.

2.3.5.1 The Ankle–Brachial Index Test

The ankle-brachial index is a simple, inexpensive, non-invasive vascular tool that correlates well with angiography disease severity and functional symptoms [259, 256]. Under normal conditions, systolic blood pressure in the legs is equal to or slightly greater than the systolic pressure in the upper limbs. In the presence of an arterial stenosis, a reduction in pressure occurs distal to the lesion [223]. The ankle-brachial index, which is calculated from the ratio of ankle to brachial systolic pressure, is a sensitive marker of arterial insufficiency. The highest pressure measured in any ankle artery is used as the numerator in the calculation of the index; a value greater than 1.0 is normal and a value less than 0.90 is abnormal [260].

Normally, an ankle-brachial index less than 0.90 is considered diagnostic of peripheral arterial disease. There are three categories; (a) mild disease correlates with an ankle-brachial index ranging from 0.70 to less than 0.90, whereas (b) moderate disease correlates with an ankle-brachial index ranging from 0.40 to less than 0.70, and finally (c) severe disease is associated with an ankle-brachial index less than 0.40 [221,261]. Patients with intermittent claudication tend to have an ankle-brachial index in the range of 0.5 to 0.9, whereas those with critical ischemia usually have an index of less than 0.50 [223]. The ankle-brachial index also has prognostic significance because of the association with arterial disease elsewhere, especially...
coronary heart disease. Recent studies indicate an association of elevated ankle-brachial index levels with increased all-cause and cardiovascular mortality [242].

2.3.5.1.1 Ankle-Brachial Index Technique

The ankle-brachial index measurement sites are displayed in figure 2.7. An ordinary blood pressure cuff is positioned over the upper arm and inflated above systolic blood pressure. Next, a Doppler ultrasonic velocity signal probe is placed over the brachial artery to detect the resumption of blood flow with cuff deflation. Measurement of systolic blood pressure is repeated on the other arm. The higher of the 2 systolic blood pressures is used in the calculation. The measurement of the ankle systolic blood pressure is detected with the Doppler probe over the posterior tibial artery and then over the dorsalis pedis artery. The higher value from the two arteries is used into the calculation. The process should be repeated for the other leg. Finally, the lowest ankle-brachial index between both legs is the ankle-brachial index that stratifies the patient’s risk [217].

2.3.5.1.2 Limitations of Ankle-Brachial Index

In some patients with diabetes, renal insufficiency, or other diseases that cause vascular calcification, the tibial arteries at the ankle become non-compressible. This leads to a false high ankle-brachial index value. In those cases, additional measures would be necessary (e.g. a toe-brachial index, pulse volume recordings or other noninvasive testing) [221].

2.3.5.2 Exercise Testing

The addition of ankle-brachial index measure with exercise testing provides more information on the dynamics of claudication [262]. Studies have reported that exercise testing is useful in those patients with claudication symptoms who have a normal ankle-brachial index or normal pulse at rest [263]. The typical treadmill protocol for persons with peripheral
arterial disease is either a graded or a constant load protocol [264]. The graded protocol utilizes a fixed speed while grade increases every two or three minutes to a symptom limited maximal (or “peak”) performance [265]. The constant load protocol has both speed and grade fixed, and it is done on a treadmill allowing the patient to walk at a standard speed and grade for a pre-determined period (2 mph at 12% incline for 5 minutes) or until claudication appears [221]. Both types of protocol have shown very good reproducibility [266].

The next section is intended to present information regarding the prognostic value of these diagnostic tools used in patients with peripheral arterial disease. This section will address the outcomes of the ankle-brachial index technique, and then, the exercise testing with the addition of ankle-brachial index. The prognostic value of exercise testing in peripheral arterial disease is as follow:

2.3.5.2.1 Ankle-Brachial Index Test

Although not a direct measure of function, the ankle-brachial index is of key importance to assess peripheral arterial disease severity [267]. The ankle-brachial index test is a non-invasive measure that is made in the peripheral arterial disease patient first while resting in the supine position, and second, post exercise (also supine) by assessing systolic blood pressure in the ankle and arm with a Doppler ultrasonic instrument [264]. In addition, many studies have reported that the ankle-brachial index decreases with age. In fact, the Rotterdam Study [227] showed that the ankle-brachial index decreased sharply with advancing age. These investigators concluded that the high prevalence of peripheral arterial disease and unfavorable cardiovascular risk profile of patients with peripheral arterial disease, illustrates the need to explore the use of the ankle-brachial index as a risk indicator in cardiovascular screening and risk profiling in medical practice. Finally, the ankle-brachial index test also provides significant information regarding;
(a) the disease progression, (b) the functional capacity, and (c) its role as predictor of cardiovascular morbidity and/or mortality among peripheral arterial disease patients [221].

2.3.5.2.1.1 Disease Progression

The changes in the ankle-brachial index values over time represent a useful tool to monitor disease progression [268]. For example, changes in the ankle-brachial index that exceed ±0.15 are considered to be outside the range of experimental error and indicate disease progression [262].

2.3.5.2.1.2 Functional Capacity

Many studies have shown that the ankle-brachial index is independently linked with impaired lower extremity functioning, even in asymptomatic patients. In that regard, the Women’s Health and Aging Study [269] revealed that decreasing ankle-brachial index values were associated with progressive worsening in functional scores, even after adjustment for age, sex, race, smoking status, and comorbidities. For example, in asymptomatic women, lower ankle-brachial index scores were associated with slower walking velocity, poorer standing balance, slower time to arise, and fewer blocks walked per week. In addition, the Study of Osteoporotic Fractures [270] showed that patients with an ankle-brachial index less than 0.90 had significantly lower hip abduction force, knee extension force, walking velocity, and number of blocks walked than those with an ankle-brachial index of 0.90 or greater. Finally, other studies have reported that progressive peripheral arterial disease is associated with muscle fiber loss, poorer leg strength, and slower walking velocity [271].

2.3.5.2.1.3 Predictor of Cardiovascular Morbidity and/or Mortality

The Cardiovascular Health Study [229] was conducted among 5888 adults 65 years of age or older in order to monitor for cardiovascular events after establishing a baseline
cardiovascular disease and ankle-brachial index measurement. The results reveal that mortality rate at 6 years was higher in those patients who had cardiovascular disease and a low ankle-brachial index (<0.90) at baseline (32.3%), in contrast it was lowest in patients with neither of these risks factors at baseline (8.7%). A study conducted by McKenna et al., [272] showed that mortality from cardiovascular disease was highest among patients with an ankle-brachial index less than 0.40 and lowest among patients with an ankle-brachial index of 0.85 to 1.50.

Interestingly, several studies had revealed the importance of the ankle-brachial index as a predictor of cardiovascular mortality among asymptomatic peripheral arterial disease patients [273,274]. The study by Criqui et al., [273] showed the survival curves based on mortality from all causes among normal subjects and subjects with symptomatic and asymptomatic peripheral arterial disease. This study confirms the overall poor prognosis with advancing age and the alarmingly high risk in asymptomatic patients whose disease would not be detected clinically. However, a hemodynamic measure such as ankle-brachial index should not be used as a primary measure of the functional effects of an intervention such as exercise rehabilitation since the correlation between ankle-brachial index and treadmill walking is often weak [264].

2.3.5.2.2 Exercise Testing

The exercise testing assessment has been considered the gold standard for evaluating exercise performance in peripheral arterial disease patients [264]. Normally, in healthy subjects ankle systolic blood pressure is maintained during moderate exercise. On the other hand, in patients with claudication their ankle systolic blood pressure falls to low or is undetectable with low level workloads and returns to baseline after a few minutes [262]. The two main measures that are obtained from treadmill testing are pain-free walking time, also called initial claudication distance and maximal walking time, also called absolute claudication distance [264,275]. Pain-
free walking time is the time a patient can walk before the onset of claudication, while maximal walking time is the amount of time a patient can walk before being stopped by severe claudication [251,264]. Finally, among patients with peripheral arterial disease the post-exercise ankle-brachial index will drop significantly [263]. However, any pain or discomfort related to the exercise while the ankle-brachial index remains normal or unchanged are not produced by peripheral arterial disease [221].

2.3.6 Classification According to the Symptom Severity

Two major classification systems have been used to rate the symptom severity and to assess the impact of intervention programs in peripheral arterial disease patients. The first one is the older Fontaine system [276]. This system assigns patients to 1 of 4 clinical stages (I-IV), depending on the manifestation of clinical signs (see table 2.8).

Table 2.8 Fontaine’s classification

<table>
<thead>
<tr>
<th>Stage</th>
<th>Clinical description and objective criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>Asymptomatic peripheral arterial disease patients</td>
</tr>
<tr>
<td>Stage II</td>
<td>Exercise-induced ischemia</td>
</tr>
<tr>
<td>IIa</td>
<td>Intermittent claudication, pain during walking</td>
</tr>
<tr>
<td>IIb</td>
<td>Relief of symptoms when standing</td>
</tr>
<tr>
<td>Stage III</td>
<td>Ischemia-driven symptoms at rest</td>
</tr>
<tr>
<td>IIIa</td>
<td>Ankle Pressure Index ( \geq 50 \text{ mm Hg} )</td>
</tr>
<tr>
<td>IIIb</td>
<td>Ankle Pressure Index (&lt; 50 \text{ mm Hg} )</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Trophic ulcers and gangrene</td>
</tr>
<tr>
<td>IVa</td>
<td>Limited gangrene</td>
</tr>
<tr>
<td>IVb</td>
<td>Extensive gangrene</td>
</tr>
</tbody>
</table>

In addition, a new classification has now been proposed by Rutherford et al., [277]. It comprises six clinical categories (see table 2.9), and its use is recommended by the Trans-Atlantic Inter- Society Consensus (TASC) Working Group for the diagnosis and assessment of the progression of peripheral arterial disease [278].
Table 2.9 Rutherford categories

<table>
<thead>
<tr>
<th>Category</th>
<th>Clinical description</th>
<th>Objective criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Asymptomatic</td>
<td>Normal treadmill test</td>
</tr>
<tr>
<td>1</td>
<td>Mild claudication</td>
<td>Treadmill exercise limited to 5 min; ankle pressure after exercise &gt;50 mmHg, but at</td>
</tr>
<tr>
<td></td>
<td></td>
<td>least 20 mmHg lower than at rest</td>
</tr>
<tr>
<td>2</td>
<td>Moderate claudication</td>
<td>Between Rutherford 2 and 3 disease</td>
</tr>
<tr>
<td>3</td>
<td>Severe claudication</td>
<td>Treadmill exercise limited to &lt;5 min; ankle pressure after exercise &lt;50 mmHg</td>
</tr>
<tr>
<td>4</td>
<td>Ischemic rest pain</td>
<td>Ankle pressure &lt;40 mmHg and/or great toe pressure &lt;30 mmHg; pulse volume</td>
</tr>
<tr>
<td></td>
<td></td>
<td>recording barely pulsatile or flat</td>
</tr>
<tr>
<td>5</td>
<td>Minor tissue loss</td>
<td>Ankle pressure &lt;60 mmHg and/or great toe pressure &lt;30 mmHg; pulse volume recording</td>
</tr>
<tr>
<td></td>
<td></td>
<td>barely pulsatile or flat</td>
</tr>
<tr>
<td>6</td>
<td>Major tissue loss (normally above</td>
<td>Ankle pressure &lt;60 mmHg and/or great toe pressure &lt;30 mmHg; pulse volume recording</td>
</tr>
<tr>
<td></td>
<td>the metatarsal level)</td>
<td>barely pulsatile or flat</td>
</tr>
</tbody>
</table>

In summary, peripheral arterial disease is a major and growing public health problem in the United States. In fact, peripheral arterial disease is a prevalent but underdiagnosed and undertreated disorder associated with significant morbidity and mortality. Peripheral arterial disease is a clinical syndrome that causes a reduction of blood flow to the lower extremities, and it produces a mismatch of oxygen delivery and metabolic demand that becomes more evident during physical activity.

The cardinal manifestation of peripheral arterial disease is intermittent claudication. Finally, the Rutherford system is currently the recommended classification used to quantify the severity of symptoms among peripheral arterial disease patients. The next section will present
information regarding the principal circulatory and muscular compensatory adaptations in peripheral arterial disease patients.

2.3.7 Compensatory Adaptations to Peripheral Arterial Disease

Peripheral arterial disease is defined as an obstruction of blood flow to the lower extremity, due to atherosclerosis. Peripheral arterial disease is mostly silent in its early stages and its symptoms progress slowly over time. Eventually, patients with peripheral arterial disease might suffer from pain at rest and/or ischemic ulceration and gangrene due to hypoperfusion, which is known as critical limb ischemia.

The primary pathophysiology of peripheral arterial disease is impaired perfusion that forces a series of functional and structural modifications in the affected limb. These compensatory modifications determine the functional ability of patients with peripheral arterial disease. Thus the purpose of this section of the review is to discuss the compensatory modifications associated with the disease. Particular focus will go toward discussing: (a) the vascular compensatory adaptations, and (b) the skeletal muscle compensatory adaptations as a result of peripheral arterial disease.

2.3.7.1 Vascular Compensatory Adaptations

Atherosclerosis is a disease of the large and medium sized arteries. The small arteries such as the digital vessels generally are not affected in atherosclerosis, but, on occasion, may become involved secondarily [279]. Traditionally, atherosclerosis research has mainly focused on investigating risk factors for plaque development and its complications. However, considerably less is known about compensatory phenomena of the vasculature in atherosclerosis. In that regard, few experimental studies encouraged the hypothesis that vessels dilate in response to atherogenesis, thereby restoring normal blood flow and delaying the onset of clinically
important stenosis [280-282]. The vasculature is recognized as a complex organ capable of sensing its environment (e.g. shear stress), transducing signals to effector cells or surrounding tissue and responding to various stimuli by releasing local regulatory mediators such as growth factors and vasoactive peptides [282-284].

Many studies have described atherosclerosis as a diffuse and progressive process with a variable distribution and clinical presentation that is dependent on the regional circulation involved [217,251,285]. Factors that may influence these differences include the size and structure of the affected artery, local and regional flow, changes in microcirculatory alterations, and end-organ damage.

Generally, the reduction of blood flow as a result of the atherosclerotic lesion would favor the installation of distal modifications far away from the original stenotic vessel [251]. The local blood flow control can be divided into two phases: (a) acute control, and (b) long-term control. Acute control is achieved by rapid changes in local vasodilation or vasoconstriction of the arterioles, metarterioles, and pre-capillary sphincters. This response occurs within seconds to minutes in order to provide a rapid means for maintaining appropriate local tissue blood flow.

The long-term control results in slow changes in flow over a period of days, weeks, or even months. These changes are the result of an increase or decrease in the physical sizes and numbers of actual blood vessels [110]. Furthermore, the understanding of atherosclerotic vascular disease has evolved most dramatically over the past 25 years with the growth of the field of vascular biology [286]. With that in mind, the purpose of this section of this review is to outline the major vascular compensatory adaptations in peripheral arterial disease. The topics included in this section are: (a) large and medium-sized arteries, (b) collateral arteries, and (c) microcirculatory disturbances.
2.3.7.1.1 Large and Medium Sized Arteries

When a large or medium sized artery becomes occluded, secondary vascular channels immediately develop around the blockage and allow, at least, partial resupply of blood to the tissue below the blockage. Vascular loops above the blockage begin to dilate, and are in part secondary to neural and/or metabolic relaxation of the smooth muscle surrounding the blood vessels. Obviously, the compensation is less than optimal, but is able to maintain cellular function under resting conditions. The compensation continues and within a few days may eventually be sufficient to normalize flow under even low levels of activity [110].

Whereas the initial compensation is more than likely the consequence of rapid neural changes and metabolic dilation, the long-term collateral formation more than likely involves structural changes in the circulatory tree that contribute to vessel growth and enlargement [110]. In fact, it has been reported that compensatory enlargement of the vessel at the site of active atherosclerosis effectively preserves normal lumen size [285]. However, whereas the compensation is clearly aimed at improving blood flow to the compromised region, vascular remodeling does alter the normal pressure/flow relationships. It is believed these alterations contribute to the degree of disability the patient suffers [287]. In fact, it is not uncommon to observe, within the same vessel, both a decrease in lumen size (due to advancing atherosclerosis) and an increase in lumen diameter [288]. Consequently, the flow profile (from laminar to more frequent turbulent flow), and vessel shear will change throughout the affected artery thereby increasing the stress on an already vulnerable vessel.

2.3.7.1.2 Collateral Arteries

The importance of the collateral arteries was described in the previous section. However, given its importance this section will describe in greater detail the underlying factors that
contribute to the formation of these vessels. Collateral arteries may already be present in the body, and are only stimulated and subsequently opened in times of need. However, collaterals also “grow” as the need remains or the extent of the disease worsens.

Many factors are well known and accepted regarding collateral arteries [279]. The collateral arteries consist of three components: the exit, mid-zone, and re-entry vessel [289]. An example of an exit collateral would be the profunda femoris with the geniculate arteries representing the reentry channel(s). Normally the mid-zone connecting the two will not be visible on an arteriogram because of their small size. However, this is the zone that will undergo the greatest change in diameter when ischemia develops. In fact, with acute arterial occlusion, limb survival will often depend on the ability of the mid-zone collaterals to increase in diameter and carry enough nutritive blood flow to permit limb survival [279]. The problems with collateral arteries is that they present an increase in resistance to flow (due to their relatively small size) which limits their capability of providing enough blood flow to reach the levels necessary to prevent claudication from occurring. Thus, they may be sufficient for resting flow needs but cannot respond to the added stress of exercise.

Another problem that is often encountered with collaterals is that when more than one set of collateral arteries are in series in a limb with multisegmental obstructions, there is a significant increase in the resistance to flow. Thus the peripheral vascular resistance rises further adding to the vascular problems. For this reason multisegment arterial disease is nearly always more severe than single-segment occlusion [287]. Indeed, it is the patient with multisegment disease who presents the greatest challenges in management. Finally, anatomically, there are profound differences in the collateral capabilities of some areas of the circulation that are important hemodynamically [279]. For instance, the aorta and iliac arteries received their collateral arteries
(called parietal) from intercostals and subcostal arteries. Visceral collaterals to supply the limb can also come from the inferior mesenteric arteries that connect with the arteries of the pelvis; however, they are not able to supply enough blood to the ischemic limb to prevent claudication from occurring [279]. Below the knee the anatomic arrangement, in terms of potential collateral flow, is much different. The blood supply to the gastrocnemius muscle is from a single source, the sural artery. Within the muscle, the arteries branch much like those of a tree; however, and most critically, there are relatively few arterial connections with the gastrocnemius muscle from outside sources. In other words, it almost has an isolated blood supply. In contrast, the soleus muscle is supplied by multiple side branches that freely communicate up and down the limb with other branches that permit inflow in cases of chronic arterial obstruction [289]. These extensive intercommunications are usually able to provide adequate levels of blood flow even in the presence of extensive chronic arterial occlusion in the arteries below the knee. They are particularly important when considering the problem of diabetic arterial disease, in which extensive pathology in this area is very common [279].

2.3.7.1.3 Microcirculatory Disturbances

The components of the microcirculation (arterioles, capillaries, venules, and the endothelial cells covering vascular structures) contribute to maintain the internal circulatory, coagulative and metabolic homeostasis [110]. The endothelial cells are in the non activated state (normal morphology) when the perfusion pressure is normal. In addition, the blood cells are in a non secretory, non adherent state [290].

As a result, the blood vessels are able to deliver the proper amount of blood flow that is plentiful in oxygen and substrates for energy production, in order to meet the metabolic requirements of the skeletal muscle. The next section will present information regarding two of
the microcirculatory disturbances seen in patients with peripheral arterial disease. The topics included are: (a) the endothelium and and (b) the blood cells.

2.3.7.1.3.1 The Endothelium

It is interesting to consider that the above-mentioned changes, in large part, aim to improve blood flow to tissue, under difficult circumstances. In contrast, a consistent finding in the literature is that patients with peripheral arterial disease have impaired endothelial function [291-294]. Indeed, Edwards et al., [295] have reported that ischemic conditions promote endothelial changes that favor platelet adhesion and subsequently activate the coagulation cascade. Furthermore, Thomson et al., [296] revealed the presence of capillary endothelial swelling in critical limb ischemia. In addition, studies in patients with peripheral arterial disease have reported an increase in adhesion molecules [297] that when interacting with their counter-receptors expressed by circulating cells, favor the cell–cell adhesion. Raised plasma levels of circulating adhesion molecules that further increase with exercise have been reported in patients with intermittent claudication [298]. Finally, Boger et al., [299] reported that patients with peripheral artery disease present impaired nitric oxide synthesis. These findings suggest that the endothelial function is compromised. Clearly, this compensation is difficult to understand if one considers that most changes aim to improve the situation. One could speculate that this in fact is more a disease related factor rather than a compensation for the atherosclerosis. Certainly, this fits with current thinking which strongly suggests that endothelial dysfunction precedes the development of atherosclerosis.

2.3.7.1.3.2 The Blood Cells

Erythrocyte mean corpuscular volume is significantly correlated with the angiographic score in peripheral artery disease patients [300]. Others also report increases in plasma viscosity
with the progression of the atherosclerotic process in patients with peripheral arterial disease and these findings correlate with the clinical stages of the disease [301]. Intuitively, this compensation makes sense as the body attempts to improve its oxygen carrying capacity in a low flow environment. However, this compensation also appears to come with negative consequences as the apparent transport of oxygen from the heme to the myoglobin and into the cell is compromised.

In addition, studies in patients with peripheral arterial disease have shown greater leukocyte and platelet activation [302,303] that could lead to physical obstruction and/or the release of toxic chemicals affecting the microcirculation. In addition, activated leukocytes and platelets may adhere to the endothelium of pre-capillary arterioles and post-capillary venules, through the action of adhesion molecules [297]. Activated leukocytes may deliver a variety of noxious substances including proteolytic enzymes, oxygen free radicals, and leukotrienes. Then, these products by inducing cell membrane peroxidation and endothelial damage, promote vascular permeability, plasma leakage and consequent interstitial oedema [302]. The activation of platelets produces the release of different products that may promote vasoconstriction and fibrin formation and inhibit fibrinolysis [303].

In addition, increased vascular permeability in patients with peripheral arterial disease has been reported by Hickey et al., [304]. Their results showed that in claudicant patients the exercise-induced ischemia in the affected limb is accompanied by microalbuminuria. Blood viscosity is an important determinant of blood flow, which in turn is determined by hematocrit, blood cell deformability, and plasma viscosity. In the normal microcirculation, under high shear stress and perfusion pressure, erythrocytes deform easily and thus pass easily through the capillaries. In contrast, erythrocytes in patients with peripheral arterial disease deform less
rapidly making their passage through the capillaries more difficult [305]. These changes would lead to red cell aggregation, increased blood viscosity and reduced blood flow. Clearly more work is needed to differentiate which of these observed changes are compensatory and which are contributory to the disease progression.

2.3.7.2.1 Histologic and Biochemical Alterations in Skeletal Muscle

The reduction in blood flow in patients with peripheral arterial disease progresses gradually and ultimately leads to severe ischemic disease. As a result peripheral arterial disease patients exhibit a variety of structural changes in skeletal muscle consisting of (a) neural modifications, specifically denervation, (b) muscle fiber atrophy, and (c) selective loss of type II fibers relative to type I fibers [306,307]. The result of these modifications is neuromuscular dysfunction, contributing to the clinical course of the disease. For instance, Hedberg et al., [306] have reported that peripheral arterial disease patients with critical ischemia have widespread destruction of muscle fibers, which are replaced with non-contractile connective tissue.

Importantly, the destruction of muscle fibers appears to be, at least, for a period of time during the disease progression somewhat selective. In fact, chronic ischemia in peripheral arterial disease patients is typically characterized by denervation and a decreased type II fiber area. Table 2.10 provides a very simplistic overview of the differences in muscle fibers [283].

Obviously; the changes are associated with muscle atrophy and a loss of muscle mass that lead to functional impairment. In that regard, England et al., [308] have reported that ischemic pain associated with peripheral arterial disease leads to lower extremity neuropathy. The neuropathy causes partial denervation-reinervation, and it results in decreased muscular strength and performance [309].
### Table 2.10 Skeletal muscle fiber types

<table>
<thead>
<tr>
<th>Features</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Red</td>
<td>White</td>
</tr>
<tr>
<td>Contractility</td>
<td>Slow twitch</td>
<td>Fast twitch</td>
</tr>
<tr>
<td>Function</td>
<td>Postural</td>
<td>Fine movement</td>
</tr>
<tr>
<td>Mitochondria density</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Oxidative capacity</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Glycolytic capacity</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Citrate synthase</td>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td></td>
<td>Succinate dehydrogenase</td>
<td>Pyruvate kinase</td>
</tr>
<tr>
<td></td>
<td>Cytochrome oxidase</td>
<td></td>
</tr>
</tbody>
</table>

These changes are seen in both motor and sensory nerves of peripheral arterial disease patients with concurrent diabetic neuropathy, and they are also reported in non-diabetic patients [310,311]. Furthermore, Eames and Lange [312] have reported sensory and motor impairment weakness in claudicants, which has a detrimental effect on the biomechanics of walking [243]. On the other hand, peripheral arterial disease patients with less severe ischemia exhibit more diverse skeletal muscle changes. For instance, selective loss of type II fibers [243] to no loss of fibers [307], or an increase in the number of type I fibers [313]. In addition, some studies have reported selective loss of type IIb fibers [314]. These changes can lead to greater strength deficits in peripheral arterial disease patients due the fact that type II muscle fibers are larger, stronger, and faster than type I muscle fibers.

Traditionally, the technique in the study of muscle fiber typing involves the examination of myofibrillar adenosine triphosphatase activity. This technique allows for the classification of muscle fibers in three distinct groups: (a) slow fibers or type I, highly fatigue resistant, (b) fast fibers or type II, specifically type IIa or intermediate, and (c) fast fibers or type IIb or high anaerobic capacity fibers [243,315,316]. Histologic changes in skeletal muscle in unilateral
peripheral arterial disease patients were examined by Regensteiner et al., [243]. Their findings indicated muscle fiber atrophy and decrease of the type II fiber cross-sectional area. Thus, the histologic changes of muscles, especially type II fiber atrophy, may contribute to the reduced exercise performance in peripheral arterial disease patients. However, there are other studies that report no clear difference in fiber distribution and in diameter between diseased and non-diseased legs of patients with unilateral peripheral arterial disease [317,318]. One reason for these conflicting results may be the heterogeneity of the muscle fiber composition. Furthermore, muscle fibers are composed of a series of contractile proteins that have different molecular and biochemical characteristics [319]. It means that more than one single myosin heavy chain isoform may exist in a single fiber [320].

These myosin isoforms determine muscle fiber type and also reflect the functional state of the ischemic muscle in peripheral arterial disease patients. The main myosin heavy chain isoform of a slow fiber is the myosin heavy chain I, the fast fiber IIb mainly consists of myosin heavy chain IIb, and the intermediate (IIa) contains mainly myosin heavy chain IIa. However, Jaschinski et al., [321] have reported that in a single fiber different myosin heavy chain isoforms exist; (a) a fiber I may be found in myosin heavy chain I and IIa; and (b) a fast fiber IIb may be found myosin heavy chain IIa and IIb.

In that regard, Steinacker et al., [316] investigated for the first time, the expression of myosin heavy chain isoforms in skeletal muscle of peripheral arterial disease patients. They findings indicate a relative decrease of myosin heavy chain IIb and increase of myosin heavy chain I, followed by a decrease of myosin heavy chain IIa with progressive ischemia (see figure 2.4). In addition, McGuigan et al., [315] have reported a significant reduction in myosin heavy chain I isoforms in peripheral arterial disease patients compared with healthy individuals. The
same study using the myofibrillar adenosine triphosphatase activity technique reported a significantly reduced percentage of type I fibers and significantly increased percentage of type IIa fibers. These investigators concluded that the conversion of type I (oxidative) to type II (non-oxidative) fibers limits the endurance capacity of peripheral arterial disease patients.

![Graph showing MHC isoform distribution](image)

**Figure 2.4.** MHC isoform distribution between control patients and patients with PAOD with clinical stage II, III, IV. In comparison with control patients, there was no change of MHC isoforms in patients with PAOD II, but there was a significant decrease of MHC IIb in PAOD III and IIa and of IIb in PAOD IV. MHC, Myosin heavy chain; PAOD, Peripheral arterial obstructive disease [316].

### 2.3.7.2.2 Capillary Density

Studies in individuals free of disease indicate that capillary density is directionally correlated with the oxidative capacity and endurance of a muscle group [322]. Patients with peripheral arterial disease exhibit significant skeletal muscle abnormalities compared with healthy controls [323]. Ironically, despite having a reduced aerobic capacity, the skeletal muscle capillary density appears to be increased in patients with peripheral arterial disease, and the
relative increase in vascular density appears to be proportional to the extent of skeletal muscle hypoxia [323,324]. This response could be viewed as a potentially beneficial adaptation and an attempt to compensate for the atherosclerotic lesions limiting blood flow upstream.

2.3.7.2.3 Metabolic Enzyme Activity

The literature appears to be quite clear in that patients with peripheral arterial disease up-regulate skeletal muscle oxidative enzyme activity. It is believed the upregulation of skeletal muscle oxidative enzyme activity in peripheral arterial disease patients is an adaptive response to the reduced skeletal muscle blood flow [307,325]. Several studies have reported an increase in the oxidative enzymes, citrate synthase and cytochrome oxidase in the calf muscles of peripheral arterial disease patients [311,313,325]. The upregulation of oxidative enzymes is further enhanced in claudicant patients who exercise regularly, suggesting the combination of hypoxia and physical activity in stimulating enzyme activity. Interestingly, the histological and biochemical features of skeletal muscle of peripheral arterial disease patients are not too dissimilar from those seen in athletes [317]. However, studies using electron microscopy have identified structural abnormalities in lower limb muscle mitochondria of peripheral arterial disease patients [326]. For instance, Kemp et al., [327] reported that peripheral arterial disease patients have twice the requirement for adenosine triphosphate than control subjects at the same workload. This metabolic imbalance or reduced metabolic efficiency may provide yet another glimpse into the mechanisms for the reduced functional ability seen in these patients. It appears part of the problem resides with alterations in fat metabolism as evidenced by studies showing an accumulation of acylcarnitine in claudicants [328]. The inability to move acylcarnitines across the mitochondrial membranes may have significant consequences on overall muscle function [329]. Finally, Hands et al., [330] have reported that peripheral arterial disease patients have
slower recovery of high-energy phosphate substrate in the muscles which lead to a poorer
tolerance of exercise and a prolonged recovery time.

2.3.7.2.4 Muscle Metabolism

Muscle contraction depends on a continuous supply of energy. The adenosine
triphosphate-creatine system supplies energy only for short periods of time. The more efficient
way to produce adenosine triphosphate is through aerobic synthesis of adenosine triphosphate,
fueled by glucose and free fatty acids as the major substrates. However, when the oxygen supply
is limited, the anaerobic system will take command. In doing that, pyruvate is converted to lactic
acid rather that Acetyl-Coenzyme A. Resting conditions or light intensity activities utilize free
fatty acids as the main source of energy (β oxidation). If the intensity of the activity increases,
glycolysis will be activated using glucose as a substrate. Although the oxidation of free fatty
acids provides more adenosine triphosphate than glucose, the amount of oxygen required for
fatty acid metabolism is higher.

In patients with peripheral arterial disease any increase in activity levels would cause an
inequality between the oxygen supply and the demand. As a result, aerobic generation of
adenosine triphosphate becomes inadequate, and it forces anaerobic metabolism that increases
lactic acid production [331]. The ischemic muscle during exercise produces high levels of
inorganic phosphate as a consequence of a high rate of adenosine triphosphate and
phosphocreatine breakdown. Hence, the ratio of inorganic phosphates to phosphocreatine
increases to a greater extent than in the normoperfused muscle. It results in a higher reliance of
glycolytic metabolism to obtain the energy needed [290]. Kemp et al., [327] using 31P magnetic
resonance spectroscopy, found an increased accumulation of lactate and depletion of cellular
adenosine triphosphate during exercise in claudicants compared with normal subjects.
The reduced vascular perfusion would be responsible for the reduction of adenosine triphosphate synthesis. As a result, both the oxygen delivery (reduced mitochondrial oxidation) and the washout of toxic metabolites from the skeletal muscle would be impaired [290]. Patients with claudication have been shown to accumulate acetylcarnitine (thus reflecting Acetyl-Coenzyme A accumulation) and lactate (as a consequence of pyruvate dehydrogenase inhibition). Interestingly, Hiatt et al., [332] found that there is an inverse correlation between both plasma and muscle levels of acetylcarnitine and subsequent assessments of peak exercise performance.

These findings were corroborated by the same Hiatt in a later study showing that peripheral arterial disease patients exhibit an accumulation of lactate and intermediates of oxidative metabolism (short-chain acylcarnitines) in skeletal muscle at rest [328]. These results strongly suggest that in patients with intermittent claudication, altered carnitine metabolism may be associated with the altered muscle energy metabolism. Furthermore, a study by Brevetti [333] reported that patients with peripheral arterial disease with mild functional impairment have low resting plasma levels of acetylcarnitine that normally increase with exercise, while the most affected patients have elevated resting levels of acetylcarnitine and do not form these esters with exercise. The plasma concentration of acetylcarnitine depends not only on the rate of formation of Acetyl-Coenzyme A but also on the availability of carnitine to remove it from the mitochondria pool.

Therefore, it is possible to assume that in peripheral arterial disease patients with moderate to severe claudication, the overutilization of carnitine at rest (as reflect by the elevated levels of acetylcarnitine) results in carnitine insufficiency. This condition would prevent to
provide sufficient carnitine to meet the increased metabolic demand induced by walking in peripheral arterial disease [290]. In fact, these patients after carnitine supplementation showed an increase in plasma acetylcarnitine as opposed to the decrease observed before treatment [333].

2.3.7.2.5 Oxygen Delivery to the Tissue

Normally, oxygen passes to the tissue via the capillaries. However, blood entering capillaries is only 50% saturated [334], thus implying that one-half of oxygen gathered by the lungs exits the circulation prior to arriving to the capillaries. Possible sinks for oxygen exiting before the capillaries include oxygen shunting from arterioles to parallel venules, arteriolar-capillary oxygen diffusional shunting and periarteriolar tissue consumption.

Those observations were first reported by Duling and Berne [334]; they found that significant amounts of oxygen exit the arteriolar network. Intaglietta et al., [335] using the phosphorescence decay technology was able to evaluate the longitudinal vessel pO2 gradients (or blood oxygen saturation gradients) and the perivascular pO2 tissue gradients that most likely determine the rate of oxygen exit from blood vessels. Their results confirm that a major portion of blood oxygen is delivered to the tissue by the arterioles.

In addition, Curtis et al., [336] reported that when endothelium is removed from the dog hind limb, oxygen consumption decreases by 34%. In fact, endothelium is capable of metabolic activity which can be 100-fold that of other cells [337]. The endothelium is responsible for the synthesis and secretion of many vasoactive products (e.g. renin, prostaglandins, collagen, nitric oxide, endothelin, and prostacyclin). Moreover, endothelial cells have an active actin/myosin-based contractile system that also may consume oxygen [338].

Therefore, the metabolic stress induced by ischemia would result in more oxygen consumed by the endothelium and less becomes available to the metabolic requirements of the
muscle. Finally, this pathophysiologic mechanism could contribute to the alteration of oxidative machinery occurring in the skeletal muscle of patients with peripheral arterial disease.

2.3.7.3 Summary of Compensatory Adaptations in Peripheral Arterial Disease

In patients with peripheral arterial disease the vascular compensatory mechanism deals more with an initial compensatory dilation and enlargement of the vessel at the site of active atherosclerosis. It is due to neurogenic or metabolic relaxation of the smooth muscle surrounding the blood vessels. Next, the opening of collateral vessels would try to normalize the blood supply. The growth of these collateral vessels follows the principles of both acute (neurogenic) and long-term (metabolic) blood flow control. Alterations in skeletal muscle include (a) selective loss of type II fibers relative to type I fibers, (b) up-regulation of the oxidative enzyme activity, and (c) increased capillary density. However, these changes seem to be proportional to the extent of skeletal muscle hypoxia.

2.3.8 The Role of Exercise in Peripheral Arterial Disease

2.3.8.1 The Acute Exercise Response in Peripheral Arterial Disease

Usually, peripheral arterial disease patients with intermittent claudication do not exhibit signs or symptoms of their disease at rest. It is not until a physiologic stress, such as exercise, is introduced that they develop pain (claudication) due to the insufficient blood flow able to reach the muscles. Therefore, the purpose of this section is to review the effects of a single bout of exercise on patients with peripheral arterial disease. In particular, this review will focus on: (a) the vascular responses and (b) the skeletal muscle responses.

2.3.8.1.1 Vascular Responses to an Acute Bout of Exercise

The change in blood flow upon the transition from rest to exercise in a normal healthy individual is regulated by multiple and redundant mechanisms, such as mechanical and/or non-
mechanical factors. The major factor that contributes to the initial blood flow change is the muscle pump set up by the contracting musculature that moves blood into the veins and away from the skeletal muscle. This is followed by a drop in pressure and an actual rapid inflow of blood in between contractions. This phenomena is known as exercise hyperemia. The more gradual rise in flow appears to be determined by the influence of metabolic and perhaps endothelial factors. Interestingly, there are no studies which have examined the flow responses in peripheral arterial disease patients. Given the stated changes in the vasculature and muscle mass it is hypothesized that the blood flow transition is in part compromised and more than likely cannot reach the same height for a given work load secondary to the blockage.

Therefore, it appears that most peripheral arterial disease patients with intermittent claudication have relatively normal resting lower limb blood flow, and blood flow could increase with exercise [339]. However, as exercise continues, arterial occlusions restrict further increases in blood flow, and consequently oxygen delivery reaches a plateau [339]. The primary effect of patients with peripheral arterial disease during a single bout of exercise is the development of claudication pain in the lower extremity musculature due to the insufficient blood flow.

Interestingly, the ankle brachial index is reduced following an exercise session because blood flow is shunted into the proximal leg musculature at the expense of the periphery and distal areas of the leg [217]. In addition, Bauer et al., [340] have reported that the oxygen consumption (VO₂) kinetics at the onset of exercise are markedly slowed in patients with peripheral arterial disease compared with control subjects. These investigators concluded that the impaired VO₂ kinetic responses in peripheral arterial disease patients appeared to be related to the presence of vascular disease. Finally, the same investigators stated that further research is needed to assess whether peripheral flow limitations or changes in the regulation of skeletal
muscle oxidative function, or both, are responsible for the observed response in patients with peripheral arterial disease.

2.3.8.1.2 Skeletal Muscle Responses to an Acute Bout of Exercise

Peripheral arterial disease leads to insufficient blood supply to the involved muscle, which is especially aggravated during exercise, inducing an imbalance between supply and demand to active muscles. Hemodynamic measures are poor predictors of symptom-limited capacity despite the major role of decreased arterial blood flow in limiting exercise performance [341]. Therefore, it indicates that alterations in skeletal muscle fibers may play an important role in exercise capacity among patients with peripheral arterial disease. Next, this section will review the major modification in skeletal muscle metabolism. Previous studies have shown that peripheral arterial disease have alterations in the regulation of oxidative adenosine triphosphate generation, abnormalities of electron transport chain enzyme activities, and an accumulation of short-chain acylcarnitines in affected skeletal muscle [328,342].

The inherent delay to increase muscle oxidative metabolism in the face of increasing adenosine triphosphate requirements has been termed “metabolic inertia,” and has been hypothesized to be an important physiologic characteristic of the exercise response [343,344]. Indeed, it has been reported that contrary to the up-regulation of oxidative enzymes seen in patients with peripheral arterial disease, these enzymes exhibit a low activity due to structural abnormalities [345]. Thus, these modifications would limit the exercise tolerance in peripheral arterial disease patients.

2.3.8.1.3 Summary of the Acute Exercise Response in Peripheral Arterial Disease

Peripheral arterial disease patients showed interplay between the metabolic and hemodynamic factors that may contribute to the exercise impairment. The exercise performance
of peripheral arterial disease is reduced because of the onset of claudication leg pain. Impaired endothelial function is one mechanism that plays a role in limiting exercise capacity in patients with peripheral arterial disease. The increase in capillary density among patients with peripheral arterial disease may follow the metabolic hypothesis by Adair et al., [346] who suggest that the blood vascular system adapts its structure to meet the maximum oxygen needs of the tissue cells rather than the average tissue requirements. Muscle performance is compromised in patients with peripheral arterial disease because of alterations in fiber type composition that limits the endurance capacity of the muscle. The hypothesized mitochondrial dysfunction may result in less efficient use of the extracted oxygen (less ATP per oxygen) and an accumulation of intermediates that further disrupt metabolism. This means oxidative ‘capacity’ may be preserved while mitochondria function under the non-optimal conditions resulting from the functional abnormality.

2.3.8.2 The Response to Exercise Training in Peripheral Arterial Disease

Many prospective studies have demonstrated the benefits of exercise training in peripheral arterial disease patients with intermittent claudication. The first randomized, controlled trial of exercise training in persons with intermittent claudication demonstrated a marked improvement in treadmill walking ability [347]. A meta-analyses [348] that examined both nonrandomized and randomized trials showed that exercise training improved pain-free walking time by an average of 180% and improved maximal walking time by an average of 120%.

Furthermore, a meta-analyses from the Cochrane Collaboration [349] that considered only randomized, controlled trials concluded that exercise improved maximal walking distance time by an average of 150%. Many trials have demonstrated the efficacy of walking
rehabilitation compared to placebo, those outcomes reported an increase in pain-free and maximal treadmill walking duration [347,350,351]. In addition, the improvement in walking ability results in improvement in routine daily activities [352]. Regensteiner et al., [353] found an improved capacity to perform routine daily activities after exercise training. Exercise training has been shown to significantly improve (a) the onset of leg discomfort, or initial claudication distance, and (b) the point at which the pain becomes unbearable, forcing the patient to stop walking, or absolute claudication distance [354,355].

Patients participating in a supervised, structured exercise program had a 180% increase in initial claudication distance, compared with a 40% increase in control groups [348]. On the other hand, unsupervised exercise rehabilitation programs, often referred to as home programs, have been carried out in addition to supervised ones. The majority of those called “home programs”, have produced a more modest benefit or even have had a lack of benefit compared to a supervised program. A recent meta-analysis by Degischer et al., [356] has reported a lack of significant benefit from these programs. However, Wullink et al., [357] have suggested that a home program with structured coaching might lead to exercise improvement. These findings suggest that exercise training programs have a clinically important impact on functional capacity among patients with peripheral arterial disease. Therefore, exercise training is one of the most effective interventions available for the treatment of claudication as a result of peripheral arterial disease. Despite its demonstrated effectiveness, supervised exercise rehabilitation has several limitations, most notably the lack of available programs.

Recently, the Centers for Medicare and Medicaid Services have established a current procedural terminology code for exercise rehabilitation in patients with peripheral arterial disease (CPT 93668) [358]. Finally, despite numerous studies demonstrating the benefits of a
supervised exercise program for treatment of claudication [359], there is a paucity of data on the durability of results. Menard et al., [360] showed that continuation of an exercise program either supervised or at home, is necessary for maintenance of the positive effects in the treatment of intermittent claudication. In addition, patients who continue to exercise maintain a higher health-related quality of life and greater daily functional status than do those who discontinue training.

2.3.8.2.1 Vascular Responses to Exercise Training in Peripheral Arterial Disease

2.3.8.2.1.1 Blood Flow

Evidence from the literature suggests that an increase in the blood flow to the lower extremity is uncommon [325,350,361-363]. The inability to increase lower limb blood leads to exercise induced pain because the muscle’s metabolic demands exceed the body’s capacity to deliver the needed energy substrate. It has been suggested that exercise in patients with peripheral arterial disease improves maximal walking distance due to an absolute increase in blood flow, possibly secondary to the growth of a collateral circulation. Studies on dogs [364] with ligated femoral arteries have shown an increase in blood flow to the lower limbs after the training intervention. However, many studies have shown that exercise training in patients with intermittent claudication does not significantly improve leg blood flow [339,350,365,366]. Furthermore, a study by Gardner and Poehlman [348] found that the increase in lower limb blood flow was small while exercise tolerance increased by over 100%. Tan et al., [367] reported that exercise training improved exercise tolerance significantly without any increase in femoral blood flow.

Finally, an increase in collateral vessel formation in relation to the leg muscle has been a postulated mechanism for improvement in intermittent claudication symptoms after exercise. Although, animal models of occlusive arterial disease [368-370] have shown that exercise
training may also cause redistribution of blood flow from inactive to active muscles, that
response has not been evaluated widely in humans. One study showed that maximal calf blood
flow increased modestly with exercise, but this small improvement was not related with the
improvement in exercise intolerance [350]. Other studies have also varied results in terms of
whether leg blood flow increased or not and whether any increase observed was correlated to
improved walking ability resulting from exercise training [325,366].

In addition, the role of angiogenesis in improving walking ability after exercise
rehabilitation has not been well investigated in patients with peripheral arterial disease. The
mechanisms underlying endogenous angiogenesis are complex, possible pro-angiogenic stimuli
include reductions in oxygen tension or related metabolic alterations in the muscle, and increased
blood flow and muscle contraction [371]. In that regard, in healthy subjects, a single session of
exercise increased vascular endothelial growth factor mRNA expression in calf muscle [371].
However, that response has not been studied in patients with peripheral arterial disease. In
addition, the finding of increased vascular endothelial growth factor mRNA expression during a
bout of exercise suggests that vascular endothelial growth factor may be related to angiogenesis
[372]. Furthermore, that increase was related in a dose-dependent fashion to the degree of
metabolic stress [372].

Exercise training may be associated with transient hypoxia in the lower extremity which
would up-regulate vascular endothelial growth factor mRNA protein expression potentially
enhancing endogenous angiogenesis. In fact, Gustafsson et al., [373] have reported similar
finding for patients with heart failure. However, content of basic fibroblast growth factor,
another important angiogenic growth factor, does not seem to be altered in response to muscle
activity in hypoxic skeletal muscle [374,375]. In conclusion, the current evidence does not
support the hypothesis that an increase in blood flow is a major mechanism to improve exercise tolerance in patients with peripheral arterial disease.

Therefore, other mechanisms must play a role in the substantial improvements in function and symptoms that occur in these patients when they receive exercise training. Therefore, it would appear that there is not sufficient evidence to suggest that muscle blood flow is positively affected.

2.3.8.2.1.2 Vascular Endothelial Function

Patients with peripheral arterial disease have impaired endothelial-dependent vasodilation [292]. Indeed, Brendle et al., [376] found that endothelial-dependent vasodilation, ambulatory function, and calf blood flow significantly improved after a 6 month exercise rehabilitation program. Furthermore, arterial flow-dependent dilation is mediated by the endothelial release of nitric oxide, which is increased with exercise training and may improve walking ability in patients with intermittent claudication [377,378]. Therefore, it would appear that there is not sufficient evidence to suggest that endothelial function is positively affected with training.

2.3.8.2.1.3 Blood Viscosity

Patients with peripheral arterial disease have a high incidence of abnormal blood rheology [379]. The study by Pararajasingam et al., [379] showed that peripheral arterial disease had a plasma viscosity in excess of the upper limit of normal (1.72). Exercise training in normal subjects leads to a reduction in plasma viscosity [380] and a decrease in packed cell volume [381]. The decrease in packed cell volume could lead to a reduction in blood viscosity and improvement in blood flow. Studies in peripheral arterial disease patients have reported no significant changes in the blood rheology after training [367,382]. Therefore, it would appear that there is not sufficient evidence to suggest that blood viscosity is positively affected.
2.3.8.2.2 Skeletal Muscle Responses to Exercise Training in Peripheral Arterial Disease

2.3.8.2.2.1 Skeletal Muscle Metabolism and Oxygen Extraction

Elevated plasma acylcarnitine levels at rest have been associated with the functional impairment in patients with peripheral arterial disease [332]. Additionally, exercise to claudication pain has been associated with an increase in the plasma concentration of acylcarnitines, which may reflect the disordered metabolic state of ischemic muscle [383]. Muscle oxidative metabolism in patients with peripheral arterial disease favors the accumulation of acylcarnitines. A controlled, randomized trial conducted by Hiatt et al., [350] reported that a 12-week exercise training program for patients with peripheral arterial disease increased peak exercise performance in all treated subjects, delayed the onset and progression of claudication pain during exercise, and improved community-based walking ability.

These investigators also reported that the improvement in performance was correlated with a change in carnitine metabolism (an index of cellular metabolism) and not to a change in blood flow. In addition, the same investigators reported that after 12 weeks of training, there were further increases in exercise performance determined from the training sessions and from treadmill testing. At this point in time, treated subjects had an increase in peak oxygen consumption possibly reflecting either an increase in muscle oxygen delivery or an improvement in muscle oxidative metabolism [350]. The same Hiatt et al., [311] reported that 12 weeks of treadmill training increased peak exercise performance in peripheral arterial disease patients. Although this study did not find the "classic" mitochondrial training response (increase in citrate synthase activity), the results confirm the relationship between skeletal muscle acylcarnitine content and function in peripheral arterial disease, demonstrating that the response to treadmill
training was associated with parallel improvements in intermediary metabolism. In addition, studies have been reported that exercise training in patients with peripheral arterial disease leads to lower lactic acid concentration than was recorded before training [351,384,385]. Many studies have reported an improvement in oxygen extraction in patients with peripheral arterial disease [311,347,350].

Exercise training is associated with an increase in muscle mitochondrial content [386], and the increase in mitochondrial content correlates with the increase in oxidative exercise capacity [387]. On the other hand, detraining or sedentary behaviors are associated with a decrease in muscle mitochondrial content [388]. Peripheral arterial disease leads to a limitation in physical activity, and an increasingly sedentary lifestyle. This level of physical activity might be considered analogous to detraining, and thus would be predicted to be associated with a decrease in mitochondrial enzyme activities. However, earliest observations have reported that the expression and activity of several mitochondrial enzymes are increased in skeletal muscle from limbs in patients with peripheral arterial disease [389]. Furthermore, the same findings have been reported in recent studies that showed an increased mitochondrial content in the muscle of patients with peripheral arterial disease [307,311,325]. This increased mitochondrial expression appears to be a direct consequence of, and is proportionate to, the severity of the occlusive disease. For instance, Jansson et al., [307] reported that bilateral gastrocnemius biopsies from patients with peripheral arterial disease primarily in one leg demonstrate increased enzyme activities in the limb with significant hemodynamic changes when compared with the less-affected limb.

One could assume that the increased mitochondrial expression could be understood in the context of compensation for impaired oxygen delivery [307]; it also suggested that the metabolic
signals leading to this enhanced expression may represent important factors in peripheral arterial disease pathophysiology.

Although peripheral arterial disease is associated with increased mitochondrial content, the regulation of mitochondrial enzyme activity may also differ from that in control subjects. In fact, exercise training is a strong stimulus for mitochondrial proliferation in normal subjects. However, exercise training in patients with peripheral arterial disease has not been uniformly associated with enhanced mitochondrial activity. Indeed, some studies have documented increased mitochondrial enzyme activities with training in peripheral arterial disease [325,363] with others showing no increase despite achieving a systemic training response [311].

This suggests that the regulation of mitochondrial expression in peripheral arterial disease may be under a different series of control factors compared with normal subjects. Despite the limitation in oxygen delivery, changes intrinsic to the skeletal muscle might also contribute to metabolic dysfunction. These findings support the concept that patients with peripheral arterial disease develop a mitochondrial myopathy [390]. Finally, mitochondria in chronically ischemic muscle have abnormal ultrastructure [391], extensively damaged DNA [392], altered expression and activities of enzymes, and abnormally high intermediates of oxidative metabolism [273,332].

2.3.8.2.2.2 Skeletal Muscle Enzymatic Changes

Using muscle biopsies, before and after training, allowed the study of possible enzymatic changes in peripheral arterial disease. In that regard, Lundgren et al., [393] have reported significant increases in the levels of oxidative enzymes such as cytochrome oxidase and succinic oxidase, with a very small rise in citrate synthase. The same study found that patients with a low level of cytochrome oxidase (compared with normal) before training have a markedly improved level on completion of an exercise [393].
Perhaps, the low enzyme activity before training may result from reduced physical activity, muscle fiber depletion or mitochondrial damage secondary to ischemia. These findings would suggest that exercise training may reverse the low oxidative capacity of the muscle in peripheral arterial disease patients.

2.3.8.2.3 Summary of the Response to Exercise Training in Peripheral Arterial Disease

Although there is little doubt that an exercise program is an invaluable first line of treatment for patients with peripheral arterial disease, the mechanisms of improvement in claudication symptoms with exercise are not well understood. The physiological, metabolic, and mechanical alteration produced by the exercise intervention presumably stimulates an adaptive response that may reduce claudication symptoms.

Some of the proposed mechanisms for an increase in exercise tolerance include: increase in leg blood flow, more favorable distribution of blood flow, improved hemorheological and fibrinolytic properties of blood (e.g. reduced viscosity), greater reliance upon aerobic metabolism because of a higher concentration of oxidative enzymes, less reliance upon anaerobic metabolism, an improvement in the efficiency of walking economy and oxygen uptake kinetics, increased free-living daily energy expenditure.

One set of mechanisms that is currently receiving attention is the role of blood flow and associated factors. There is the possibility that angiogenesis, adaptations in peripheral blood flow or changes in blood flow distribution may be a cause of improvement in walking after exercise rehabilitation [371,372]. The role of angiogenesis in improving walking ability after exercise rehabilitation has not been well investigated in patients with peripheral arterial disease and provides an exciting possibility. Although animal models of occlusive arterial disease have
shown that exercise training enhances collateral-dependent blood flow to the hind-limb muscles [369], studies of that nature are limited in humans.

2.4 Protection vs. Optimization

The conditions so far, heart failure and peripheral arterial disease, have a common underlying problem of reduced muscle blood flow and subsequent exercise intolerance. However, the manner in which the body responds to the reduced blood flow is remarkably different and warrants careful consideration. This review has outlined in great detail the different physiological and morphological compensatory adaptations for both conditions. It is very important that we understand these compensatory adaptations properly in order to provide the most effective exercise prescription and avoid just prescribing a generic recommendation for exercise that cannot be as effective.

The main purpose of this next section is to compare heart failure and peripheral arterial disease in regard to the different pathophysiologic pathways to a common problem, the reduction of blood flow to skeletal muscle. In doing so, this section of this review includes: (a) differential adaptation to reduced blood flow, (b) indication for protection and optimization, (c) the underlying mechanisms, and (d) the implication of an exercise program.

2.4.1 Differential Adaptation to Reduced Blood Flow

In the early stages of both diseases, the majority of patients are able to still perform normal daily activities. However, the symptoms and signs are more evident when these patients are exposed to stressful conditions, such as physical activity. Heart failure, as defined previously, is a syndrome in which cardiac output is insufficient to meet metabolic demands. Although the natural history of heart failure is punctuated by occasional episodes of acute decompensation with overt systemic volume overload and pulmonary edema, the primary chronic symptoms in
patients with heart failure are exertional fatigue and dyspnea. These symptoms are the primary determinants of their health-related quality of life. Heart failure is characterized by a series of compensatory mechanisms designed to maintain cardiac output and arterial pressure at a level compatible with life.

The cardiac compensatory changes include (a) ventricular dilation to stretch the sarcomeres (fast adaptation); (b) ventricular hypertrophy (slow adaptation) and aim to preserve cardiac output. The circulatory changes include (a) autonomic nervous system activation (fast adaptation); (b) humoral activation (intermediate to slow adaptation); (c) impaired vascular responsiveness (slow adaptation), and aim to maintain blood pressure. Alterations in skeletal muscle include (a) skeletal muscle atrophy (slow adaptation) and (b) impaired oxidative metabolism (slow adaptation). Although speculative, it is thought that those mechanisms with an intermediate to long-time constant eventually contribute to the severe clinical manifestations.

In contrast, peripheral arterial disease results from atherosclerotic occlusion that impairs perfusion to the lower extremity. The two major clinical presentations of peripheral arterial disease are intermittent claudication and critical limb ischemia. Normally, in patients with intermittent claudication, arterial occlusive disease is manifested by insufficient blood flow during exercise. On the other hand, in patients with critical limb ischemia, blood flow is inadequate to meet the resting demands of the limb. Furthermore, peripheral arterial disease is characterized by a series of compensatory mechanisms designed to increase the peripheral blood flow. They include an initial compensatory dilation and enlargement of the vessel at the site of active atherosclerosis. These changes are thought to be due to neurogenic or metabolic relaxation of smooth muscle surrounding the blood vessels. Next, the opening of collateral vessels would try to normalize the blood supply. The growth of these collateral vessels follows the principles of
both acute (neurogenic) and long-term (metabolic) blood flow control. Alterations in skeletal muscle include: (a) selective loss of type II fibers relative to type I fibers, (b) up-regulation of the oxidative enzyme activity, and (c) increased capillary density. However, these changes seem to be proportional to the extent of skeletal muscle hypoxia.

2.4.2 Indication for Protection and Optimization

In terms of the heart failure condition, one can argue that the reduction in physical performance secondary to the compensatory changes is an effort to protect the organism from putting too much stress on a weakening organ. In other words, if the heart is failing, the body does not want to add an additional stress to cause the heart to fail even more. Thus, exercise intolerance in heart failure is a protective mechanism. Removal of these compensatory changes could therefore be disastrous. Therefore, the patient is dealing with a double-edged sword. By not being active the condition gradually worsens, by being potentially too active the patient will cause further harm. In contrast, patients with peripheral arterial disease also aim to protect themselves, but do so by optimizing blood flow and oxygen transport, in an effort to maintain functionality. However, in peripheral arterial disease exercise intolerance is also a protective mechanism, as the claudication pain with exercise is a warning signal that the tissue is underperfused and at risk for damage and deterioration.

Therefore, two interesting questions come to mind that are relevant to this review and my focus area. First, how does a muscle cell recognize that reduced blood flow is a consequence of a failing heart or a femoral lesion? Second, what are the clinical implications of these differential compensations, and how can this be addressed by the exercise physiologist?

These are clearly challenging questions. In regards to the first, there certainly must be a series of mechanisms that need to be identified to help understand the phenomena. In addition,
there clearly must be interplay between organ systems to orchestrate such compensations, thereby re-establishing the importance of the integrative physiologist. There are probably many candidates that may help understand the compensations for heart failure. These include (a) the neurohormonal system and (b) an altered balance between catabolism and anabolism. Without a doubt the neuroendocrine milieu in heart failure is significantly altered, and in most cases hyperactive in comparison to controls.

In heart failure, the impaired contraction and/or relaxation of the heart and disturbance of central hemodynamics activate compensatory mechanisms, including the neuroendocrine reaction, also called the neurohumoral response, which creates the self-perpetuating vicious circle of heart failure. The low blood pressure reflexly stimulates the adrenergic and renin-angiotensin-aldosterone systems, with increased peripheral vascular resistance and afterload. Then, aldosterone excess promotes fluid retention, peripheral congestion and edema so that both the preload and afterload also increase. Among these neuroendocrine responses, the ergoreflex response is quite important in heart failure. In fact, these ergoreceptors, small myelinated and unmyelinated afferents of the skeletal muscle (group III and group IV afferents), play the role of a feedback control mechanism to maintain the homeostasis between muscle work and energy supply to perform it. Stimulated by the metabolic state of the muscle, the ergoreceptors monitor the effectiveness of the blood flow, and adjust the cardiocirculatory, ventilatory, and autonomic responses to meet the increased metabolic needs of the contracting muscle. In addition, it has been reported an enhanced activity of these receptors, which contribute to the excessive neuroendocrine response [84,92,142]. Some of the possible metabolic products of a discrepancy between blood flow and metabolism in the contracting muscle that have been shown to activate the muscle afferents are potassium [394], a decrease in pH [395], prostaglandins [396], and
blood flow itself [397]. This information is clear evidence of the role of the ergoreceptors as the muscle cell that is able to recognize the reduction of blood flow as result of a failing heart.

In contrast, these neuroendocrine changes are typically not observed in peripheral arterial disease. The main physiological cause for the manifestations of peripheral arterial disease is linked to the insufficient oxygen supply secondary to reduced blood flow. It seems that the candidate is the skeletal muscle itself. Many studies have reported that peripheral arterial disease is associated with a skeletal muscle metabolic myopathy [325,398]. In fact, patients with peripheral arterial disease with severe ischemia had the most defective oxidative function [399]. In addition, the combination of factors related to intensity and frequency of “ischemic insult,” and possibly the ischemia reperfusion associated with it, may be responsible for defective mitochondria [400]. The mitochondrial dysfunction may have two major negative consequences in patients with peripheral arterial disease; (a) peripheral arterial disease mitochondria do not have the capacity to produce as much adenosine triphosphate as normal skeletal muscle mitochondria do, (b) dysfunctional mitochondria are a source of abnormally high levels of reactive oxygen species, which may be independently injurious to the mechanisms that sustain normal cellular structure and function. The end result of this process is a significant decrease in energy production and a potentially increased level of destructive oxygen radicals in the skeletal myocytes of patients with peripheral arterial disease [401]. This evidence would suggest that independent of where the reduced blood flow is coming from, the major problem is the defective mitochondrial function as a pathophysiologic component of peripheral arterial disease.

The next section will try to address the second question: what are the clinical implications of these differential compensations, and how can this be addressed by the exercise physiologist? In heart failure, these compensations are present in every step of the oxygen transport system,
from the center (the heart, lung, central neural control) to the periphery (circulation, neurohumoral status, reflexes, autonomic nervous system, and muscle metabolism). Therefore, the complete system is under permanent stress due to the compensatory responses that are trying to maintain function in these patients. As an exercise physiologist, it is important to have a better understanding of these modifications in order to provide the best and appropriate exercise program without producing further cardiovascular stress in these patients.

In contrast, in peripheral arterial disease, the greater compensations are in the periphery. These compensations are trying to optimize blood flow and oxygen transport in order to maintain and preserve function in these patients. The role of the exercise physiologist is also very important in patients with peripheral arterial disease. It is necessary to know these modifications in order to recognize how these patients will respond to the different exercise activities. In summary, it is very important that we understand these compensatory adaptations in heart failure and peripheral arterial disease, to provide the best recommendation for exercise that fit the condition of these individuals.

2.4.3 Underlying Signals and Mechanisms

This section will attempt a discussion to further understand the underlying signals and mechanisms contributing to, and differentiating compensations between the disease states. The section will also attempt to present information regarding the underlying mechanisms of exercise intolerance in heart failure and peripheral arterial disease.

Heart failure is characterized by general muscular weakness, muscle atrophy, and exercise intolerance that lead to reductions in functional capacity, ability to perform activities of daily living, and health-related quality of life [402]. The cardinal symptoms limiting exercise capacity in patients with heart failure are fatigue and/or dyspnea. The severity of exercise
intolerance can be quantified by a variety of methods, semi-quantitative assessments, such as interview (New York Heart Association classification) and surveys (Minnesota Living with Heart Failure or Kansas City Cardiomyopathy questionnaires), and quantitative methods, including timed walking tests (6-minute walk distance) and graded exercise treadmill or bicycle exercise tests. However, the cardiopulmonary exercise testing on a motorized treadmill or a bicycle ergometer provides the most accurate and reliable assessment of exercise tolerance [22].

Normally, heart failure patients exhibit reduced peak exercise oxygen consumption when compared to normal individuals. Heart failure causes exercise-limiting changes in every step of the oxygen transport system, from the center (the heart, lung, central neural control) to the periphery (circulation, neurohumoral status, reflexes, autonomic nervous systems, and muscle metabolism). The assessment of peak oxygen consumption gives insight into the pathophysiology of exercise intolerance. Exercise intolerance will be closely related to one or both of the parameters that influence peak oxygen consumption (i.e. cardiac output and arteriovenous oxygen (a-vO₂) difference). Indeed, it has been shown that fatigue arises from the impaired cardiac pumping capacity delivering inadequate amounts of oxygen, leading to a buildup of products of anaerobic metabolism within the exercising muscle [142]. The association of heart failure and muscular function might propose that a reduction of blood flow to exercising skeletal muscle would cause a demand-perfusion mismatch, leading to the symptoms and reduced exercise capacity. In fact, patients with severe heart failure distribute only approximately 50% to 60% of total cardiac output to the exercising skeletal muscles, compared with approximately 90% of total cardiac output in healthy individuals [151].

However, in patients with more established heart failure, the major determinants of diminished work capacity appear to be reduced skeletal muscle perfusion during exercise and
alteration in skeletal muscle histology and biochemistry. In these patients, reduced exercise capacity cannot be explained by clinical measures of heart failure (e.g. left ventricular ejection fraction) [173]. In fact, improvement of cardiac output with an infusion of dobutamine improves the femoral vein flow of patients with heart failure, but does not alter muscle performance [403]. These results suggest that nutrient delivery to skeletal muscle may not simply equate to total blood flow, and abnormal nutrient delivery may be caused by maldistribution of blood during exercise. Muscle metabolism and contractile performance correlate with the extent of nutritive flow and inversely with non-nutritive flow [404]. In addition, alterations in the regulation of vascular activity by the sympathetic nervous system and increased noradrenaline may contribute to abnormal nutritive delivery [142]. Therefore, the problem appears to reside in the periphery. Indeed, the problem may be secondary to the inability to shunt blood to the working muscle, to distribute the blood within muscle, or to utilize the available oxygen. A recently proposed hypothesis argues that peripheral skeletal muscle is the source of the signals which disrupt normal patterns of cardiorespiratory control [141]. This hypothesis is known as the muscle hypothesis.

In patients with heart failure the neurohormonal activation and the altered balance between catabolism and anabolism (in favor of catabolism) contribute to disease progression and the transition from non-wasted heart failure to cardiac cachexia [309]. In addition, the ergoreflex (a peripheral reflex originating in skeletal muscle sensitive to products of muscle work) is overactive in heart failure patients and contributes significantly to impaired exercise tolerance [405]. Indeed, a clear association exists between symptom severity, New York Heart Association functional class, muscle wasting, [309] and ergoreflex overactivity [406]. In addition, a recent study by Piepoli et al., [152] has reported: (a) a significant association between peripheral muscle
wasting and enhanced muscle reflex, (b) a strong relationship between more advanced cardiac cachexia and muscle reflex overactivity, which is consistent with the concept that syndrome progression is related to peripheral maladaptive changes in the muscles, and (c) the ergoreflex assessment in 1 limb (arm) is associated with lean tissue loss measured in a different limb (leg), consistent with a systemic activation of the muscle reflex system in heart failure patients. These authors concluded that the downward spiral of dysregulation in exercising skeletal muscle suggested by the muscle hypothesis is similar to the larger picture of other vicious cycles in heart failure in which the physiological responses initially appear adaptive but turn maladaptive over time.

In contrast, patients with peripheral arterial disease suffer exercise-induced limb pain, or claudication. Indeed, claudication is associated with significant functional disability and impairment of quality of life. Claudication typically limits maximal walking capacity to approximately 50% of that in age-matched controls [332]. As a result peripheral arterial disease patients face inability to perform a variety of occupational and recreational physical activities. Despite the clear role of decreased arterial flow in claudication, hemodynamic limitations in the limb are poor predictors of the degree of claudication-associated impairment [331,341]. Furthermore, invasive revascularization does not completely normalize exercise performance [407], and interventions such as exercise rehabilitation (which do not alter hemodynamics) have established efficacy [350]. Therefore, one could suggest that additional factors distal to the arterial obstruction may contribute to the pathophysiologic pathway seen in patients with peripheral arterial disease.

Many investigators have reported evidence that peripheral arterial disease is associated with a skeletal muscle metabolic myopathy [325,326,342,408-410]. Their findings suggest that
in addition to reduced oxygen supply, suboptimal energy production from defective mitochondria may be a factor in peripheral arterial disease pathogenesis. Over the last decade a number of studies have documented that mitochondria in chronically ischemic muscle have abnormal ultrastructure [326], damaged DNA [411], altered enzyme expression and activity, and abnormally high intermediates of oxidative metabolism [325,342]. These studies evaluated the mitochondria characteristics using fixed or frozen muscle samples. Other laboratories have used phosphorus $^{31}$ magnetic resonance spectroscopy [398,409,412] to study the bioenergetics of chronically ischemic skeletal muscle during various types of exercise. These in vivo studies of mitochondrial function demonstrate an intrinsic defect in mitochondrial respiration, similar to that in the mitochondrial myopathies [323,413]. In summary, the skeletal muscle abnormalities include: (a) alterations in mitochondrial expression, (b) accumulation of metabolic intermediates, (c) altered control of mitochondrial respiration, (d) enhanced oxidative stress, and (e) somatic mitochondrial DNA mutations [319].

2.4.4 The Implication of an Exercise Program

Patients with heart failure have poor clinical status and impaired exercise capacity due both to cardiac limitations (significant left ventricular impairment) and to peripheral maladaptations of the skeletal musculature. The latter include the reduction of peripheral blood flow and impaired perfusion as well as deficiencies in skeletal muscle function, morphology and metabolism. These skeletal muscle disturbances increase cardiovascular stress, deteriorate symptoms and reduce further exercise capacity [156,414]. Since the late 1970s, several studies have reported that exercise training can induce favorable physiological adaptations and improve symptoms, clinical outcome and quality of life [70,195,415,416]. The majority of these studies were based on aerobic exercise, which was traditionally the main exercise mode in rehabilitation.
programs. Recently, it has been reported that muscle strength \([417]\) was a better predictor of long-term survival than workload or peak oxygen consumption (\(\text{VO}_2\text{peak}\)) in patients with severe congestive heart failure, indicating the importance of maintaining a normal skeletal muscle mass in order to improve the outcome of these patients. Therefore, resistance training programs would allow training the peripheral muscles effectively without producing great cardiovascular stress. In that regard, dynamic resistance training, based on the principles of interval training, alone \([200-202,418]\) or in combination with aerobic exercise \([203-205]\) is beneficial and counteracts the negative peripheral side effects seen in patients with heart failure.

In contrast, peripheral arterial disease is an obstructive condition where the flow of blood through peripheral arteries is impeded. During periods of increased oxygen demand (e.g. exercise), peripheral limb ischemia occurs, resulting in the sensation of muscle pain termed claudication. As a result of claudication, subjects’ ability to exercise is greatly reduced affecting their quality of life \([252,419]\). Many treatment options for patients with peripheral arterial disease exist; however, exercise training is an effective and low-cost means of improving functional ability and quality of life. Recently, a Cochrane Review supported the role exercise training has in the symptoms of peripheral arterial disease \([349]\), specially improving walking ability in these patients. In addition, Gardner and Poehlman \([348]\) published a meta-analysis of training studies conducted in patients with peripheral arterial disease. Their recommendations appear to have formed the basis of exercise prescription for patients with peripheral arterial disease. These investigators concluded that the most effective training program included training sessions \(>\) 30 minutes in duration, at least three times a week, over a period of 27 weeks or more \([348]\). In regard to the intensity they stated that the program must include training to near maximal pain levels. In addition, few research studies have compared the use of different
training modes in improving walking ability in patients with peripheral arterial disease. The use of resistance [351,353], stair-climbing [420], pole-striding [421] and less specific modes of training including flexibility [422] and gymnastics [423] have been complemented with, or in place of, treadmill walking in an attempt to improve walking ability in patients with peripheral arterial disease. The effectiveness of each type of training appears to vary; however, few statistical comparisons analyzing the effectiveness of walking training to other training modes have been made. However, the use of different modes of exercise training has become an important research topic. Indeed, some elderly people are not able to, or are not comfortable with, exercising on treadmills [424]. In addition, the use of training modes other than walking could improve the adherence of subjects to training programs by removing the discomfort of claudication during exercise.

2.5 Conclusion

In conclusion, in both diseases, heart failure and peripheral arterial disease, which essentially have a common underlying problem of reduced muscle blood flow and subsequent exercise intolerance, the manner in which individuals respond, adapt, and compensate to the disease is highly variable. This review was intended to outline how the different physiologic systems attempt to compensate for the problems incurred by the pathophysiologic pathway of heart failure and peripheral arterial disease. These “compensatory changes” are critical to understand as they to a large degree contribute to the clinical course of patients afflicted by these two diseases. Finally, this is critical as we need to move from generic exercise programs to individualized targeted treatment plans that may be more effective in their delivery, and subsequently help to maintain and preserve functional ability in these patients.
Chapter 3
High and Low Volume Resistance Training and Vascular Function

3.1 Introduction

The addition of resistance training guidelines to the 1998 American College of Sports Medicine (ACSM) position stand was the result of overwhelming evidence of the health and functional benefits associated with this type of training [425]. Resistance training not only impacts strength and endurance, but also contributes to the maintenance of functional ability, prevents osteoporosis, sarcopenia and disabilities [425-427]. Resistance training also reduces cardiovascular risk factors [426]. The mechanisms through which resistance training reduces cardiovascular disease risk are currently not clear. One hypothesis is that resistance training improves vascular function. Data from our laboratory and others show regional vascular adaptations when a localized training stimulus is used [5,428,429]. Importantly, these adaptations occur within 4 weeks of training.

Few studies have examined the effects of whole-body resistance training on vascular function. Those that have studied this issue reveal mixed results. For example, Rakobowchuk et al., [430] reported peripheral arterial remodeling following intense resistance training. However, they did not find changes in the reactivity of the artery. Maiorana et al., [431] did not find any vascular modifications following resistance/aerobic training. In contrast, Green et al., [432] reported improvements in nitric oxide-mediated vascular function, following 8 weeks of resistance/aerobic training in patients at risk for and with coronary artery disease. Those findings were further confirmed in type II diabetics [433]. One study reported a decrease in vascular function following high-intensity resistance training [434]. The reasons for the mixed results are probably multifactorial and include: training duration, intensity, volume, and specificity. We
hypothesized that vascular function would be modified following short-term, whole-body circuit weight training (CWT) and that the improvements would be greater in individuals performing high volume compared to low volume training.

Consequently, the aim was to examine the influence of two volumes of CWT on reactive hyperemic blood flow (RHBF), venous capacitance (VC) and outflow (VO) in younger individuals.

3.2 Methods

3.2.1 Subjects and Experimental Protocol

Thirty-five individuals were recruited. The majority of participants were untrained and/or novices to weight lifting. Those with disease were excluded. Following explanation of the details, participants signed an informed consent approved by the Institutional Review Board of the host institution. The study was a prospective, randomized trial, consisting of 5 weeks of CWT. The experimental procedures consisted of two separate visits performed before and after training.

3.2.2 Experimental Procedures

3.2.2.1 Visit 1: Strength Measures

Handgrip strength was examined in the non-dominant arm, using a dynamometer (model 78010, Lafayette Instruments; Lafayette, IN) and determined from the average of 3 maximum voluntary contractions (MVC). A one repetition maximum (1RM) was used to determine knee extension and bench press strength, using standard nautilus equipment. To eliminate inter-observer variability the same investigator performed these procedures before and after training. Prior to the measures participants were familiarized with the procedures and instructed on the correct techniques.
3.2.2.2 Visit 2: Vascular Function

Participants were refrained from food, alcohol or caffeine for 12 h, and rigorous physical activity for 24 h before the procedures. Prior to the measures, blood pressure cuffs were positioned around the non-dominant upper arm and wrist, and a mercury-filled strain gauge placed around the forearm 10 cm distal to the olecranon process. The strain gauge was connected to a plethysmograph (EC-5R system, Hokanson; Bellevue, WA, USA). The forearm was extended and slightly supinated and supported by a foam block. Following the set-up the participant was allowed to rest for 15 min. The technique is noninvasive, and based on the assumption that alterations of pressures in strategically placed cuffs allows for examination of the rate of change in limb volume thought to reflect vascular function [435].

Resting forearm blood flow was obtained as follows: First, hand circulation was occluded for 1 minute by inflating the cuff at the wrist to 240mmHg. Forearm blood in-flow was obtained using a venous occluding pressure of 7 mmHg below diastolic blood pressure [4]. Forearm VC and VO were measured after an additional 5 min of venous occlusion. Then, RHBF was examined after 5 min of forearm arterial occlusion achieved by inflating the upper arm cuff to 240mmHg [4]. Vascular indices were then determined as described above. Throughout the experiment heart rate and blood pressure were obtained.

Data analysis was performed as follows: Resting blood flow was recorded at a paper speed of 5 mm/sec and values were derived from the slope drawn at a best-fit tangent. Calculations were made as a function of 60 sec divided by the horizontal distance (mm) needed for the slope to rise vertically from baseline to the top of the recording paper and multiplied by the full chart range. Forearm VC was calculated as the vertical distance (mm) representing the change in volume graph following 5 min of venous filling [436]. Forearm VO was derived from
a tangent line representing the vertical drop in the volume-graph from the excursion line and drawn at 0.5 second and 2 sec following the release of the venous occlusion pressure [436,437]. Forearm RHBF was recorded at a paper speed of 25 mm/sec.

Analyses were performed using a slope drawn at a best-fit tangent to the curves of the first 3 pulse flows post cuff release. The RHBF was then calculated from 60 sec multiplied by the paper speed (25 mm/sec) divided by the horizontal distance (mm) needed for the volume slope to increase by 20 mm vertically [4]. All vascular indices are expressed in ml · 100 ml tissue⁻¹ · min⁻¹. Previous work from our laboratory indicated adequate stability and reproducibility of this technique [4].

3.2.2.3 Training Intervention

The CWT was performed in 1 hr sessions, 3 days/wk for 5 wks, consisting of 8 stations. The high volume group was encouraged to complete 3 sets/station, while the low volume group performed 1 set/station. For each station, participants performed 2 or 3 exercises, using sets of 8 to 12 reps performed to voluntary muscular fatigue.

Initial intensity was set at 60% of 1RM or as tolerated. After each station, passive static stretches were performed. Training load was increased by 5-10% for the next session when participants were able to perform 12 reps. Training load, number of reps, and ratings of perceived exertion (Borg scale) were recorded, to document the tolerance and progression of training.

The circuit included: Station 1: biceps curl, triceps extension, and punch-outs; Station 2: knee flexion, knee extension, and calf raises; Station 3: pull backs, pull over; Station 4: squats, and step lunges; Station 5: bench and military press; Station 6: row, and pull down; Station 7: pull and jerk, and sit-ups; and Station 8: Back extension: McKenzie exercises [438].
### 3.2.3 Statistical Analysis

Statistical analyses were performed using SPSS (11.0). Group values are expressed as mean ± SD. Pre-training group differences were examined using independent t-tests. Differences in training volume across the 5 wks were examined using a 2 (high and low volume) * 5 (wks of training) analysis of variance with repeated measures. The influence of training on vascular function and strength was examined using two separate multivariate analyses (MANOVA). Alpha was set a priori at 0.05.

### 3.3 Results

#### 3.3.1 Participant Characteristics and Intervention Results

The average age of the participants was 22±1.89yrs. Weight, height, BMI, resting systolic and diastolic blood pressure, and resting heart rate were not statistically different between groups. The participants’ characteristics are presented in Table 3.1.

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Low Volume</td>
<td>21</td>
<td>1.84</td>
</tr>
<tr>
<td></td>
<td>High Volume</td>
<td>22</td>
<td>1.85</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Low Volume</td>
<td>74</td>
<td>14.39</td>
</tr>
<tr>
<td></td>
<td>High Volume</td>
<td>85</td>
<td>28.45</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Low Volume</td>
<td>168</td>
<td>9.64</td>
</tr>
<tr>
<td></td>
<td>High Volume</td>
<td>173</td>
<td>9.38</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Low Volume</td>
<td>26.2</td>
<td>4.44</td>
</tr>
<tr>
<td></td>
<td>High Volume</td>
<td>27.9</td>
<td>7.00</td>
</tr>
<tr>
<td>Rest SBP (mmHg)</td>
<td>Low Volume</td>
<td>118</td>
<td>10.31</td>
</tr>
<tr>
<td></td>
<td>High Volume</td>
<td>120</td>
<td>9.48</td>
</tr>
<tr>
<td>Rest DBP (mmHg)</td>
<td>Low Volume</td>
<td>65</td>
<td>9.04</td>
</tr>
<tr>
<td></td>
<td>High Volume</td>
<td>65</td>
<td>9.86</td>
</tr>
<tr>
<td>HR rest (bpm)</td>
<td>Low Volume</td>
<td>61</td>
<td>11.49</td>
</tr>
<tr>
<td></td>
<td>High Volume</td>
<td>65</td>
<td>7.07</td>
</tr>
</tbody>
</table>

BMI, body mass index; Rest SBP, resting systolic blood pressure; Rest DBP, resting diastolic blood pressure; HR rest, heart rate at rest.
One participant from the low volume group did not finish the training due to lack of compliance. No injuries or significant problems were reported. The progression and volume of training are presented in Figure 3.1. Over the 5 wks of CWT the high volume group increased the training volume by 62%, from 3219 (Wk 1) to 5236 lbs (Wk 5). The low volume group increased the training volume by 68%, from 2707 (Wk 1) to 3997 lbs (Wk 5).

**Table 3.2** Changes in strength measures (pre and post) in both groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Test</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handgrip (kg)</td>
<td>Low volume</td>
<td>Pre</td>
<td>22.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>25.4*</td>
</tr>
<tr>
<td></td>
<td>High volume</td>
<td>Pre</td>
<td>28.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>33.37*</td>
</tr>
<tr>
<td>Bench press (kg)</td>
<td>Low volume</td>
<td>Pre</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>70.91*</td>
</tr>
<tr>
<td></td>
<td>High volume</td>
<td>Pre</td>
<td>60.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>71.97*</td>
</tr>
<tr>
<td>Knee extension (kg)</td>
<td>Low volume</td>
<td>Pre</td>
<td>79.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>112.44*</td>
</tr>
<tr>
<td></td>
<td>High volume</td>
<td>Pre</td>
<td>94.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>123.53*</td>
</tr>
</tbody>
</table>

Pre, pre-training measures; Post, post-training measures; *p<0.05 vs. Pre

3.3.1.1 Visit 1: Strength Measures

The MANOVA revealed significant increases in all strength measures. However, no significant differences in strength gains were observed between the groups. The strength measures are presented in Table 3.2. The mean absolute and percent change in strength for handgrip strength was 3.89±4.57 kg (+15.59%), knee extension 30.62±12.52 kg (+35.31%), and bench press 12.4±8.81 kg (+21.03%).

3.3.1.2 Visit 2: Vascular Function

Results for forearm vascular measures are presented in Table 3.3. The MANOVA revealed no significant changes in forearm RHBF, VC and VO.
3.4 Discussion

The aim was to examine the influence of different volumes of CWT on forearm vascular function including RHBF, VC and VO among young individuals. Our findings indicate significant and similar increases in strength for both groups. In contrast, group averages for vascular function did not change following five weeks of CWT. Overall changes in strength for this study averaged 24%. These improvements are consistent with previous studies. In fact, the 2002 ACSM [439] position statement on progression models in resistance training indicate that muscular strength increases approx. 40% in untrained and 20% in moderately trained individuals over periods ranging from 4 wks to 2 yrs. The initial gains in strength are believed to be due to neural changes [439]. These adaptations include: improvements in muscle activation and coordination, enhanced muscle fiber recruitment, and improved muscle contraction synergism [440,441]. It is not until ~6 wks into training that muscle hypertrophy becomes obvious and contributes to the strength gains [442]. This study was not designed to measure muscle mass or size, but, forearm circumference did not change.

The average values for the vascular measures were similar to those found in previous studies [4,5], and similar to a low fitness group studied in our laboratory [4]. In addition, the forearm RHBF values are in agreement with others [429,443]. It was hypothesized that vascular function would be modified following CWT and that the improvements would be greater in individuals performing higher volume training. It is important to understand the rationale for the hypothesis. First, the selection of the training duration stems from studies conducted in our laboratory and by others indicating short-term handgrip exercise training results in improved vascular function [5,428]. For example, Alomari et al., [5] reported a unilateral improvement in vasodilatory responsiveness after 4 wks of handgrip training. The training protocol consisted of
20 min/session performed 5 days/wk at 60% of MVC. After training, RHBF improved by ~21% [5]. Studies conducted by others report similar increases (~ 20% to 30%) in RHBF following short-term localized forearm training [429,443]. Given these findings we hypothesized that whole-body CWT for 5 wks would be a sufficient stimulus to induce vascular changes.

Table 3.3 Results for the pre and the post forearm blood flow measures

<table>
<thead>
<tr>
<th>Group</th>
<th>Test</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest BF (ml/100ml/min)</td>
<td>Low volume</td>
<td>Pre</td>
<td>2.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>2.76</td>
</tr>
<tr>
<td></td>
<td>High volume</td>
<td>Pre</td>
<td>3.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>3.15</td>
</tr>
<tr>
<td>Rest VC (%)</td>
<td>Low volume</td>
<td>Pre</td>
<td>3.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>3.48</td>
</tr>
<tr>
<td></td>
<td>High volume</td>
<td>Pre</td>
<td>3.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>3.34</td>
</tr>
<tr>
<td>Rest VO (ml/100ml/min)</td>
<td>Low volume</td>
<td>Pre</td>
<td>38.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>40.78</td>
</tr>
<tr>
<td></td>
<td>High volume</td>
<td>Pre</td>
<td>36.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>39.29</td>
</tr>
<tr>
<td>RHBF (ml/100ml/min)</td>
<td>Low volume</td>
<td>Pre</td>
<td>20.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>21.67</td>
</tr>
<tr>
<td></td>
<td>High volume</td>
<td>Pre</td>
<td>23.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>22.71</td>
</tr>
</tbody>
</table>

Rest BF, resting blood flow; Rest VC, resting venous capacitance; Rest VO, resting venous outflow; RHBF, reactive hyperemia blood flow.

Second, the issue of volume of training stems from the lack of predictability in the vascular responses among the available studies. For example, Rakobowchuk et al., [430] noted an increase in mean brachial artery diameter following intense whole body resistance training. Their participants completed 60 sessions (2/3 sets of 10-12 repetitions, and finished with 4-6 reps with loads exceeding 90% of 1 RM) conducted 5 days/wk for 12 wks. In addition, they reported an increase in RHBF thought to be the result of increased capillarization. However, they did not find changes in the reactivity of the peripheral artery. Maiorana et al., [431] did not find any
vascular modifications following 8 wks of resistance/aerobic training in middle-age men. Their training consisted of aerobic activities (70 to 85% of peak heart rate) and 7 strength training exercises (55 to 65% of MVC), designed to minimize the involvement of the arms. Training was conducted 3 days/wk. Finally, Bond et al., [434] reported a decrease in leg RHBF following 4 wks of training consisting of 6 sets*15 MVC’s of the calf, 4 days/wk. The authors attributed the decrease in peripheral hemodynamics to an increase in calf volume. Clearly the lack of predictability in the vascular responses between studies could, in part, be a consequence of different training volumes.

The present study is the first to examine the influence of different training volumes on vascular function. As shown in figure 3.1, the training volume increased significantly, with both groups noting a 60% change. In addition, the training volume was approx. 25% greater for the

![Figure 3.1](image-url)
high volume group from wk 1 through 5. Despite the evidence in training progression and
difference in volume, no group changes were apparent for any of the vascular measures under
study. Thus, the stated hypothesis was rejected. Several factors may have contributed to the
present findings. These factors will be discussed briefly.

The first factor may be the training stimulus and localization. Our previous handgrip
studies [5,6] included protocols that were quite intensive (20 min sessions, 4 or 5 days/wk) and
very specific to a region. The current study may not have been able to achieve the specificity or
localization needed to trigger vascular adaptations.

A second factor concerns the length of training. Despite our previous work and
expectations, the relative short period of training may not have been enough to promote changes
in muscle mass and/or capillarity density in the region of interest. Arguably, vascular adaptations
may not occur until there is evidence of a change in muscle mass. In fact, changes in the cross-
sectional area of muscle appear to contribute to an increase in capillary density [442]. Perhaps,
the length of training was not enough to allow the structural changes that accompany muscle
hypertrophy to occur. Moreover, Rakobowchuk et al., [430] noted peripheral arterial remodeling
at 6 wks into training. Thus, the present study may have been on the verge of peripheral changes,
but fell short.

A third factor may be the population under study. The participants were healthy students
without overt disease. Generally, improved vascular function following training is observed in
individuals who have reduced vascular function prior to training. For example, Green et al.,
[432] reported that an 8 wk resistance/aerobic training improved endothelium-dependent nitric
oxide-mediated vascular function in both conduit and resistance vessels in patients at risk for and
with coronary artery disease. Those findings were further confirmed in type II diabetics [433]
and patients with heart failure [204]. Interestingly, in this study those individuals with RHBF’s below the 50th percentile (i.e. <23 ml/100ml/min, n=19) before training had a 17% greater RHBF response after training (Pre: 17.41±4.03; Post: 20.30±4.55 ml/100ml/min, p=0.006), independent of group assignment. Similarly, those with the lowest values for VO (i.e. <39.5 ml/100ml/min, n=17) also had significant improvements after training (Pre: 30.58±6.46; Post: 36.11±8.38 ml/100ml/min, p=0.013). We are cautious in the interpretation of these findings due to: (1) paired t-tests were used for these sub analyses, (2) there was no control group, as this was not the focus of the study, and (3) there were no associations between the observed strength gains and vascular changes. Despite this, the improvements in this sub-group are not too dissimilar from previous reports [5,429]. Thus, it appears individuals with evidence of low vascular function may respond favorably to CWT.

3.4.1 Conclusion

In conclusion, short term CWT using high and low volume training resulted in significant, yet, similar increases in strength. In contrast, group averages for vascular function did not change following CWT. Interestingly; those with the lowest pre-training vascular measures did have significant increases in forearm RHBF and VO, independent of group assignment.
Chapter 4

Relationship between Vascular and Physical Function in Individuals with Peripheral Neuropathy

4.1 Introduction

Peripheral neuropathies are a wide range of diseases affecting the peripheral nerves. According to the 108th Congress report, 20 million Americans suffer from the disease [7]. In fact, peripheral neuropathy affects more people than several other well-publicized pathologies, including diabetes mellitus (17-18 million), coronary heart disease (13.2 million), and asthma (15 million) [444]. The prevalence of peripheral neuropathy increases significantly in the older adult population and nearly one-third of all diabetics suffer from this condition [7].

Irrespective of the etiology, it appears that pathological changes associated with the disease are in many cases consistent with ischemia, secondary to vascular insufficiencies [8]. In fact, the literature has clearly established that chronic ischemia contributes to demyelination or axonal degeneration [445], which consequently, gives rise to a variety of symptoms including reduced or altered sensation, pain, muscle weakness, and chronic fatigue. The progression of peripheral neuropathy, generally, follows a distal to proximal pattern, with symptoms usually beginning in the toes before the fingers. Eventually, patients with peripheral neuropathy develop gait abnormalities and subsequent decrements in balance [446]. As a result, a large number of individuals with peripheral neuropathy suffer from a reduction in daily physical activity and reduced quality of life [447].

Importantly, in a recent 2-year follow-up on individuals with lower-extremity peripheral arterial disease (PAD), baseline leg symptoms and ankle brachial index (ABI) were strong predictors of the degree of functional decline [219]. Moreover, PAD patients reportedly have
worse nerve conduction velocity in the legs, considered a clinical marker of peripheral neuropathy, than individuals without PAD [243], suggesting a link between vascular deficiencies and neural function. Thus, it appears that vascular impairments contribute to the functional decline in patients with PAD. Ultimately, it is thought that functional decline leads to loss of independence and disability [448].

Interestingly, despite evidence of functional deterioration and increased risk of falls in patients with peripheral neuropathy [449,450], and studies reporting nerve damage in patients with chronic ischemia [445], few studies have examined the link between vascular and motor function in peripheral neuropathy patients. In one such study, patients with PAD coupled with diabetes exhibited greater lower extremity dysfunction compared to PAD patients without diabetes. The apparent differences were, at least, in part secondary to poorer neuropathy scores [256]. Given the association between physical function and independence, understanding those factors that may contribute to a loss of functional ability and mobility in individuals with peripheral neuropathy is critical.

Therefore, the purpose of the present investigation was to examine the relationship between measures of vascular (both arterial and venous) and physical function (mobility and lower extremity strength) in individuals with peripheral neuropathy. It was hypothesized that individuals with evidence of greater vascular function also would exhibit greater physical function among this population.

**4.2 Methods**

**4.2.1 Participants**

Individuals with peripheral neuropathy were recruited from the greater Baton Rouge metropolitan area. The participants were referred to us with the diagnosis of peripheral
neuropathy by their respective physicians. No distinction was made for the types of peripheral neuropathy. The inclusion criteria for the study was (a) ability to maintain an upright posture for at least one minute voluntarily; (b) ability to walk at least 20 yards independently; (c) willingness to participate in the study; and (d) a diagnoses of bilateral peripheral neuropathy. Individuals were excluded from participation if there was (a) a history or evidence of central nervous system dysfunction; (b) musculoskeletal deformity which could influence gait and balance; (c) a history or evidence of vestibular dysfunction; (d) a history of angina; (e) evidence of plantar ulcer; (f) unstable disease (e.g. uncontrolled diabetes mellitus, Arthritis, coronary artery disease, etc.); and cognitive impairment defined as a score of less than 25 on the mini-mental state exam [451].

Following explanation of all the details of the study, each participant signed an informed consent approved by the Institutional Review Board.

4.2.2 Measurements

4.2.2.1 Peripheral Sensory Testing

Plantar cutaneous pressure sensitivity was assessed using the 5.07 gauge Semmes-Weinstein monofilament (North Coast Medical, Inc). The monofilament exerts 10 g of force at the tip upon buckling and has been used in both clinical and research settings. While there is no widespread consensus as to the optimal protocol, reduced sensitivity as measured by this monofilament has been correlated to common symptoms of peripheral neuropathy [452].

The fiber was applied to 5 sites on the plantar surface (i.e. the great and fifth toes, the first and fifth metatarsal, and the heel). The fiber was applied for 1.5 s and pushed to an angle of 90°. The order of the test sites was randomized to help prevent anticipation of the test by the participant. The participant indicated when he or she felt the fiber. If the participant exhibited any sign of foot ulcers, the testing sites were moved to the perimeter of the ulcer. All testing was
performed by the same technician. Data was recorded as a “0” for no sensation, or a “1” if the monofilament was sensed.

4.2.2.2 Vascular Assessment

Vascular function of the lower leg was assessed using strain gauge plethysmography at rest and after 5 min of arterial occlusion. Specific measures included resting arterial inflow (AI), vascular capacitance and conductance, venous outflow volume and half time (VO\text{t1/2}), and reactive hyperemic blood flow (RHB).

Prior to the vascular assessments participants were instructed to refrain from food, alcohol or caffeine for 12 h, and rigorous physical activity for 24 h. Upon arrival to the vascular laboratory, the participants were placed on an examination table and segmental pressure cuffs were positioned around the lower thigh and ankle [453]. In addition, a blood pressure cuff was positioned in the standard position around the upper arm to obtain blood pressure measures continuously. The data was collected using an automatic blood pressure device (Datascope Accutorr 4, Datascope Corp. Paramus, NJ).

Subsequently a mercury-filled strain gauge was positioned around the widest girth of the lower leg. The position was marked and kept constant throughout the experiments. The strain gauge was connected to a plethysmograph (EC-5R system, Hokanson; Bellevue, WA). The leg was extended and slightly supinated and supported at least 10 cm above the level of the heart by foam blocks, but ensuring the strain gauge was not impeded, in order to provide the most comfortable arrangement for the participant. Following the set-up procedures, participants were allowed to rest for 25 minutes. Resting in-flow was measured in triplicate as follows. First, foot circulation was occluded for 1 minute prior to the assessment by inflating the cuff at the ankle to 240 mmHg. Then, the segmental pressure cuff on the thigh was inflated to 7 mmHg below
diastolic blood pressure, measured at the arm. Arterial in-flow was then recorded three times for 10 seconds with 10 seconds between each recording. Following an additional 2 minutes of maintaining the segmental thigh cuff at the previous determined pressure, venous capacitance was obtained. Subsequently, venous outflow was measured after release of the thigh pressure cuff for 20 seconds.

Patients were then given another 5 minutes of rest prior to initiating the hyperemic test. Following this rest period the thigh cuff was inflated to at least 60mmHg above systolic blood pressure for 5 minutes. In the fourth minute foot circulation was again occluded for 1 minute by inflating the cuff at the ankle to 240mmHg. Upon release of the thigh pressure cuff to a pressure equal to 7 mmHg below diastolic blood pressure, reactive hyperemic blood flow was measured in triplicate for 10 seconds, with 10 seconds between each measure.

Data were analyzed using a customized computer program (Matlab; Mathworks, Natick, MA, U.S.A.). Resting arterial in-flow was calculated from the entire slope, 10 seconds in length, of the volume change over an interval of 10 seconds and the mean of three consecutive measurements was used to determine the average arterial in-flow. Recordings with significant movement artifact were excluded. Resting vascular resistance was calculated as mean arterial pressure / average in-flow. Resting vascular conductance was the reciprocal of vascular resistance or average in-flow / mean arterial pressure. For both assessments, vascular resistance and conductance, a blood pressure measure taken at that point was used for the analysis. Venous capacitance was determined through visual inspection of the position of the recording needle on the plethysmograph. Using a standard scale developed by the manufacturer an overlay is placed over the recording paper. The distance from the base of the graph to the position of the mark at the end of 2 minutes represents the relative venous capacitance.
Reactive hyperemic blood flow was calculated from the slope of the volume change over each 10 seconds interval. This allows for the determination of peak and the average reactive blood flow response across the three measurements. In order to assess the timing characteristics of the venous outflow, venous outflow data were fitted to an exponential curve using the least mean square optimization method and processed by using Solver plug-in within the Microsoft Excel environment. The Venous outflow half time (VO_{1/2}) were calculated based on the time constant of the simulated exponential curve of each testing session.

4.2.2.3 Physical Function Assessment

On a separate day, participants were evaluated in a series of tests for functional capacity, mobility, and lower extremity strength. At least five minutes of rest was provided in between each test, while at least two minutes of rest was provided between trials of applicable tests. The reliability of the functional testing protocol in this study has been reported elsewhere, and was found to be high for patients with peripheral neuropathy. The protocol used is outlined below.

The Six-Minute Walk test was administered using a standardized protocol outlined by the American Thoracic Society [73]. Briefly, cones were placed 30m apart along a well-lit indoor hallway. Participants walked as far as possible in 6-min by walking back and forth around the cones. The distance covered was recorded to the nearest meter. Specific pre- and within-test participant instructions can be found in the American Thoracic Society statement [73]. Maximum walking distance traveled in 6 minutes (6MWD), to the nearest meter, was recorded and used for analysis.

In order to examine balance and coordination a timed up- and-go test (TUG) was implemented, using a previously established protocol [454]. The TUG-test is a useful test in detecting mobility impairment [455]. Briefly, a firm chair with arm rests was placed in the
middle of a well-lit, indoor hallway. In front of the chair, a distance of 3 m was marked on the floor and a large cone was placed on the opposite end from the chair. The test began with the participant seated with their back against the chair, arms in lap, and feet just behind the distance-marker on the floor. Instructions were to stand up using the arm rests if needed, walk safely to and around the cone, walk back to the chair, and sit all the way back in the chair as fast as possible. The timer was started on the work “go,” and stopped when the participant’s back touched the back of the chair. The average time to complete each of 2 separate trials was recorded and used for analysis.

Isokinetic knee extensor peak torque and knee flexor peak torque were measured with the Biodex isokinetic dynamometer (Biodex Medical, Shirley, New York). Participants sat in the Biodex chair with the back rest fixed at 85 degrees. Subjects were secured using pelvic and torso straps to minimize extraneous body movements that may influence testing performance. To further minimize such effects, participants were instructed to hold the Biodex handgrips during the procedure. The lateral femoral epicondyle was used as the reference point for matching the axis of rotation of the knee joint with that of the dynamometer resistance adapter. The lower limb was attached to the resistance adaptor to allow full knee extension and flexion to approximately 100 degrees.

A warm-up procedure consisting of five isokinetic knee extension and flexion movements at 60 °/s was initially completed. A 10s rest period was given in between each trial. Participants were instructed to kick and pull their leg lightly during the first trial, and increase the amount of produced force with each trial so as to generate at or near maximal force during the fifth trial. Following a 3-min rest period, the maximal strength test was completed. Participants repeated warm-up procedures (5 trials, 10s rest between trials), only with specific
instructions to “push/pull your lower limb as hard as you can, remembering to breathe out normally during the trial.” During all maximal trials, verbal encouragement and concurrent visual feedback from a computer monitor were provided to facilitate maximal values. Peak knee extension (KE) and knee flexion (KF) torque (N·m) were recorded for each trial. Values from the three best trials for each movement were averaged and used for analysis.

4.2.3 Statistical Analyses

Statistical analyses were performed using SPSS for Windows (version 11.0). Data are presented as means and SD. To examine the associations between age, measures of vascular and physical function, Pearson product moment correlation coefficients were calculated. Associations between the monofilament scores and various measures were examined using a Spearman rank correlation. To further appreciate the influence of the vascular indices on motor function, analyses of variance were used to compare participants classified according to tertiles on the performance scores, or in the case of the six minute walk test the clinically relevant score of 400m. Finally, potential differences in vascular and motor function based on certain groupings (e.g. etiology, gender, duration of disease) were determined using analyses of variance or independent t-tests. Significance was tested at the 95% confidence level ($p \leq 0.05$).

4.3 Results

4.3.1 Participant Characteristics

A total of 59 individuals with peripheral neuropathy participated in all aspects of this study. Participant characteristics are presented in Table 4.1. Forty-one percent of the participants were women, and 59% men. Seventy percent of the participants were over the age of 65. The oldest participant tested was 87 years old, and the youngest was 39 years old. The duration of the diagnosis ranged from 6 months to 20 years, with ~60% of the participants
suffering from peripheral neuropathy less than 5 years. The etiology of peripheral neuropathy was idiopathic in approximately 56% of the subjects. Thirty-one percent had Type II Diabetes Mellitus, 7% of patients suffered from chronic low back pain, and 5% of patients with neuropathy were cancer survivors. Thirty percent of the patients had a BMI greater than 30kg·m⁻², and 40% had stage I hypertension on the basis of systolic blood pressure (SBP > 130mmHg).

Table 4.1 Participant characteristics

<table>
<thead>
<tr>
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<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
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<td>87</td>
<td>69</td>
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<td>21.78</td>
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<td>BMI(kg·m⁻²)</td>
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<td>47.94</td>
<td>28.78</td>
<td>6.29</td>
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<td>Systolic Blood Pressure (mmHg)</td>
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<td>175</td>
<td>130</td>
<td>17</td>
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<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>52</td>
<td>95</td>
<td>72</td>
<td>11</td>
</tr>
<tr>
<td>Resting Heart Rate (bpm)</td>
<td>42</td>
<td>116</td>
<td>71</td>
<td>12</td>
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<tr>
<td>Pulse Pressure (mmHg)</td>
<td>34</td>
<td>97</td>
<td>57</td>
<td>12</td>
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<tr>
<td>Monofilament Test Score</td>
<td>0</td>
<td>5</td>
<td>2.47</td>
<td>1.43</td>
</tr>
</tbody>
</table>

m = meters; kg = kilograms; bpm = beats per minute

4.3.2 Peripheral Sensory Test

The majority of patients reported partial sensation on the peripheral sensory tests. Ten percent of patients reported no sensation at any of the sites tested, and only 5% of participants reported no diminished sensation at any of the sites. The sites that yielded the greatest sensitivity were the big toe and fifth toe (~64% of participants reported sensing the monofilament). The site of the heel yielded the lowest sensitivity, with ~16% of patients sensing the monofilament. The duration of disease was significantly associated with the sum of the scores on the peripheral sensory test (r=−0.48, p=0.002).

No significant relationships were found between the reactive hyperemic responses or VO₂₁/₂ and the sum of the sensory test scores. However, there were significant associations
between vascular conductance (r=0.44, p=0.01), the TUG (r= -0.37, p=0.03) and the sum of the sensory tests.

4.3.3 Vascular Assessment

The average values for the blood flow indices at rest and following occlusion are presented in Table 4.2. The average resting inflow was 2.48±1.17 mL·100mL⁻¹·min⁻¹. Post occlusion reactive hyperemia values significantly decreased (p=0.0001) with each measure from a peak hyperemic response of 26.93±13.44 mL·100mL⁻¹·min⁻¹, immediately after cuff release, to 17.23±8.58 mL·100mL⁻¹·min⁻¹ at the second and 13.72±7.59 mL·100mL⁻¹·min⁻¹ at the third time point.

Group comparisons based on etiology, gender, or disease duration did not reveal significant differences in any of the blood flow indices, even when the diabetic patients were compared to all others. In addition, there were no correlations between age and any of the inflow measures. In contrast there was a significant association between age and VO₂t/2 (r=0.31, p=0.02). Finally, Table 4.3 reveals several significant associations between RHBF, venous volume, VO₂t/2 and vascular conductance.

4.3.4 Physical Function Assessment

The average scores for the physical function tests are shown in Table 4.4. No differences in performances scores were noted for the different etiologies and disease duration. Men were significantly stronger for KE (Men: 141±73; Women: 80±20 N·m, p=0.001) and KF (Men: 70±30; Women: 39±10 N·m, p=0.001), but no differences were noted for the 6MWD and TUG.

In addition, there were significant associations between age and each of the functional scores (Age vs. 6MWD: r= -0.34, p=0.01; Age vs. TUG: r= 0.33, p=0.01; Age vs. KE: r= 0.51, p=0.001; and Age vs. KF: r= 0.50, p=0.001). Finally, Table 4.3 shows several associations
between the performance scores. A particular strong association was noted between 6MWD and TUG ($r=0.79$, $p=0.001$), whereas there were no significant relationships between TUG and measures of leg strength.

**Table 4.2 Blood flow indices at rest and following occlusion**

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial Resting Inflow (mL·100mL·min⁻¹)</td>
<td>0.96</td>
<td>6.29</td>
<td>2.48</td>
<td>1.17</td>
</tr>
<tr>
<td>Vascular Resistance (AU)</td>
<td>13.46</td>
<td>109.85</td>
<td>45.43</td>
<td>22.01</td>
</tr>
<tr>
<td>Venous Capacitance (mL·100mL·min⁻¹)</td>
<td>0.00</td>
<td>4.00</td>
<td>1.57</td>
<td>0.90</td>
</tr>
<tr>
<td>Venous Volume (mL·100mL·min⁻¹)</td>
<td>8.00</td>
<td>58.00</td>
<td>31.26</td>
<td>12.37</td>
</tr>
<tr>
<td>VO_t/2 (s)</td>
<td>0.60</td>
<td>3.25</td>
<td>1.31</td>
<td>0.51</td>
</tr>
<tr>
<td>RHBF Peak (mL·100mL·min⁻¹)</td>
<td>8.46</td>
<td>73.62</td>
<td>26.93</td>
<td>13.44</td>
</tr>
<tr>
<td>RHBF Average (mL·100mL·min⁻¹)</td>
<td>7.52</td>
<td>51.18</td>
<td>19.36</td>
<td>8.58</td>
</tr>
<tr>
<td>Peak Vascular Conductance (mL·100mL·min⁻¹)</td>
<td>0.08</td>
<td>0.69</td>
<td>0.30</td>
<td>0.15</td>
</tr>
</tbody>
</table>

AU = arbitrary units of resistance; VO_t/2 = venous outflow halftime; s = seconds; RHBF = reactive hyperemia blood flow

4.3.5 Associations between Vascular and Physical Function

The associations between the vascular indices and physical function are presented in Table 4.3. In particular the associations between peak RHBF responses and the TUG (Peak RHBF = 37.88 - 1*TUG; $r=-0.31$, $p=0.024$) and the 6MWD (Peak RHBF = 8.65 + 0.05*6MWD; $r=0.37$, $p=0.007$) are of interest. Specifically, as shown in Figure 4.1 individuals in the top tertiles of the TUG had significantly higher peak RHBF responses compared to the lowest tertile [$≤9.02$, RHBF: 30.97±12.56 mL·100mL⁻¹·min⁻¹; 9.02-10.88s, RHBF: 28.88±15.99 mL·100mL⁻¹·min⁻¹; >10.88s, RHBF: 19.88±8.10 mL·100mL⁻¹·min⁻¹, $p=0.04$].

Those with a 6MWD score $<400$m had lower RHBF compared to those who walked $>400$m (see Figure 4.2) [$<400$m, RHBF: 23.47±10.52 mL·100mL⁻¹·min⁻¹; $>400$m, RHBF: 30.65±15.05 mL·100mL⁻¹·min⁻¹, $p=0.05$]. In addition, peak vascular conductance was also significantly related to the TUG (Peak Vascular Conductance = 0.50 – 0.02*TUG; $r=-0.38$,}
p=0.007) and 6MWD (Peak Vascular Conductance = 0.50 + 0.001*6MWD; r=0.44, p=0.001), showing similar differentials based on the tertiles and 400m threshold as for the peak RHBF response (see Figure 4.3 and 4.4). Finally, a significant association between VO\(_{t/2}\) and KF (VO\(_{t/2}\) = 1.67 - 0.01*KF; r=-0.37, p=0.01) and KE (VO\(_{t/2}\) = 1.67 - 0.01*KF; r=-0.37, p=0.01) was observed, with those in the top tertile exhibiting faster VO\(_{t/2}\) (see Figure 4.5) [>77N·m, VO\(_{t/2}\): 1.01±0.31s; 36-77N·m, VO\(_{t/2}\): 1.54±0.64s; <36N·m, VO\(_{t/2}\): 1.38±0.40s, p=0.004].

**Table 4.4 Physical function scores**

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>6MWD (m)</td>
<td>98</td>
<td>570</td>
<td>373</td>
<td>106</td>
</tr>
<tr>
<td>Timed up and go (s)</td>
<td>6.20</td>
<td>33.25</td>
<td>11.00</td>
<td>4.26</td>
</tr>
<tr>
<td>KE (N·m)</td>
<td>23.67</td>
<td>327.17</td>
<td>104.71</td>
<td>56.83</td>
</tr>
<tr>
<td>KF (N·m)</td>
<td>10.20</td>
<td>151.57</td>
<td>51.38</td>
<td>25.09</td>
</tr>
</tbody>
</table>

6MWD = 6 minutes maximal walking distance; m = meters; s = seconds; KE = knee extension; N·m = newton-meter; KF = knee flexion

### 4.4 Discussion

The purpose of the present investigation was to examine the relationship between measures of lower leg vascular and physical function in individuals with peripheral neuropathy. The present data, indeed, support a link between measures of vascular and physical function in individuals with peripheral neuropathy. Specifically, individuals in the lowest tertile of the TUG and those with a 6MWD score <400m had significantly lower peak RHBF responses and peak vascular conductance compared to the other groups.

In addition, individuals in the top tertile of KE and KF exhibited faster VO\(_{t/2}\) than the other tertiles. These findings fit “The Disablement Pathway” and suggest that lower motor function may, in part, be a consequence of deterioration of peripheral vascular function in individuals with peripheral neuropathy.
4.4.1 Vascular Assessment

The use of venous occlusion plethysmography is a powerful tool to study limb blood flow in humans. In general, the study of blood flow responses following occlusion indicates that younger, fitter, and healthier individuals exhibit greater flow responses, suggesting better vascular function.

![Graph showing association between peak reactive hyperemia blood flow (peak RHBF) responses and the timed up and go (TUG) tertiles. * indicates significant difference from other tertiles (p=0.01)](image)

**Figure 4.1.** Association between peak reactive hyperemia blood flow (peak RHBF) responses and the timed up and go (TUG) tertiles. * indicates significant difference from other tertiles (p=0.01)

To that extent, peak RHBF responses in the present study were ~17% lower than values reported for a group of healthy individuals in the same age range, and ~18% and 29% higher than a group of heart failure and PAD patients [153,456], using the same analyses techniques.

Although beyond the scope of this paper, a lower than expected peak RHBF response suggests alterations in the main factors that govern reactive hyperemia. More recently,
Table 4.3 Correlations between vascular and physical function

<table>
<thead>
<tr>
<th></th>
<th>6MWD</th>
<th>TUG</th>
<th>KE</th>
<th>KF</th>
<th>Venous Cap</th>
<th>Vasc Con</th>
<th>Venous Vol</th>
<th>Venous Outflow</th>
<th>RHBF avg</th>
<th>RHBF peak</th>
<th>Peak Vasc Con</th>
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<tr>
<td>6MWD (m)</td>
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<td></td>
<td></td>
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<tr>
<td>TUG (s)</td>
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<td></td>
<td></td>
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<tr>
<td>KE (Torque)</td>
<td>0.29</td>
<td>-0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.029</td>
<td>0.321</td>
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<tr>
<td>KF (Torque)</td>
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<td>0.088</td>
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<td>Venous Cap (mL·100mL⁻¹·min⁻¹)</td>
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<td>Vasc Con (mL·100mL⁻¹·min⁻¹)</td>
<td>0.938</td>
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<td>Venous Outflow t₁/₂ (s)</td>
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<td>RHBF avg (mL·100mL⁻¹·min⁻¹)</td>
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<td>RHBF peak (mL·100mL⁻¹·min⁻¹)</td>
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<td></td>
</tr>
</tbody>
</table>

6MWD = 6 minutes maximal walking distance; m = meters; TUG = timed up and go; s = seconds; KE = peak knee extension torque; KF = peak knee flexion torque; Venous Cap = venous capacitance; Vasc Con = vascular conductance, Venous Vol = venous volume; RHBF avg and RHBF peak = reactive hyperemia blood flow average and peak; Peak Vasc Con = peak vascular conductance.
researchers have also used peak limb vascular conductance immediately after a period of cuff arterial occlusion as an indirect measure of the structural limitations placed on limb muscle blood flow [457]. The values for peak vascular conductance, in the present study, were considerably lower than those reported for a group of men free of overt disease [457].

Figure 4.2. Association between peak reactive hyperemia blood flow (peak RHBF) responses and the maximal walking distance cut-off points. Difference is significant at p=0.05

Thus, a lower than expected peak vascular conductance in these patients could reflect, in part, structural changes related to the arterial vasculature of the limbs (e.g. a reduction in vascular density and or number) that limit the extent of vasodilation. Together, these findings suggest that the arterial vasculature in individuals with peripheral neuropathy is compromised. Such a compromised system could certainly contribute to the progression of the disease. In fact, the literature provides significant evidence that conditions of acute and chronic ischemia
contribute to changes in peripheral nerve function and structure [445]. In fact, Laghi Pasini et al., [445] reported a strong correlation between severity of nerve damage and stage of vascular insufficiency. It is not currently known what underlying mechanisms may contribute to deterioration of peripheral nerve function, but an increase in intracellular calcium [458], and/or reduced oxygen species [459] could disrupt myelin and lead to axonal fiber degeneration.

In regards to venous circulation, several investigators have reported a reduction in venous function with advancing age [460] and in patients with heart failure [461]. It is not clear what mechanisms are involved in this reduced function, but impaired muscle activation and generalized deconditioning may be involved. In addition, it is important to consider the changes in the structure and function of the vasculature and peripheral nerves as a result of the aging process. In that regard, Fu et al., [460] reported an age-related decline in venous filling and emptying. It is not known if the progression of peripheral neuropathy may accelerate the decline in venous function, or if a decrease in venous function contributes to the progression of the disease. Given the important contribution of the venous system in metabolic waste removal and end-diastolic volume, changes in this part of the circulation may be quite significant in terms of overall vascular function. The present data confirms an association between venous outflow half time and age in individuals with peripheral neuropathy.

4.4.2 Physical Function Assessment

A decline in functional performance in individuals with peripheral neuropathy is widely accepted, although the rate of decline and contributing mechanisms are much less understood. The six-minute walk test is an easily implemented test that determines the maximum distance a person can walk in 6-min. This test is a valid measure of “functional capacity” as it evaluates the integrated response of all systems involved during physical activity [73].
It is therefore frequently used in both elderly and diseased populations, and was therefore chosen for the current study. The total group average for the 6MWD (373±106m) in this study appears to be quite typical for those seen in patients with heart failure [64], indicating significant functional limitations. Importantly, based on the literature indicating that a walking distance less than 400 meters is associated with higher prevalence of institutionalization, morbidity, mortality, hospitalization and transplantation [462], the results of the present study suggest 50% of the neuropathy patients are at elevated risk for complications.

\[\text{Figure 4.3. Association between peak vascular conductance and the timed up and go (TUG) tertiles. * indicates significant difference from slowest tertile (p=0.003). † indicates significant difference from middle tertile (p=0.05)}\]

The TUG test has been suggested to be a measure of basic mobility skills [454]. The test assesses the time it takes a participant to stand up from a chair, walk around a cone 3 m away, walk back to the chair, and sit down. Data regarding norms and threshold scores for the TUG-test are available but are somewhat conflicting. For example, Steffen et al.,[463] reported average
TUG-test times on a group of 60 to 69 years old men and women around 8 sec. Bischoff et al., [455] recommended the need for early evaluation and intervention for community-dwelling elderly women between 65-85 years of age, who scored above 12 s on the TUG-test. Others identified a cutoff point of 24 s as a parameter that significantly predicted falls during a 6-month follow-up period. In the present study, 26% of participants scored above the 12s mark, suggesting these patients may be at elevated risk.

![Figure 4.4. Association between peak vascular conductance and the maximal walking distance cut-off points. Difference is significant at p=0.007](image)

Maximal isokinetic knee strength is a common measure of muscular strength and thus chosen for the current study. It has been shown to decline with advancing age [464, 465]. The average KE for this cohort (104.71±56.83 N·m) is approximately 10% lower than those reported for 60 to 69 year old individuals participating in NHANES 1999-2000 [465]. In addition, the average KF in the present study (51.38±25.09 N·m) is 17% lower than those reported for men and women aged 70 to 79 years from the Health, Aging, and Body Composition Study [464].
These data suggest that patients with peripheral neuropathy have lower than expected leg strength. Lack of measurement of muscle mass, composition, or more sophisticated tests to examine neural recruitment, prevents speculation regarding the underlying mechanisms involved.

![Graph](image)

**Figure 4.5.** Association between venous outflow halftime (VOt\(1/2\)) and peak knee flexion torque (KF) tertiles. * indicates significant difference from other tertiles \(p<0.05\)

4.4.3 Associations between Vascular and Physical Function

The major focus of this study was to examine the relationship between measures of lower leg vascular and physical function in individuals with peripheral neuropathy. The data are consistent with the stated hypothesis that individuals who have greater peak RHBF responses and vascular conductance following occlusion have higher scores on the TUG and 6MWD tests. In contrast, no significant associations were noted between the measures of vascular reactivity and knee flexion and extension.
It is important to notice the relationship between peak RHBF responses, vascular conductance and the 6MWD. Both vascular indices studied provide evidence of the ability of the vasculature to respond to an increase in metabolic demand (i.e. 5 minutes of occlusion). Arguably, the physiologic demands associated with the walking test would also serve to challenge the vasculature, and consequently individuals who have evidence of vascular impairments would have a reduced motor capacity. Importantly, this study used 400 m as the differentiating point. Studies have indicated the prognostic value of this threshold as it relates to institutionalization, morbidity, mortality, hospitalization and transplantation [462]. It is interesting to speculate that the increased risk in patients with peripheral neuropathy, who score below 400m on the walk test, may in part be secondary to diminished vascular function.

It is also important to notice the relationship between peak RHBF responses, vascular conductance and the TUG-test. The TUG-test is a basic test used to examine mobility and balance. Arguably performance of the TUG-test is a more complex interplay between physiological systems than the walk test. It is generally accepted that the TUG-test does not focus on independent effects of organ impairments, such as low muscle strength, decreased balance and other impairments, but measures the interaction of these factors on the performance of activities of daily living. It is not clear what influence a diminished vasculature has during the TUG-test in the peripheral neuropathy patients. However, we speculate that the initiation and subsequent continuation of a movement pattern is very much dependent on the ability to immediately increase blood flow to the working muscle. A major factor in the acute rise in muscle blood flow is the muscle pump, and subsequent distribution of cardiac output toward the working muscle. Thus, if the factors involved in the initial rise in blood flow with the onset of activity are impaired this could result in the increase time needed to perform the TUG-test. We
are unaware of studies that have examined blood flow at the onset of exercise in peripheral neuropathy, but do recognize that such responses are blunted in the elderly. Thus, we encourage further studies to explore the link between muscle blood flow and functionality in the peripheral neuropathy patient.

The lack of significant associations between peak RHBF responses, vascular conductance and the isokinetic strength tests was not surprising. The strength tests probably are more specific in regards to the physiologic systems involved in the movement (e.g. muscle mass and neural innervation), and more than likely does not rely heavily on the delivery of blood to perform the test. In contrast, both KE and KF were significantly related to the VO\textsubscript{1/2}. These findings further confirm data from our laboratory suggesting that muscular fitness is related to venous function. The associations suggest the contribution of the venous system to motor function is beyond the typical role as a “passive volume reservoir”. Importantly, the venous system is controlled primarily by the sympathetic nervous system. Generally, patients with chronic degenerative conditions tend to be hyperadrenergic. It is certainly possible that muscle deconditioning in some patients with peripheral neuropathy contributes to modifications in venous function.

We remain cautious in our interpretations considering the limitations inherent to a cross sectional design and the lack of causality presented. It is also recognized that the lack of longitudinal data regarding vascular and physical function and many other important factors in individuals with peripheral neuropathy are not accounted for in the present study. However, the present observations are unique in a sense that it is the first study to establish a link between vascular and physical function in patients with peripheral neuropathy, and consequently contributes to the existing literature in identifying several unique aspects that warrant further discussion and research.
It is also recognized that the present data do not elucidate possible mechanisms why individuals with the variables under interest are linked. The lack of a mechanistic approach in the current study prevents more sophisticated speculation regarding the development of possible preventive or compensatory interventions. However, given data indicating that exercise training can improve vascular function [433] we hypothesize that physical training may be an excellent way to preserve functional ability and independence in certain patients with peripheral neuropathy.

In summary, the present findings strongly suggest that diminished vascular function contributes to a decrease in physical function in individuals with peripheral neuropathy. These findings are particularly interesting in light of recent data that clearly indicates that lower cardiorespiratory fitness is a significant predictor of dependence [466] and that ABI is a strong predictor of the degree of functional decline in patients with PAD [219].

The newly published “International Classification of Impairments, Disabilities, and Handicaps” (ICF) by the World Health Organization (www.who.int/classification/icf) emphasizes the influence of human body function and structure on the level of functioning, activity and participation. People with peripheral neuropathy have little activity and participation due to limited by mobility, and this in turn have a negative effect on their quality of life. The recognition of a relationship between vascular and physical functions enables us to further investigate the mechanisms that underlie the functional limitations. These future studies may provide us the information necessary for the development of effective interventions.

4.4.4 Conclusion

The purpose of the present investigation was to examine the relationship between measures of leg vascular and physical function in individuals with peripheral neuropathy. The
results support a relationship between measures of vascular and physical function where individuals with greater physical function scores exhibited more favorable measures of arterial inflow and venous outflow. The lower physical function may, in part, be a consequence of deterioration of peripheral vascular function in individuals with peripheral neuropathy.
Chapter 5

Effects of an Acute Bout of Exercise on Nitric Oxide Bioavailability, Oxidative Stress, and Anti-oxidant Defense Markers in Trained Individuals

5.1 Introduction

The endothelium controls vascular tone, coagulation, and inflammatory responses. Indeed, normal endothelial function includes control of antithrombotic and thrombolytic activity, vascular architecture and permeability, leukocyte interactions with the vessel wall, and vascular tone regulation during rest and exercise [237]. Vascular endothelial cells play an important role in maintaining cardiovascular homeostasis primarily through the release of protective bioactive substances, the most widely studied being nitric oxide [467]. The capacity of the endothelium to release nitric oxide has been recognized as an important surrogate marker of general endothelial health [468]. Cardiovascular diseases, diabetes, aging, and smoking, among other factors, have been associated with reduced vascular endothelial nitric oxide function [469]. In addition, disturbances of nitric oxide bioavailability have been suggested to play a key role in vascular dysfunction and the development of atherosclerotic lesions [470].

Nitric oxide is a labile, lipid soluble gas synthesized in endothelial cells from amino-acid L-arginine through the action of endothelial nitric oxide synthase [471]. Three distinct nitric oxide synthase isoforms have been identified: (a) neuronal nitric oxide synthase (type I), (b) inducible nitric oxide synthase (type II), and (c) endothelial nitric oxide synthase (type III) [470].

There are two major compartments for nitric oxide metabolism; the blood plasma and the red blood cells compartment. The major immediate breakdown product of nitric oxide in human plasma is nitrite. Plasma nitrite can be taken up by red blood cells, where it is oxidized in a hemoglobin-dependent manner to nitrate, which may subsequently redistribute into plasma...
The term ‘free radical species’ summarizes a variety of highly reactive molecules that can be divided into different categories, e.g. reactive oxygen species, reactive nitrogen species, and reactive chlorine species [473]. In addition, oxidative stress species, more generally, reactive oxygen species, as well as reactive nitrogen species, are products of normal cellular metabolism [474]. Indeed, reactive oxygen species and reactive nitrogen species are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems [475].

The beneficial effects of reactive oxygen species occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia; for example in defense against infectious agents and in the induction of a mitogenic response [474]. On the other hand, the harmful effect of free radicals causing potential biological damage is termed oxidative stress and nitrosative stress [476]. Therefore, oxidative stress refers to the situation of an imbalance between the production of free radicals and the antioxidant defense mechanisms.

In addition, Davies et al., [477] have reported that free radicals are formed also in response to physical exercise. In fact, there is significant evidence that an acute bout of exercise results in a significant increase in oxidative stress markers [478]. Interestingly, we are aware of only one study that has examined markers of oxidative stress and anti-oxidants molecules in conjunction with nitric oxide bioavailability [479]. This is particularly important as nitric oxide can neutralize or become neutralized by reactive oxygen species. The neutralizing capabilities of nitric oxide may to some extent protect the endothelial environment, but excess reactive oxygen species may also reduce the other functions of nitric oxide, thereby increasing vascular vulnerability [470]. The study by Di Massimo et al., [479] evaluated the ratio between nitrite/nitrate or NOx, as a marker of nitric oxide bioavailability. Their results indicated an
increase in NOx and oxidative stress markers, and a decrease in total antioxidant systems from baseline to peak exercise, returning to baseline values within 24 hrs. These findings suggest that endothelial balance may be altered with acute exercise. However, it is well known that plasma nitrate levels are influenced by a variety of nitric oxide synthase-independent factors (dietary intake, saliva formation, denitrifying liver enzymes, and inhaled atmospheric gaseous nitrogen).

Furthermore, Lauer et al., [470] have reported that nitrate and/or NOx do not generally represent useful markers of endogenous nitric oxide production. According to Lauer et al.,[470] a better marker of nitric oxide bioavailability is the nitric oxide stored on the hemoglobin of the red blood cells; nitrosylated hemoglobin. Indeed, this marker has been suggested to have a potential role in transport and delivery of nitric oxide [480].

Given the current shortcomings in the literature regarding our understanding of endothelial balance, the purpose of the present study was to examine the effects of an acute bout of exercise on nitric oxide bioavailability, oxidative stress markers, and anti-oxidants markers at three time points, before, at peak exercise, and at 20 minutes into the recovery phase in aerobic trained individuals. It was hypothesized that an acute bout of exercise would result in a decrease in nitric oxide bioavailability and an increase in oxidative stress markers from baseline to peak exercise, with a gradual return to baseline in the recovery phase. In addition, on the basis of previous investigations [479] it was hypothesized that the total anti-oxidants markers would decrease from baseline to peak exercise, and return to pre-exercise levels in recovery. It was speculated that the changes in the biological markers of nitric oxide, oxidative stress, and anti-oxidant defenses, in part, reflect the endothelial responses to an acute bout of exercise and represent a marker of the ability to maintain endothelial balance during and following physical stress. In addition, this study also examined the effects of acute exercise on vascular reactivity,
defined as brachial artery flow mediated dilation. It was hypothesized that vascular reactivity would decrease from baseline to immediately after 30 minutes of high intensity cycling, and return to pre-exercise levels within 20 minutes of recovery.

5.2 Methods

5.2.1 Participants

Aerobically trained individuals were recruited to participate in this study. Individuals with any unstable manifestations of cardiovascular, metabolic, orthopedic, neurological disease, or at risk for adverse responses to exercise, and/or taking any medication which may affect cardiovascular function were excluded from participating. The current study was reviewed and approved by the Louisiana State University Institutional Review Board.

5.2.2 Experimental Design

The study was a prospective design consisting of two separated visits. Both visits involved a bike time trial of 30 minutes. During the first visit, prior to, at peak exercise (30min) and at 20 minutes in recovery blood samples were obtained for the purpose of examining reactive oxygen species, anti-oxidants, and nitrosylated hemoglobin. In addition, gas analyses were used for the determination of oxygen consumption, carbon dioxide production, and ventilation. During visit two, vascular reactivity of the brachial artery was assessed prior to, immediately following peak exercise and at 20 minutes in recovery.

5.2.3 Experimental Protocol

5.2.3.1 Visit 1

Participants reported to the laboratory, and were provided with the details, risks, and potential benefits of the study. Following explanation and signing of the informed consent the participant was provided a short questionnaire to determine the training status of the individual.
Given the participants were all trained the questions dealt specifically with their average volume of training per week, and schedule of competition, and their rankings.

After the assessment a nurse clinician placed an indwelling catheter in an antecubital vein for the purpose of blood sampling. After securing the catheter the participant was given the opportunity to relax for approximately 15 minutes before the initial sample was obtained.

Subsequently, the participant was asked to mount their bicycle which was placed in a high performance, microprocessor controlled indoor trainer (Race Mate Compu Trainer). The participant was then fitted with head-gear for the purpose of gas analyses and a Polar heart rate monitor. After a brief calibration period heart rate and blood pressure were assessed, and the breath-by-breath respiratory gas analyses system (Sensormedics software, Yorba Linda, CA) started, and baseline measures obtained for five minutes. Then the time trial commenced with the participant pedaling at a speed of 80 to 90 revolutions per minute. Resistance was increased over the first 5 minutes to achieve 80 to 85% of the participant’s estimated heart rate reserve.

Throughout the test breath-by-breath measures of oxygen consumption, carbon dioxide production, and pulmonary ventilation were obtained. In addition, before, during and after the test, heart rate, blood pressure, and ratings of perceived exertion were obtained at five minute intervals using a standard sphygmomanometer, Polar heart rate monitor, and Borg’s perceived exertion scale (RPE, Borg’s 20-point scale) [481], respectively.

Blood sampling was performed prior to, at peak exercise (30min) and at 20 minutes in recovery, from an antecubital vein. Each sample was approximately seven milliliters (7 mL). Following each draw the sample line was flushed with saline. Prior to the second and third draw approximately 2 to 3 ml were drawn and discarded to prevent any saline in the sample to be analyzed. Following each draw approximately 3 ml of blood was immediately treated with
specific spin trap probes and subsequently frozen in liquid nitrogen and kept at -80°C for analyses. The remaining sample was centrifuged and separated and subsequently frozen for the analyses.

5.2.3.2 Visit 2

Participants reported to the laboratory and were placed on an examination table in preparation for the assessment of brachial artery reactivity prior to, immediately following peak exercise and at 20 minutes in recovery. Following a 15 minute resting period, ultrasound images were obtained using high resolution ultrasonography (Hewell Packer, Sonos 2000, 7.5-MHz linear array transducer). Images were obtained in the longitudinal view, approximately 4 cm proximal to the olecranon process, in the anterior/medial plane, with the participant's arm immobilized and slightly supinated. Image depth was initially set at 4 cm, and gain settings were adjusted to provide an optimal view of the anterior and posterior intimal interfaces of the artery and kept constant throughout. Subsequently, brachial artery reactivity was tested by inflating a blood pressure cuff, positioned approximately 1 cm distal to the olecranon process, to 240 mm Hg for five minutes. Images were obtained immediately before forearm occlusion, and continuously from the final 30 seconds of occlusion until three minutes following the release of the blood pressure cuff.

After the vasoreactivity test, the participant was asked to mount their bicycle which was again placed in a high performance, microprocessor controlled indoor trainer (Race Mate Compu Trainer) and the bike was properly calibrated. The time trial commenced with the participant pedaling at a speed of 80 to 90 revolutions per minute. Resistance was increased over the first 5 minutes to achieve 80 to 85% of the participant’s estimated heart rate reserve. Before, during and after the test, heart rate, blood pressure, and ratings of perceived exertion were obtained at five
minute intervals using a standard sphygmomanometer, Polar heart rate monitor, and Borg’s perceived exertion scale (RPE, Borg’s 20-point scale) [481], respectively.

Immediately following the time trial the participant was again re-positioned on the examination table, and the exact same procedures were used to examine brachial artery vasoreactivity. This test was then again conducted at 20 min in recovery.

5.2.3.3 Data Analyses

The blood markers that were analyzed include: (a) reactive oxygen species, including peroxynitrite, (b) specific endogenous anti-oxidants such as the enzymes glutathione, glutathione peroxidase and catalase, (c) nitrosylated hemoglobin, a method for in vivo nitric oxide detection. It is important to emphasize that nitrosylated hemoglobin have been reported as indicator of the potential role in transport and delivery of nitric oxide [480]. The biochemical variables were analyzed using the electron paramagnetic resonance (EPR) spectroscopy.

EPR has the potential to provide unique information on free radicals and other species with unpaired electrons, because of its unique ability to detect unpaired electron species (reactive oxygen species, nitric oxide) [482]. In fact, EPR is the most direct and unambiguous method to measure free radicals and thiols [483]. Under some circumstances the concentration of these species or their magnetic properties may make it difficult to measure them directly with EPR. Consequently, a technique termed “spin trapping” has been developed in which a detecting molecule (usually termed a “spin trap”, e.g., a nitrone or nitroso compound) is added to the system, which, upon reaction with a free radical, can form a product that is another free radical that is considerably more stable that the initial radical [484]. The concentration of nitrosylated hemoglobin was recorded with an X-band EPR spectrometer. The amount of detected nitric oxide was determined from the calibration curve for intensity of the EPR signal of erythrocytes.
treated with known concentrations of nitrite (1–25 µM) and Na2S2O4 (20 mM) [485].

The quantification of reactive oxygen species was as follow: cyclic hydroxylamine 1-hydroxy-3-carboxy-pyrrolidine (CP-H) was used for the quantification of reactive oxygen species. During the reaction of CP-H with reactive oxygen species (lifetime more than 4 h), reducing agent-resistant nitroxide radicals are formed. The amount of trapped reactive oxygen species was obtained by quantifying the concentration of the corresponding nitroxide radical 3-carboxyproxyl (CP). Quantification of reactive oxygen species released from blood samples (non-stimulated reactive oxygen species formation) was performed after incubation at 37°C for 30 min using 500 µM CP-H and the low-field component of electron spin resonance spectra in 50 µl quartz capillaries (Noxygen Science Transfer & Diagnostics GmbH, Elzach, Germany) [486].

All brachial artery imaging and analyses were conducted in accordance with the Guidelines set forth by the Brachial Artery Reactivity Task Force [487]. Images were analyzed using specifically developed edge-detecting software (Medical Imaging Applications, LLC). Visual inspection was used to ensure consistent selection of the blood-vessel interface. Arterial diameters were calculated as the mean distance between the anterior and posterior wall at the blood vessel interface, with the image in diastole, defined as the peak of the r-wave. Brachial artery flow mediated dilation was defined as the absolute (mm) and percent change in vessel diameter from rest to peak dilation, as seen at 45, 60 and 90 seconds following cuff release. The resolution of the brachial image in the current study allowed for a caliper accuracy of 0.028mm. The reproducibility of this technique in our laboratory has yielded average mean differences in brachial artery diameter change for days, testers, and readers of 1.91%, 1.40%, and 0.21mm respectively, with intra-class correlation coefficients of 0.92, 0.94, and 0.90, respectively [488].
5.2.4 Statistical Analysis

Data was analyzed using SPSS for Windows (version 15.0). Data was expressed as mean ± SD. To examine the changes in nitrosylated hemoglobin, total reactive oxidative stress, peroxynitrite and anti-oxidant molecules between baseline, after exercise and during recovery data were analyzed using ANOVA with repeated measures. A value of p<0.05 was considered statistically significant.

5.3 Results

5.3.1 Participants Characteristics

Ten aerobically trained men (Age 33.3 ±7.3 years; Height 73.38 ± 2.72 inches; Weight 188.75 ± 17.32 lbs) participated in the study. The majority of the athletes were either regionally or nationally ranked triathletes. Two of the athletes were strictly cyclists, one was an ultradistance runner. All athletes had been involved in regular training for at least 3 years, but the majority had over 5 years of competitive training experience.

5.3.2 Exercise Measures

The group averages for oxygen consumption, ventilation, and respiratory exchange ratios during the final 10 minutes of the bike trial conducted in visit 1, were 38.51±6 ml/kg/min, 106.55±25.89 l/min, and 1.05±0.04U, respectively. In addition the average heart rate over the course of the bike trial was 160±12 bpm, and the target heart rate was achieved approximately 5 minutes into the trial for all participants. The average heart rate over the course of the second bike trial was 157±13 bpm, and was not significantly different from the bike trial in visit 1.

5.3.3 Biochemical Variables

The group averages for all the biochemical variables are presented in Table 5.1. Group averages for production rate of reactive oxygen species increased from baseline (0.06±0.02
Units/uL) to peak exercise (0.10±0.05 Units/uL), and return to pre-exercise values after 20 minutes or recovery (0.05±0.03 Units/uL). The group average for peroxynitrite increased from baseline (0.24 ±0.81 Units/min) to peak exercise (0.31±0.11 Units/min) and returned to pre-exercise levels after 20 minutes of recovery (0.23±0.10 Units/min).

**Table 5.1 Biochemical variables**

<table>
<thead>
<tr>
<th>Biochemical variable</th>
<th>Baseline</th>
<th>Peak exercise</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive Oxygen Species (Units/uL)</td>
<td>0.06±0.02</td>
<td>0.10±0.05*</td>
<td>0.05±0.03</td>
</tr>
<tr>
<td>Peroxynitrite (Units/min)</td>
<td>0.24 ±0.81</td>
<td>0.31±0.11*</td>
<td>0.23±0.10</td>
</tr>
<tr>
<td>Glutathione Peroxidase (nmoles/mg protein/min)</td>
<td>97.63±19.28</td>
<td>71.94 ±15.93*</td>
<td>100.16 ±25.81</td>
</tr>
<tr>
<td>Glutathione assay (nmol es/mg protein)</td>
<td>13.58±1.52</td>
<td>10.62±4.69</td>
<td>18.96±4.37</td>
</tr>
<tr>
<td>Catalase assay (Units/mg protein/min)</td>
<td>5.90-05±2.30</td>
<td>3.27-05±0.84</td>
<td>5.40-05±1.76</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD., *p<0.05

The average for the anti-oxidant glutathione peroxidase decreased from baseline (97.63±19.28 nmoles/mg protein/min) to peak exercise (71.94 ±15.93 nmoles/mg protein/min) and returned to pre-exercise values at 20 minutes after recovery (100.16 ±25.81 nmoles/mg protein/min). The average for the anti-oxidant glutathione was 13.58±1.52 nmole/mg protein at baseline, 10.62±4.69 nmole/mg protein at peak exercise, and 18.96±4.37 nmole/mg protein after 20 minutes of recovery. In addition, catalase average was 5.90-05±2.30 Units/mg protein/min at baseline, 3.27-05±0.84 Units/mg protein/min at peak exercise, and 5.40-05±1.76 Units/mg protein/min into the recovery phase. However, there were no significant changes in glutathione and catalase.

Unfortunately at this moment we are not able to report the results for the nitrosylated hemoglobin measurements as was intended due to technical difficulties when the samples were
analyzed using the electron paramagnetic resonance spectrometry. The major reason was the insufficient sensitivity of the electron paramagnetic resonance spectrometry used to detect nitrosylated hemoglobin under basal conditions. The same equipment was used previously to detect easily approximately 400 nM of nitrosylated hemoglobin in rodent blood. However, in order to detect nitrosylated hemoglobin in human blood (40 - 100 nM) it is required to increase the sensitivity of equipment up to 10 times. Several strategies were attempted to allow for a representation of this variable but as of yet to no avail. In particular red blood cells’ packing through a second centrifugation period was attempted but the sensitivity remained too low for accurate detection.

5.3.4 Brachial Artery Diameter and Vascular Reactivity

Group averages for the brachial artery diameters before, immediately after and 20 minutes in recovery are depicted in Table 5.2. Group averages for the combined averages for brachial artery flow-mediated dilation (BAFMD) at 45, 60 and 90 seconds post release are also presented in Table 5.2.

The repeated measure ANOVA for brachial artery diameters yielded a significant main effect (p=0.0001) indicating a significant increase in brachial artery diameter after forearm occlusion. However, the ANOVA also revealed a factor by condition effect (p=0.009) indicating that the increase in brachial artery diameter post-occlusion was only seen in the pre-bike trial and at 20 minutes after the bike-trial. In contrast, no significant changes in brachial artery diameter were observed immediately after the time trial, even though there were no significant changes in the pre-occlusion diameters between conditions.

The ANOVA for brachial artery reactivity yielded a significant condition effect (p=0.002), with a significant blunted response immediately post bike trial (BAFMD = 0.38%).
compared to pre bike trial values (BAFMD = 5.24%), and 20 minutes in recovery (BAFMD = 3.93%), indicating a temporal pattern of reactivity.

Table 5.2 Brachial artery diameter and vascular reactivity

<table>
<thead>
<tr>
<th>Vascular measure</th>
<th>Pre Bike Trial</th>
<th>Immediately post Bike Trial</th>
<th>20 min after Bike Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Diameter (mm)</td>
<td>4.43±0.36</td>
<td>4.68±0.21</td>
<td>4.58±0.23</td>
</tr>
<tr>
<td>45sec Post release (mm)</td>
<td>4.60±0.38*</td>
<td>4.64±0.24</td>
<td>4.69±0.27*</td>
</tr>
<tr>
<td>60sec Post release (mm)</td>
<td>4.64±0.40*</td>
<td>4.70±0.22</td>
<td>4.79±0.17*</td>
</tr>
<tr>
<td>90sec Post release (mm)</td>
<td>4.67±0.38*</td>
<td>4.71±0.20</td>
<td>4.77±0.23*</td>
</tr>
<tr>
<td>BAFMD (%)</td>
<td>5.24±2.78</td>
<td>0.38±1.17**</td>
<td>3.93±1.47</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD., *p<0.05 vs. base; **p<0.05 vs. Pre Bike and 20min Post Bike trial

The ANOVA for brachial artery reactivity yielded a significant condition effect (p=0.002), with a significant blunted response immediately post bike trial (BAFMD = 0.38%), compared to pre bike trial values (BAFMD = 5.24%), and 20 minutes in recovery (BAFMD = 3.93%), indicating a temporal pattern of reactivity.

5.4 Discussion

The major purpose of this study was to examine the effects of an acute bout of exercise on nitric oxide bioavailability, oxidative stress and anti-oxidants markers, and brachial artery reactivity. Specifically these dependent measures were examined before, at peak exercise, and at 20 minutes into the recovery phase, in aerobically trained individuals. The two major unique outcomes of the present study indicate a significant increase in the production rate of reactive oxygen species and peroxynitrite, and a decrease in the anti-oxidant glutathione peroxidase at peak exercise. In addition, brachial artery reactivity revealed a temporal pattern, with a blunted response immediately after exercise, but near complete recovery after 20 minutes of recovery. These findings support the a-priori hypotheses, and suggest an acute bout of exercise contributes
to a significant rise in reactive oxygen stress on the vasculature, which can in part be buffered by anti-oxidants systems, but may cause temporary blunting of arterial reactivity.

5.4.1 Exercise Measures

The present study utilized a time trial to examine the effects of exercise on pro- and anti-oxidants and vascular function. The exercise protocol in the present study was similar to the study by Shing et al., [489]. The average oxygen consumption (VO$_2$) during the bike trial in visit 1 was 38.51±6 ml/kg/min. This average was lower than the subjects in the study by Shing et al., [489] who reported a VO$_2$ average of 44.60 ml/kg/min among their participants. The differences in the VO$_2$ average may in part be due to an older population in the present study (Age= 33 ±7.3 years), compared to Shing et al., [489] (Age= 25±4 Years). Interestingly, a study by Laursen et al., [490] aimed to compare the cycling performance of cyclists and triathletes found that cyclists may be able to perform better than triathletes in cycling time-trial events. This finding is important in trying to explain the VO$_2$ differences. As mentioned before, the participants in the study by Shing et al., [489] were all trained cyclists, whilst the majority of the participants in the present study were triathletes.

Regardless whether the VO$_2$ averages are somewhat lower than perhaps expected, the heart rate responses were consistent with an 80 to 85% of the participant’s heart rate reserve. In doing so, all the participants were exercising at the same intensity, which mimicks a competitive ride as reported by most of the riders. The decision to use the heart rate reserve method in this population is based on work done by Lounana et al., [491]. These investigators stated that the use of percentage heart rate reserve is a better indicator of exercise intensity among cyclists. Finally, the findings that the averages heart rate did not show significant differences between the bike time trials; provides strong evidence that both tests were performed equally as planned.
5.4.2 Reactive Oxygen Species

This study found that the production rate of reactive oxygen species and the pro-oxidant peroxynitrite increased from baseline to peak exercise and then returned to pre-exercise values after 20 minutes of recovery. Previous studies that have investigated oxidative stress markers among trained individuals report similar trends. For example, Shing et al., [489] found an increase in biomarkers of oxidative stress among trained cyclists after high-intensity exercise. The major aim of the study by Shing et al., [489] was to determine the influence of consecutive days of high-intensity aerobic bike exercise on oxidative stress markers. The blood samples were taken 10 minutes before and 5 minutes after the exercise. Their results indicated a significant increase (18%) in plasma malondialdehyde post-exercise. In addition, Lovlin et al., [492] investigated the relationship between exercise intensity and lipid peroxidation in moderately trained males. These investigators reported that maximal exercise resulted in a 26% increase in plasma malondialdehyde post-exercise. These previous studies clearly show a similar pattern, albeit to a lower magnitude, as reported in the present study, where the production rate of reactive oxygen species increased 66%, and peroxinitrite 29%, from baseline to peak exercise. Interestingly, the production rate of reactive oxygen species and peroxinitrite were not different from baseline values at the 20 minutes mark in recovery. This stands in contrast with several studies which report that secondary measures of oxidative stress remain elevated for a period of time after exercise. For example, Michailidis et al., [493] reported that lipid peroxidation, as measured by thiobarbituric acid reactive substances was elevated immediately and up to 3 hours after 45 minutes of running on a treadmill at an intensity corresponding to 70-75% of subjects’ VO$_{2}$max. The subjects in the study by Michailidis et al., [493] were low to moderately trained. Further confirmation that secondary measures of oxidative stress remain elevated comes from a
study by Alessio et al., [494]. These investigators also reported an elevated (46%) in plasma thiobarbituric acid reacting substances among moderately trained individuals after 30 minutes submaximal exercise. Participants in a study by Watson et al., [495] utilized trained individuals who ran for 30 minutes at a submaximal speed corresponding to 60% of their VO$_{2\text{max}}$. Again, Watson et al., [495] reported that plasma lipid peroxidation, as measured by F$_2$-isoprostanes concentration (marker of oxidative stress) was significantly higher after the submaximal bout of exercise (38%), and remained significantly elevated one hour into recovery (31%). However, the findings in the current study were similar to those observed by Steensberg et al., [496]. Those investigators reported that the concentration of plasma F$_2$-isoprostanes increased approximately 1.6-fold in response to a treadmill exercise, but declined towards pre-exercise values within the first hour of recovery. Obviously the differences in observations between studies are often secondary to study design, study population and methodological issues, and are therefore difficult to reconcile. However, the present study is particularly unique in that the production rate of reactive oxygen species was a direct measure rather than a secondary marker of oxidative stress.

In fact, it is important to recognize that many of the referenced studies have indeed used secondary biomarkers to report oxidative stress (e.g. plasma malondialdehyde, thiobarbituric acid reactive substances, and F$_2$-isoprostanes). Arguably a major problem with those studies and methods is that the measures of oxidative stress are indirect and reflect tissue damage rather than production of oxidative stress. Consequently, many of those studies lack specificity. For example, it is well-known that thiobarbituric acid reactive substances react with a wide variety of compounds, including sugars and DNA [497]. Indeed, there is concern about the validity of thiobarbituric acid reactive substances assay in detecting lipid peroxidation in vivo, because
thiobarbituric acid reactive substances assay is not specific to malondialdehyde, and lipid peroxidation is not the exclusive source of malondialdehyde [498]. Furthermore, oxidation products are typically present at low levels in biological material. Finally, normal concentrations of F_{2}-isoprostanes in human plasma have been proposed to range from 5 to 40 pg·mL^{-1} [499]. However, the clinical significance of concentrations outside this range has not been described because no direct casual relationship between F_{2}-isoprostanes and exercise performance has been found [499]. Such assays therefore are prone to artifacts and cannot provide strong evidence of the actual oxidative stress in vivo [497].

The unique contribution of the present study is that the production rate of reactive oxygen species was examined before, at peak exercise, and 20 minutes after recovery. This is the first such study in exercising humans, and may represent a more direct method of determining the total pool of reactive oxygen species produced under stressful conditions, such as an acute bout of exercise. However, some caution is needed at this point. The present study reports the production rate of reactive oxygen species as measured from the red blood cell. Obviously future studies must determine if a change in the production rate in the red blood cell is indeed a reflection of other physiological systems that may also produce oxidative species at a high rate during exercise. In addition, the rate of production of the reactive oxygen species is not taken in real-time but is measured after obtaining a blood sample and immediately adding the sample to a buffer and frozen in liquid nitrogen.

Given these possible limitations, it must also be appreciated that reactive oxygen species can be produced during exercise from several potential cellular sources. Some sources may be more important that others in a certain organ, at a specific time, or with a specific exercise mode. However, these sources are not mutually exclusive and can be activated simultaneously [500]. In
fact, extensive reviews regarding the sources of reactive oxygen species during exercise [501] clearly suggests a major source of free radical production during exercise is the mitochondrial electron transport chain [497]. Indeed, superoxide is produced during the oxidation of reduced nicotinamide adenine dinucleotide to oxidize nicotinamide adenine dinucleotide [502]. Under normal metabolic conditions, it has estimated that for every 25 oxygen molecules reduced by normal respiration, one free radical is produced [503]. Given that the rate of whole body oxygen consumption during exercise may increase 10–15 fold, and oxygen flux in an active muscle may increase about 100 fold [504], the rate of production of several oxidative species is sure to rise. With this rise in production a greater number of these species are sure to leak out of the electron transport chain [503] and leave surrounding tissues vulnerable to their damaging effects. Several candidates of oxidative species have been identified, including hydrogen peroxide, hydroxyl radicals, and others [505]. Approximately 4% of electrons that enter the respiratory chain lead to the formation of superoxide, whereas hydrogen peroxide is produced after the spontaneous or superoxide dismutase-catalyzed dismutation of superoxide as well as many others enzymatic reactions. Unlike superoxide, which remains at the site of production, hydrogen peroxide can diffuse across membranes and through the cytosol [506].

More recently, nitric oxide has also been linked to the formation of pro-oxidants. In fact, at higher levels of production, nitric oxide reacts with radicals such as superoxide to form peroxynitrite, a powerful pro-oxidant [507]. Interestingly in the present study a significant rise in the production rate of peroxynitrite was seen from pre- to peak exercise, and returned to pre-exercise levels at 20 minutes into recovery. This provides direct evidence of a significant rise in pro-oxidants during exercise, probably subsequent to a higher production of both nitric oxide and superoxide.
In addition to mitochondrial production of oxidative species, some studies have reported an extramitochondrial source of free radicals. There is significant amount of information on putative reactive species that could contribute to exercise-induced oxidative injury or post-exercise vascular injury [497]. Central to such oxidative damage are xanthine dehydrogenase and oxidase and calcium. For instance, Vina et al., [508] have hypothesized that activation of xanthine oxidase could be important in the generation of free radicals during exercise. Xanthine dehydrogenase is found mainly in endothelial cells of blood vessels and in skeletal and cardiac muscle [497]. Xanthine dehydrogenase may convert nicotinamide adenine dinucleotide to reduced nicotinamide adenine dinucleotide, whereas xanthine oxidase utilizes oxygen and xanthine, a purine degradation product, to produce superoxide and hydrogen peroxide. In addition, xanthine oxidase catalyzed reactions have been well established as one of the major sources of free radical generation in the ischemia and reperfused heart [509]. Duarte et al., [510] pointed out that the endothelium might contribute to muscle damage induced by exercise. These investigators stated that the xanthine oxidase located in capillary endothelium is one of the possible sources for the production of reactive oxygen species. In addition, Vina et al., [511] showed that inhibition of xanthine oxidase with allopurinol protects against exercise-induced oxidation of glutathione in humans.

Finally, an increase in the release of calcium by pro-oxidants stimulates calcium-dependent enzymes such as calcium-dependent proteases, nucleases, and phospholipases, which subsequently trigger apoptosis of the cells [512]. Once calcium efflux has been triggered, a series of common pathways of apoptosis are initiated, each of which may be sufficient to destroy the cell [512]. Given the rise in the production rate of pro-oxidants from the red blood cell as shown in this study, this is yet another extramitochondrial source of free radicals. Future studies must
determine if the production rate of free radicals from the red blood cell is indeed similar, or at least a reflection of other free radical generating sources.

It is important to reflect on the necessity of the production of reactive oxygen species in the human body. Certainly not all effects of these pro-oxidants are negative. In fact they can be beneficial to living systems [475]. The beneficial effects of reactive oxygen species occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia; for example in defense against infectious agents and in the induction of a mitogenic response [474]. On the other hand, the harmful effect of free radicals may contribute to biological damage and is termed oxidative stress [476]. Indeed, these radicals can cause damage to cardinal cellular components such as lipids [513] proteins [514] and nucleic acids [515].

In summary the present study indicates a significant rise in pro-oxidants with exercise in trained individuals, which is quickly resolved within 20 minutes of recovery. These data suggest a potential increase in vulnerability to oxidative stress with exercise, but only for a limited time perhaps indicating a significant ability to buffer against oxidative species.

5.4.3 Anti-oxidants

It is, indeed, imperative to emphasize that oxidative status is controlled by a wide spectrum of exogenous and endogenous anti-oxidants [473]. In general, the body has adequate anti-oxidant reserves to cope with the production of reactive oxygen species under most normal physiological conditions. For example, the system consists of anti-oxidant vitamins, glutathione and thiols, and anti-oxidant enzymes [502]. Each of these anti-oxidants plays a unique role in the cell and complements each other functionally. Furthermore, inter-organ transport of certain anti-oxidants is likely to occur. These anti-oxidant protection systems preserve homeostasis for normal cell functions at rest and perhaps during mild exercise [501]. However, when reactive
oxygen species production is excessive, or anti-oxidant capacity insufficient, oxidative stress will develop, potentially leading to cellular damage. Indeed, the extent of oxidative damage during physical exercise is determined not only by the level of free radical generation, but also by the defense capacity of anti-oxidants.

The possible mechanisms by which anti-oxidants protect against pro-oxidants are complex and multifactorial [516], and they may include; (a) prevention of reactive oxygen species formation, (b) interception of reactive oxygen species attack by scavenging the reactive metabolites and converting them to less reactive molecules and/or by enhancing the resistance of sensitive biological targets to reactive oxygen species attack, (c) avoiding the transformation of less reactive oxygen species (e.g., superoxide) to more deleterious forms (e.g., hydroxyl), (d) facilitating the repair of damage caused by reactive oxygen species and triggering the expression of genes that encode anti-oxidant proteins, and (e) providing a favorable environment for the effective functioning of other anti-oxidants (e.g., as a cofactor or by acting to maintain a suitable redox status) [517]. Therefore examination of a disturbance in the equilibrium status of pro-oxidant/anti-oxidant reactions in living organisms is quite important to determine the organism’s vulnerability [474].

As discussed above, the present study clearly indicates that the oxidative stress increases with exercise, but quickly returns to pre-exercise levels suggesting the overall exposure to the pro-oxidants is short. Importantly this study also reports that some anti-oxidant systems are modified during and after exercise. In fact, glutathione peroxidase concentrations decreased from baseline to peak exercise and then returned to pre-exercise values at 20 minutes of recovery. However, no such changes were noted for catalase and glutathione. The manner in which anti-oxidants operate will be briefly discussed in this next section. Glutathione (gamma-
glutamylcysteinylglycine) is a tripeptide of glycine, glutamic acid and cysteine that plays a central role in metabolic pathways, as well as in the anti-oxidant system of most aerobic cells. Indeed, glutathione is the most abundant non-protein thiol source in the cell and serves multiples functions in protecting tissues from oxidative damage and keeping the intracellular environment in the reduced state [518]. Glutathione acts as an anti-oxidant both intra- and extracellularly and is required as a coenzyme by a variety of enzymes including glutathione peroxidase, glutathione S-transferase and thioltransferase [502]. In the red blood cell, the reduced form of glutathione is vital in maintaining hemoglobin in a reduced state and hence protecting the cells from oxidative damage [519]. In addition, glutathione is involved in detoxification of hydrogen peroxide through glutathione peroxidase. Glutathione peroxidases are selenoenzymes which catalyze the reduction of hydroperoxides in the presence of glutathione. Hydrogen peroxide, a reactive oxygen species, is a harmful by-product of many normal metabolic processes. In order to prevent damage, it must be quickly converted into other, less dangerous substances [520]. Both, glutathione peroxidase and catalase (an intracellular enzyme) are frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules [520]. In addition, glutathione peroxidase and catalase provide the primary defense against reactive oxygen species generated during exercise, and activities of these enzymes are known to increase in response to exercise in both animal and human studies [521].

In regard to our findings, previous studies [522] have also reported a decrease in glutathione peroxidase after an acute bout of exercise. For example, the study by Bloomer et al., [523] reported a decrease in glutathione peroxidase immediately following exercise, and it returned to pre-exercise values at 60 minutes into the recovery phase. This study was conducted among trained individuals performing an aerobic cycling activity for 30 minutes. The same
investigators [523] suggested that after exercise, hepatic glutathione (coenzyme for glutathione peroxidase formation) supply may not be sufficient to match the enhanced use resulting in reduction of blood glutathione concentration. In addition, the decrease in anti-oxidant enzyme activity observed may reflect allosteric downregulation of the enzymes in addition to enzyme inactivation attributable to overwhelming oxidative stress. Indeed, Elosua et al., [524] have also shown that there is a transient decrease in whole-blood glutathione peroxidase after 30-min exercise cycling bout, and a tendency to recover the basal values within two hours. These investigators [524] concluded that exercise-induced reactive oxygen species production leads to the consumption of enzyme activity with a subsequent rebound recovery. Clearly, the above studies reported a decrease in glutathione peroxidase immediately after an acute bout of exercise.

Furthermore, in the present study, the anti-oxidant catalase did not exhibit significant changes after the acute bout of exercise. This finding is consistent with most of the previous studies concerning muscle catalase [500]. However, studies that have examined the effect of a single bout of aerobic exercise on blood catalase activity have reported mixed results. Some of them have reported an increase [522], whilst others reported no differences [478]. It is important to notice that our study was performed by aerobically trained individuals that did not exhibit significant changes in the anti-oxidant catalase. On the contrary, those studies that have reported an increase in catalase after an acute bout of exercise [522] were done among untrained individuals performing exhaustive aerobic exercise of moderate duration. The discrepancy among the results may be explained by the trained status of the participants. Indeed, several studies have found that trained individuals responded differently to oxidative stress induced by an acute exercise bout compared with untrained subjects [478]. In that regard, Vollaard et al., [498] suggested that the degree of oxidative stress may be attenuated by chronic aerobic and
anaerobic training, through an increase in endogenous anti-oxidant production, a decrease in free radical generation, or a combination of both processes. Furthermore, individuals engaged in long-term heavy exercise become more resistant to oxidative stress mainly due to the adaptation of their anti-oxidant defense systems [521].

In addition, Chakraborti et al., [525] have reported that an acute bout of exercise increases the activities of anti-oxidant enzymes, including glutathione peroxidase and catalase in skeletal muscle, heart and liver, which may result in a transient reduction in their blood concentration. Indeed, plasma and muscle glutathione peroxidase content may be decreased eventually during prolonged exhaustive exercise when hepatic glutathione reserve is diminished and glutathione peroxidase use exceeds glutathione uptake [526]. Finally, Chakraborti et al., [525] concluded that the threshold and magnitude of activation appear different among enzymes and tissues. The mechanisms by which anti-oxidant enzymes are activated within a relatively short period of time during exercise are largely unknown. However, reactive oxygen species have long been postulated to stimulate the expression of anti-oxidant enzymes [504].

The findings of the present study suggest that the anti-oxidant defenses among aerobically trained individuals are critical in the efforts to buffer the increase in the rate of production of pro-oxidants. In addition, the present findings clearly show a significant rebound effect following exercise in aerobically trained individuals. However, understanding the mechanisms involved in the increased anti-oxidant enzyme activity during exercise remains a great challenge. Obviously, further studies are needed to determine if this apparent rebound is similar in less trained individuals or those who are older or suffering from chronic disease. Evidence of the beneficial effect of physical exercise on minimizing oxidative stress might be related to increased anti-oxidant defenses [527]. In the same manner, Franzoni et al., [528] have
demonstrated that chronic endurance training is associated with increased plasma anti-oxidant activity in young and older men.

In summary, the current study suggests that some anti-oxidant defenses in aerobically trained individuals are critical in the efforts to buffer the increase in the rate of production of pro-oxidants. However, it appears that some anti-oxidant systems are depleted at peak exercise, but rebound to pre-exercise levels within 20 minutes of recovery in aerobically trained individuals.

5.4.4 Brachial Artery Diameter and Vascular Reactivity

A unique objective of this study was to determine the effects of an acute bout of exercise on brachial artery diameter and reactivity. The basis for this examination was evidence from other studies that an acute assault to the vasculature results in a transient reduction in vascular function as defined by brachial artery flow mediated dilation. For example, several studies indicate that a single high fat [529] and or high glucose meal [530], cigarette smoke [531], and mental stress [532] transiently blunt vascular function.

Interestingly, no studies have examined the effects of an acute bout of exercise on vascular function in trained individuals, despite significant evidence that chronic exercise training improves vascular health. So the idea that chronic consumption of high fat, high glucose, tobacco, and mental stress may have a chronic detrimental effect on the vasculature, chronic exercise training appears to benefit vascular health. Thus the examination of potential factors that may be produced during an acute bout of exercise may eventually provide insight about the manner in which exercise may protect the vasculature. To that extent the discussion so far has centered on the effects of an acute bout of exercise on pro- and anti-oxidants. This section of the discussion will center on the vascular responses. The unique finding of the study is a transient reduction in brachial reactivity at peak exercise which is restored within 20 minutes.
This is the first study to show a blunted response in brachial vascular reactivity immediately following acute exercise in aerobically trained individuals. In fact, only one other study [533] has reported on post exercise vasoreactivity. In that study the authors report a 2.3 fold reduction in brachial vascular reactivity among persons with intermittent claudication following a single bout of maximal treadmill exercise. Interestingly along with the reduction in vasoreactivity (from 8.5±0.9 to 3.7±0.8%, p<0.01), the authors found an increased plasma levels of thiobarbituric acid-reactive substances (from 1.93±0.06 to 2.22±0.1 nmol/ml, P<0.02) and of soluble intercellular adhesion molecule-1 (from 282±17 to 323±19 ng/ml, P<0.01). More importantly, vitamin C prevented the acute, systemic impairment in endothelial function induced by maximal exercise, suggesting a role for anti-oxidant therapy [533]. In fact, these investigators recommend that there is a need for future studies aimed at assessing whether chronic oral treatment with anti-oxidant agents improves systemic endothelial function in patients with intermittent claudication.

The potential mechanism that a change in pro-oxidant/anti-oxidant balance could mediate vascular reactivity is obviously pertinent to the current study as well, and will be discussed in greater detail in the next section of the paper. However, prior to this discussion it is also critical to discuss additional factors which could have contributed to the temporal pattern, of vasoreactivity during and following exercise.

Generally vasoreactivity as defined by brachial artery flow mediated dilation is thought to be in part controlled by endothelial derived vasodilators (esp. nitric oxide), local metabolites, autonomic influences, and structural components of the vessel [534]. To that extent, a change in or utilization of nitric oxide immediately after exercise could indeed affect vasoreactivity. However, it is important to appreciate that previous data from this laboratory consistently reports
a significant inverse relationship between arterial diameter and vasoreactivity [535]. Given the
evidence that the brachial artery diameter after exercise was larger than the pre-exercise
diameters (p<0.05) would in part explain why the reactivity would be less. However, this does
not fully explain the loss of reactivity as the brachial diameters immediately after exercise were
not significantly different from the 20 minute recovery mark.

Probably the major factor that contributed to a larger artery immediately after exercise is
a dramatic increase in blood flow and/or flow velocity. In fact, Tanaka et al., [536] reported that
the brachial artery is exposed to an increase in blood flow even though work is performed by the
lower extremities. This augmented blood flow, through local regulatory mechanisms, causes
vasodilation; which can persist for at least one hour after cessation of exercise [537], depending
on the exercise intensity. Thus one could argue that the reason for the reduced vasoreactivity
immediately after exercise is due to the artery being close to its “maximal” physiological value.
The lack of response may therefore be a consequence of a “ceiling effect”, rather than other
factors.

In summary, the present study indicates that brachial artery reactivity revealed a temporal
pattern, with a blunted response immediately after exercise, but near complete recovery after 20
minutes of recovery. These findings are consistent with other studies that report that certain
stressors may contribute to decreased vascular performance for a period of time. Future studies
are necessary to further determine why exposure to some stressors (e.g. high triglyceride, and
hyperglycemic meals, and cigarette smoke) causes vascular erosion, and other stressors (such as
exercise) contributes to improved vascular function. Within such an exploration it is
hypothesized that the maintenance and/or return to a favorable balance of pro- and anti-oxidant is
critical.
In a recent provocative review by Deanfield et al., [9] perturbations to endothelial homeostasis was suggested as a major factor in the development of arterial disease. A major point of discussion within the review was endothelial activation. Endothelial activation [9] was defined as a switch from a quiescent phenotype toward one that invokes activation of molecular machinery that result in the expression of chemokines, cytokines, and adhesion molecules designed to interact with leukocytes and platelets and target inflammation [538]. The fundamental change involved in this process is a switch in signaling from a nitric oxide-mediated silencing of cellular processes toward activation by redox signaling. Reactive oxygen species in the presence of superoxide dismutase, lead to generation of hydrogen peroxide, which, like nitric oxide, can diffuse rapidly throughout the cell and react with cysteine groups in proteins to alter their function [9]. However, because of the different chemistry involved, this results in very different consequences, such as phosphorylation of transcription factors, induction of nuclear chromatin remodeling and transcription genes, and protease activation [9]. It is intriguing that endothelial nitric oxide synthase, which normally helps maintain the quiescent state of the endothelium, can switch to generate reactive oxygen species in appropriate circumstances as part of endothelial activation [9]. This is termed endothelial nitric oxide synthase uncoupling, and results in superoxide formation if the key cofactor tetrahydrobiopterin is not present or generation of hydrogen peroxide if the substrate L-arginine is deficient [539]. The ability of endothelial nitric oxide synthase to regulate both the quiescent and activated endothelial phenotype puts this enzyme at the center of endothelial homeostasis [9], and the maintenance of vascular health. In fact, it is hypothesized that prolonged and/or repeated exposure to factors that ultimately exhaust the protective effect of endogenous anti-inflammatory systems within
endothelial cells, will contribute to endothelial dysfunction, loss of endothelial cell integrity, accelerated cell senescence, and detachment into the circulation.

Central to this paper is the manner in which an acute bout of exercise can alter endothelial homeostasis. This information is thought to be critical in developing a better understanding of how chronic exercise may help maintain vascular health as implied from the available literature [540]. The present study clearly shows an increase in the production rate of reactive oxygen species and to some extent a reduction of anti-oxidant systems at peak exercise. In fact, examination of the ratio between glutathione peroxidase and reactive oxygen species (Pre Exercise: 1701±485; Peak Exercise: 669±221; 20 min Recovery: 2093±1543, p<0.05), and glutathione peroxidase and peroxynitrite (Pre Exercise: 515±415; Peak Exercise: 225±71; 20 min Recovery: 471±143, p<0.05) shows a significant decline in these ratios at peak exercise, suggesting a significant change of the balance of pro-oxidant/anti-oxidants, in favor of oxidative stress. Purportedly such a change in oxidative stress may be sufficient to trigger endothelial activation, and contribute to a more vulnerable endothelial environment. Obviously, it is recognized that the derived ratio within this study, is merely a gross measure of the balance of pro-oxidant/anti-oxidants, and only serves as a point of illustration. However, the fact the present study also shows an association between the levels of glutathione peroxidase and brachial artery flow mediated dilation (r=0.578, p<0.05), and reactive oxygen species and brachial artery flow mediated dilation (r=-0.431, p<0.05) certainly keeps the hypothesis alive that the pro-oxidant/anti-oxidant balance is a critical component in maintaining vascular reactivity.

Theoretically the ability of an acute bout of exercise to trigger endothelial activation seems to defy intuition; in the face of a plethora of evidence of the benefits of exercise on the cardiovascular system is undeniable [541]. Thus, the current finding beckons the question how
chronic exercise protects the vasculature. Obviously, the present study was not designed to answer this question, but future study designs should attempt to examine this directly. However, it is perhaps within the discretion of this paper to speculate how chronic exercise may eventually produce a protective endothelial environment. Using this discretion, it is perhaps important to revisit Selye’s theory of stress-response-adaptation [542]. Applied to vascular physiology this theory suggests that an acute exercise stress, when applied to the vascular system, will initially contribute to a period of reduced function. However, if the exercise stress is repeated over a period of time, the vasculature adapts to allow for an increased delivery of blood flow to active skeletal muscle, thereby contributing to a greater functional ability. Thus the initial decline in vascular performance with acute exercise could be a consequence of a perturbation of endothelial homeostasis. In fact, the exercise-induced endothelial activation may serve as a significant trigger for vascular modification, similar to exercise-induced mRNA activation, which may lead to skeletal muscle hypertrophy [543]. Typically, when exercise-induced activation is too frequent or infrequent, too excessive or not intense enough, or too long or not long enough the process may lead too insufficient adaptations or maladaptation. It is subsequently no surprise that the optimal dose-response for exercise training among different populations is not entirely understood.

However, it is interesting to consider studies that reported less evidence of oxidative damage among trained individuals [516]. Moreover, Leeuwenburgh et al., [497] reported that an acute bout of exercise in untrained subjects’ increased oxidative stress considerably. These studies imply that chronic exercise training may in fact create an environment that allows for the maintenance of endothelial balance. Future studies are needed to further appreciate this speculation. It is intriguing that the subject’s in the present study showed a considerably rebound
effect in recovery from exercise, in terms of pro-oxidant/anti-oxidant balance and vascular reactivity. This suggests that the exposure to oxidative stress was significant but short-lived in these subjects. Further data is needed to appreciate whether this rebound is different in less trained individuals. In addition, it is intriguing to speculate what might be seen in individuals who already have endothelial activation pre-exercise, due to risk factors, or disease, or in older individuals. Thus this study may have set the stage for a series of additional experiments with endothelial homeostasis as its focus.

In that regard, a review by Lum et al., [544] acknowledged that acute and chronic oxidant stress to the vascular endothelium is a serious causative factor of vascular endothelial dysfunction (or “endothelial activation”), and plays an important role in the pathophysiology of several vascular diseases. Indeed, endothelial activation may endanger normal synthesis and release of nitric oxide by endothelial cells. However, under ‘normal’ physiological conditions, reactive oxygen species production is low and the endogenous anti-oxidant systems are sufficient to maintain the pro-oxidant/anti-oxidant balance. This vital action would uphold superoxide production and elimination under tight control, thus preventing the breakdown of nitric oxide [545]. On the other hand, in disease states in which the production of reactive oxygen species is increased and/or the anti-oxidant capacity of the vessel is decreased, nitric oxide is transformed to peroxynitrite, resulting in inhibition of endothelial-dependent relaxation [546].

Interestingly, it has been reported that reactive oxygen species are also frequently elevated in patients with one or more cardiovascular risk factors, for example in smokers or in patients with hypercholesterolemia [545]. Thus one could argue that the increased production of reactive oxygen species in individuals at risk for disease or already with a disease is a key component in the pathogenesis of endothelial activation. In addition, one could speculate that
any further overwhelming exposure to oxidative stress may compromise seriously the already tenuous pro-oxidant/anti-oxidant balance in these individuals.

Likewise, there is a growing amount of evidence that aging is associated with increasing systemic imbalance between the generation and the scavenging of pro-oxidants [546]. Furthermore, it has been proposed that increased oxidative stress may play a role in premature aging [547]. It is important also to emphasize that a progressive impairment of endothelial vascular function, nitric oxide related, is a peculiar aspect of the physiological aging process [548]. Thus it seems to be an important interaction between age-associated pro-oxidant/anti-oxidant balance and the ability of vasculature to preserve nitric oxide bioavailability. Once again, one could suggest that endothelial activation, as reported in disease states, is also a keystone in the age-associated changes in humans.

Arguably, it could be speculated that conditions where endothelial activation is already present (e.g. end-stage chronic diseases) oxidative stress on the vasculature is chronic rather than acute. Such a condition could lead to a chronic imbalance and place individuals at great risk for the consequences of such imbalance. In such situations the use of exercise may in fact be counterproductive as it would merely add to the oxidative stress rather than serve as a stimulus for adaptation. However, given the plethora of evidence of the beneficial effects of chronic exercise in many disease states [549] the threshold where exercise training is harmful appears to be set quite high.

In summary, the present study reports a significant increase in pro-oxidants, a decrease in anti-oxidants, suggesting a change in the environment that may trigger endothelial activation. Moreover, the study reports a blunted brachial artery reactivity at peak exercise. Interestingly, the decline in pro-oxidants and return to pre-exercise levels for anti-oxidants and vasoreactivity
implies a rapid return of endothelial homeostasis, within 20 minutes after exercise within these trained individuals. Therefore, the present study provides an excellent model to study the dynamic balance between pro-oxidant challenge and anti-oxidant defense in the biological system.

5.4.6 Limitations of the Study

As with all studies, certain limitations prevent stronger inferences at this time. Generally, limitations are a consequence of the study design, study population and methodologies. The present study limitations within each of those categories will be briefly discussed.

In regards to the study design, there is no doubt that the use of only three measures to detect a pattern in biological systems is a limitation. A true detection of a temporal pattern of any system must involve more data points. However, due to feasibility issues this first ‘look-and-see’-study elected to examine three time points. It is recognized future studies are needed to examine the inferred pattern discussed within this paper with greater care.

In regards to the selected participants, this study is certainly limited in scope. A stronger design may have been one that compared individuals with different fitness levels or a wider age range. The basis of the selection was one of convenience in terms of recruitment, finances, and internal review board concerns. Despite this limitation, the current study did find consistency within the responses among the subjects. Clearly this consistency in the response gives the study a lot more strength. It is also important to understand that the selection of the participants was done with the knowledge that the participants were willing to push themselves through an arduous protocol that involved blood sampling at high levels of exercise. It was thought that this strategy may allow for the greatest chance to see a consistent response as these athletes are generally used to such high levels of performance. Obviously, it is important to take the next step
and examine these responses among a greater number of populations where vascular issues can be of major concern as it relates to physical performance.

Finally, the inability to accurately quantify the changes in nitrosylated hemoglobin, due to technical difficulties experienced with the electron paramagnetic resonance spectroscopy equipment, merely allows us to indirectly discuss what is believed to be happening with endothelial balance. Several strategies were attempted to allow for a representation of this variable but as of yet to no avail. Nevertheless, it is important to reinforce that this study is reporting peroxynitrite, (interaction of superoxide with nitric oxide). This biochemical variable may in some way provide some insights of the activities of superoxide and nitric oxide. Although these limitations, the present findings regarding the dynamics of the pro-oxidant /anti-oxidant are quiet exciting and deserve future follow up.

5.4.7 Conclusion

The major purpose of this study was to examine the effects of an acute bout of exercise on nitric oxide bioavailability, oxidative stress and anti-oxidants markers, and brachial artery reactivity. Specifically these dependent measures were examined before, at peak exercise, and at 20 minutes into the recovery phase, in aerobically trained individuals.

The two major unique outcomes of the present study indicate a significant increase in reactive oxygen species and peroxynitrite, and a decrease in the anti-oxidant glutathione peroxidase at peak exercise. In addition, brachial artery vasoreactivity revealed a temporal pattern, with a blunted response immediately after exercise, but near complete recovery after 20 minutes of recovery.

These findings are quiet important if we take in consideration that increased vascular oxidative stress represents a major cause of reduced endothelial nitric oxide bioavailability, and
it compromises the pro-oxidant/anti-oxidant balance. Clearly, the present study provides an excellent model to study the dynamic balance between pro-oxidant challenge and anti-oxidant defense in the biological system.
Chapter 6

Conclusion

Cardiovascular diseases remain the leading cause of death in the United States and most western countries [11]. Indeed, cardiovascular disease is the underlying cause of death for 36.3% of all deaths in 2004, or 1 of every 2.8 deaths in the United States. Among these cardiovascular diseases, coronary heart disease caused 1 of every 5 deaths in the United States in 2004 [11]. An estimated 79 million American adults (1 in 3) have 1 or more types of cardiovascular disease. Of these, 37 million are estimated to be age 65 or older [11]. Finally, the economic burden of cardiovascular disease is enormous [12], with the costs of cardiovascular disease totaling an estimated $352 billion dollars in the United States, in 2004 [12].

Early identification of risk factors and subsequent risk factor modification represents an important tool to fight back and try to decrease the incidence and prevalence of cardiovascular diseases. Among the modifiable behaviors that increase risk of cardiovascular disease are cigarette smoking, high blood pressure, obesity, and physical inactivity. Interestingly, exercise training is an excellent and beneficial strategy to improve overall health. In fact, epidemiologic studies have shown that physical activity reduces cardiovascular morbidity and mortality in the general population [549-551], and in patients with coronary artery disease/myocardial infarction [13,14]. The evidence clearly shows that regular exercise has a favorable effect on many established risk factors for cardiovascular disease. For example, exercise training is associated with beneficial changes in blood pressure, lipid metabolism, glucose metabolism, neurohormonal factors, weight loss, etc. [552]. In addition, exercise training improves muscular function and strength, the ability to utilize oxygen (“maximal oxygen consumption or aerobic capacity”), muscle blood flow, and many other physiologic systems. Furthermore, although the effect of an
exercise program on any single risk factor is generally small, the effects of continued, moderate exercise training on overall cardiovascular risk, when combined with other lifestyle modifications (e.g. proper nutrition, smoking cessation), can be dramatic [553].

Nevertheless, it is important to emphasize that despite the fact that exercise training is beneficial; the mechanisms underlying the protective effect of physical activity on cardiovascular disease are still not fully understood. It is, however, clear that there are distinct changes in the vasculature, which could contribute to the protective effect of physical activity on cardiovascular disease [554]. In that regard, the vascular benefits of long-term physical activity appear to be mediated in part by improvements in endothelial structure and endothelial function in the systemic, coronary, and pulmonary beds [528,555], as well as by increased bioavailability of nitric oxide [556,557]. Indeed, several studies have shown that regular physical training enhances endothelium-dependent vasodilation in the forearm and coronary circulation in patients with hypertension and patients with coronary heart disease as well in healthy individuals [13,194,558].

Thus one could argue that preserving healthy endothelial function may play a major role in vascular tone, vascular smooth muscle proliferation and migration, inflammation, innate immunity, and anticoagulation [557]. In fact, impaired endothelial function has been associated with several risk factors for the development of atherosclerosis [554], and is associated with a significantly higher incidence of cardiovascular events [559]. Given the changes in the endothelium are associated with these events serial measures of markers of endothelial function may prove to be important in monitoring individuals’ risk. In that regard, high-resolution ultrasound has been used as a non-invasive technique to assess endothelial function in peripheral arteries [560]. Indeed, non-invasive evaluation of brachial artery flow-mediated dilation has
emerged as a useful tool to study vascular function. This method assesses the ability of the endothelium to dilate in response to shear-induced increase in nitric oxide. The dilatory response associated with increased flow is thought to be endothelium dependent, and is subsequently used as a marker of endothelial function. For example, it has been reported that brachial artery flow mediated dilation is reduced in the presence of numerous cardiovascular risk factors [561-563] and holds predictive value for cardiovascular events [564]. In addition, aging is associated with a progressive decline in endothelial-dependent vasodilation [565]. Finally, there is also evidence of a link between decreased vascular reactivity and physical function [292,376].

Clearly, the assessment of vascular function in clinical research has emerged as a powerful tool on vascular biology. Indeed, its determination might provide significant insight about vascular “health”. It is this underlying assumption that has led to the overall idea of this dissertation. Indeed, this work was motivated with the idea to continue to explore the effects of exercise on the vasculature. The outcomes within each of the projects within this dissertation in some unique way have contributed to a slightly better understanding of the possible underlying mechanisms and/or controllers that govern vascular function. Clearly, this information is not only important from a research point of view, but also may help health professionals from different background to improve their delivery as health providers. Ultimately, the main focus of this work was to determine how the vasculature may be influenced or may influence physical function.

The first step in the research process started with a follow-up study of previous work from this laboratory. Indeed, those studies showed several associations between strength and vascular measures [4] and improvements in vasoreactivity following isolated resistive types of exercises [6]. Importantly, these adaptations occurred within four weeks of training. These
studies were used as a foundation to keep exploring the effects of training on the vasculature, specifically the effects of resistance training on blood flow responses. Resistance training not only impacts strength and endurance, but also contributes to the maintenance of functional ability [425]. Thus the initial study was aimed to investigate the influence of two volumes (high and low) of whole-body circuit weight training on forearm vascular function in younger healthy college individuals. The findings revealed significant and similar increases in strength for the high and low volume groups. However, despite the increases in strength, vascular changes were only seen in the subjects who had lower vascular function prior to training [566]. Thus the study finding indicated that individuals with evidence of low vascular function may respond favorably to whole-body circuit weight training.

So it is perhaps not a surprise that the second project in this dissertation focused on individuals who may have lower vascular function due to some sort of pathologic condition. The population selected for the second study was a group of individuals with peripheral neuropathy. The major reason for that selection is the fact that irrespective of etiology, pathological changes associated with the disease are in many cases consistent with ischemia, secondary to vascular insufficiencies [8]. The main focus of the second study was to examine the link between vascular and physical function. The importance of such a link is to recognize that a large component of physical decline could be secondary to a loss of vascular function. If such a link can be found the development of strategies aimed at preserving vascular function could eventually help individuals remain physically able, even in the face of certain pathologic conditions. The findings of the second study confirmed a relationship between measures of vascular and physical function in peripheral neuropathy. Indeed, individuals with greater physical function scores exhibited more favorable measures of arterial inflow and venous outflow. Specifically,
individuals in the lowest tertile of the time up and go and those with a 6 minute walk distance score <400m had significantly lower peak reactive hyperemia blood flow responses and peak vascular conductance compared to the other groups. In addition, individuals in the top tertile of knee extension and knee flexion exhibited faster venous outflow half-time than the other tertiles. These findings fit “The Disablement Pathway” and suggest that lower physical function may, in part, be a consequence of deterioration of peripheral vascular function in individuals with peripheral neuropathy. Future longitudinal studies are needed to confirm the relationship between vascular and physical function and to determine whether exercise interventions can maintain and/or improve vascular function and contribute to the preservation of functional ability in individuals with peripheral neuropathy.

Although the previous studies were able to provide important information on how the vasculature may be influenced or may influence physical function, these findings also demonstrated how complex the study of vascular function could be. In fact, the initial studies could only speculate regarding the true “controllers” that could mediate vascular function. So the last step in this process was to begin the exploration in some putative factors thought to control vascular function. Within this last step the work by Deanfield et al., [9] was particularly motivating and stimulating. Indeed, Deanfield et al., [9] have speculated the vascular function is very much influenced by the balance of pro- and anti-oxidants. In fact, a negative balance, which is termed oxidative stress, has been implicated in a number of human diseases as well as in accelerated aging [544,567].

Interestingly, it appears that the balance is very much controlled by a molecule that is released by the vascular endothelium under different conditions, which is nitric oxide. Thus the third project was the initial development of the idea that the balance between pro- and anti-
oxidants would be very much influenced by the nitric oxide status of an individual. If the balance is in favor of oxidative stress this might mean that the vasculature would be more vulnerable as defined by less reactive.

Clearly this step was a significant extension from the previous studies, and because of that it was thought that these investigations should first be conducted in healthy trained individuals which hopefully would allow for the establishment of a data set to which other populations could ultimately be compared. Thus the last project was intended to examine the effects of an acute bout of exercise on nitric oxide bioavailability, oxidative stress and anti-oxidants markers, and brachial artery reactivity before, at peak exercise, and in recovery. The two major unique outcomes of this final study indicated a significant increase in reactive oxygen species and peroxynitrite, and a decrease in the anti-oxidant glutathione peroxidase at peak exercise. In addition, brachial vasoreactivity revealed a temporal pattern, with a blunted response immediately after exercise, and near complete recovery after 20 minutes of recovery. These findings support the a-priori hypotheses, and suggest an acute bout of exercise contributes to a significant rise in reactive oxygen stress on the brachial artery, which can in part be buffered by anti-oxidants systems, but may cause temporary blunting of the reactivity of the brachial artery.

In summary the findings of this dissertation further add to the current understanding of the role of the vasculature in physical function. It appears that physical function is very much influenced by the vasculature even in individuals with significant disease. Moreover, the vasculature can be acutely modified, and may improve over time in individuals involved in physical training. The acute modification of exercise appears to be at least in part negative (i.e. reduced vascular reactivity) due to oxidative stress. However, the ‘negative’ effects of acute exercise on the vasculature are short-lived in highly trained athletes suggesting a significant
buffering capacity. Obviously future studies will have to determine if these effects are in part a component of the signaling pathways that may enhance vascular function as is evident within the literature. The improvements in vascular function following training were again noted in this dissertation, although those benefits were seen only in those who had evidence of low function prior to training. Futures studies will continue this line of research in order to improve the current understanding of vascular function. In the same manner, it is imperative to recognize that the implementation of proper exercise programs is necessary to keep blood vessels healthy. Clearly, these findings have set the stage for a series of additional experiments with endothelial homeostasis as its focus.
References


Appendix: Consent Form

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

1. Title of Study: Effects of an Acute Bout of Exercise on Nitric Oxide Bioavailability, Oxidative Stress, and Antioxidant Defense Markers in Trained Individuals

2. Performance Sites:
LSU-Department of Kinesiology
Room 54 and 58, Exercise Laboratory and Vascular Laboratory
112 Huey P. Long Fieldhouse
Baton Rouge, LA 70803

3. Contacts:
The following investigators are available for questions at the telephone numbers listed below:

   Principal Investigator: Michael A. Welsch, Ph.D.
   Department: Kinesiology
   Telephone Number: (225)578-9143

   Co-Investigator Arturo Arce, M.D.
   Department: Kinesiology
   Telephone Number: (225)578-20

4. Purpose of the Study:
The purpose of the present study is to examine the effects of an acute bout of exercise on nitric oxide bioavailability, oxidative stress markers, and antioxidants markers, before, immediately and 20 minutes after, in trained individuals.

5. Subjects:
A. Inclusion Criteria You must be at least 18 years of age. Apparently healthy highly trained cyclist.

B. Exclusion Criteria If you have unstable manifestations of cardiovascular, metabolic, orthopedic, and/or neurological disease; active infection; risk for adverse responses to exercise; taking any medication which may affect cardiovascular function.

C. Maximum number of subjects: 20

6. Study Procedures:

If you are selected to participate in this study you will be involved for a total of 2 distinct visits. They include a series of tests at the beginning, during and the end of the study.

On the first visit you will be asked to perform a bike time trial and we will take some pictures of an artery in your arm.

The bike time trial will be performed on a stationary bike. You will begin pedaling at a speed that would allow you to regulate your workload by pedaling cadence, just as you would on a real cycle in a real competition. During the test we will measure the amount of oxygen you consume and carbon dioxide you produce to allow us to more precisely determine your fitness. We will measure oxygen and carbon dioxide using a metabolic analyzer. You will breathe regular room air, but when you exhale, a machine will measure how much air you are actually breathing out, and how much oxygen and carbon dioxide is in your breath. We will also measure your heart rate and blood pressure at the beginning, during and after the exercise.

In addition to the bike time trial, we will also take some pictures of an artery in your arm. This will be done right before and immediately after the exercise test. To perform this test
you will be asked to lie down on an examination bed. A laboratory technician will place 4 electrodes and 2 blood pressure cuffs on your chest and both arms, respectively. The electrodes are used to examine your heartbeat. The cuffs are used to measure blood pressure in the one arm and change blood flow to the hand in the other arm to allow us to test how your arteries react. After all the equipment is in place an ultrasound technician will be taking pictures of an artery in your arm before and after inflating a blood pressure cuff on the forearm for 5-minutes. Throughout the procedure, heart rate, and blood pressures will also be recorded. This test is called the brachial artery dilation test.

On the second visit you will again be asked to perform a bike time trial similar to the test on the first visit. However, prior to the test a certified phlebotomist will place a Heparin-Lock in the antecubital vein of the left arm for the purpose of blood sampling prior to the start of the exercise, at peak exercise, and 20 minutes after. The amount of blood drawn at each time point will be about 10ml. The blood samples will be processed and prepared for analyses. The purpose of the blood samples is to examine the influence of the exercise test on factors that have an effect on the health of the blood vessels.

7. **Benefits:**

We cannot promise any benefits from being in the study. However, participation in this study may assist the investigators in gathering information leading to better treatments for cardiovascular disease. You will have the opportunity of having the required tests and procedures performed during your participation in this study.

All clinic visits, physical examinations, study medication, laboratory tests, electrocardiograms, and any other procedures associated with the conduct of this study are free if you participate.
8. **Risks/Discomforts:**

The possible risks and discomforts for this trial are detailed in this informed consent. Blood sampling will be conducted by a certified and trained phlebotomist. The discomforts and risks of a blood test are the pain of a needle going into the vein, possible bruising and the remote risk of infection. However, if venipuncture is done properly, there should be little pain and little risk for adverse reactions. The phlebotomist will constantly monitor your condition during the course of the test. Inflation of the blood pressure cuff on the forearm may cause some temporary discomfort in the forearm and hand. If the discomfort is too severe you may terminate the procedure immediately by asking the technician to stop the test. Temporary numbness and tingling in the hand similar to the sensation of having your hand “fall asleep” may occur. There is no known risk to the use of ultrasound to measure the size of the blood vessel. There is no theoretical risk to blocking blood flow in the artery through inflation of the blood pressure cuff. Not a single permanent adverse event has been associated with this procedure in over 100,000 examinations reported in the medical literature. In addition to the risks listed above, the procedures may have unknown, unforeseen or unanticipated side effects. There is always the possibility that you will have a reaction that is currently not known and not expected. The risks of the bike time trial test using the methods described are minimal in this population but include: muscle soreness, stiffness or tightness, or in some rare cases muscle injuries or more severe complications.

9. **Measures taken to reduce risk**

The risk in this study will be minimized as a result of proper evaluation, education, and appropriate assessment of exercise responses and the presence of well trained personnel.
capable of monitoring exercise equipment.

10. **Right to Refuse:**

Your participation is voluntary. If you decide not to participate in this study you will not suffer a penalty or loss of benefits to which you are otherwise entitled. If you decide to participate in this study you may discontinue your participation at any time, without penalty or loss of benefits.

11. **Privacy:**

Every effort will be made to keep the information we learn about you private. Records of your participation in this study will be kept confidential except as required by law. Any publication of the data will not identify you.

12. **Financial Information:**

You will have no financial obligations.

13. **Withdrawal:**

If you decide to participate in this study you may discontinue your participation at any time, without penalty or loss of benefits and it will have no effect on the quality of your medical care, academic standing, job status, etc.

14. **Removal:**

You will have completed the study after finishing the two visits. The length of each visit will be approximately one hour.

The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. If I have questions about subjects' rights or other concerns, I can contact Robert C. Mathews, Chairman, LSU Institutional Review Board, (225)578-8692. I agree to participate in the
study described above and acknowledge the researchers’ obligation to
provide me with a copy of this consent form if signed by me.

Subject Signature: ________________________________ Date: ___________

The study subject has indicated to me that he/she is unable to read. I certify that I have read this
consent form to the subject and explained that by completing the signature line above, the subject
has agreed to participate.

Signature of Reader: _______________________________ Date: ___________
Arturo Arce was born in Arequipa, Peru, on January 31, 1968. He received his Medical Degree (M.D.) from Universidad Nacional de Cordoba, Argentina, in December of 1993. He currently is a post-doctoral fellow at the University of Missouri-Columbia. At the May 2008 commencement ceremony he will be awarded the Doctor of Philosophy in kinesiology from the graduate school at Louisiana State University.