

EFFECTS OF RESISTANCE EXERCISE ON MARKERS OF INFLAMMATION AND
ANTIOXIDANT DEFENSES IN HEART TRANSPLANT RECIPIENTS

By

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This document is dedicated to my parents, Vern and Carole Hamlin, who have continually supported me and pushed me to succeed in all of my scholastic and professional endeavors.

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Chronic heart failure (CHF) is a worldwide epidemic with over 400,000 new patients in the United States alone, each year. More than 750,000 hospitalizations per year are attributed to CHF, amounting to over eleven billion dollars in hospital expenses. Since 1974, heart transplantation (HT) has evolved into an accepted life-extending procedure for end-stage heart-failure patients. With 5-year survival rates exceeding seventy percent, the desired procedural outcome has shifted from patient survival to improving patient function and quality of life. This study was conducted to determine if a progressive resistance-training regimen is an efficacious method for decreasing resting levels of inflammation and oxidative stress, as well as increasing antioxidant defenses in heart transplant recipients (HTR).

A total of 15 subjects completed the study. Five subjects were randomized to the control group receiving standard care, while ten subjects were randomized to the resistance-training group, which exercised two days/week for six months post-transplant.

Blood samples were taken from both groups at baseline (2-months post-HT), and post-intervention (8-months post-HT), and stored for later biochemical analysis.

Resting levels of the inflammatory markers CRP, TNF- α , and TNFR did not differ between groups. However, resting IL-6 was significantly reduced in the training group, suggesting a training effect. We did not see significant alterations in the resting levels of SOD activity, our marker of antioxidant defense. Also, we did not see significant alterations in resting levels of 8-iso-PGF, our marker of oxidative stress.

Our data suggest that a resistance-training regimen consisting a single set of eight exercises performed two days/week provides insufficient stimulus to decrease all markers of inflammation. However, IL-6 was decreased by this regimen. We cannot exclude the possibility that immunosuppression therapy needed for anti-rejection in these patients may have blunted inflammation and skewed results. Our data also suggest that the resistance-training regimen was insufficient stimulus to increase antioxidant defenses or decrease oxidative stress in this population.

CHAPTER 1 INTRODUCTION

Statement of Problem

Chronic Heart failure (CHF) is a worldwide epidemic with over 400,000 new patients in the United States alone, each year[1]. More than 750,000 hospitalizations per year are attributed to CHF, amounting to over eleven billion dollars in hospital operating expenses[2]. Although these numbers are startling, the incidence of CHF is, in part, due to scientific advances, which have increased life expectancy and also survival after acute myocardial infarction.

Since 1974, heart transplantation (HT) has evolved into an accepted life-extending procedure for end-stage heart-failure patients. With 5-year survival rates exceeding seventy percent, the desired procedural outcome has shifted from patient survival to improving patient function and quality of life. Successful outcomes are often compromised by peripheral phenomena, including skeletal muscle atrophy, weakness, and fatigue. Although cardiovascular function is markedly improved with restoration of heart function, the persistent skeletal muscle myopathy severely limits the activity and functionality of heart transplant recipients (HTR).

There are two distinct stages in the etiology of skeletal muscle myopathy in HTR. First, prior to heart transplantation, CHF patients display antecedent skeletal muscle myopathy, which has developed and progressed throughout years of heart failure. Poor pump function leading to decreased cardiac output in CHF patients may be a principle factor in the gross skeletal muscle wasting observed in the periphery. The ‘Muscle

Hypothesis' of CHF, as described by Andrew Coats[3], postulates that a decreased oxygen delivery by peripheral vasculature, as well as a decreased oxygen uptake by skeletal muscle tissue, is not only causal of exercise intolerance, but further serves to propagate loss of skeletal muscle tissue in heart failure patients.

Secondly, after transplant, HTR experience further *de novo* skeletal muscle loss due to immunosuppressive therapy. It is well documented that administration of bolus glucocorticoids, as part of the anti-rejection strategy, causes skeletal muscle wasting via increasing protein catabolism, amino acid efflux, and decreasing protein synthesis[4-12].

Justification of Research

The current standard of care (SC) provided to HTR has achieved limited success in reversing the loss of skeletal muscle size, strength, and endurance. Studies of the skeletal muscle ultrastructure in CHF patients have revealed a strong relationship between exercise intolerance and skeletal muscle myopathy[8]. The sequelae of myopathic disease in CHF patients includes: skeletal muscle atrophy, fiber type shifts, decreased mitochondria and enzymatic reserve, and increased global and local inflammatory responses that lead to persistence of the aforementioned changes[3,13-20].

Unfortunately, the improved cardiac output achieved through HT does not result in an immediate reversal or normalization of skeletal muscle myopathy[6,7,21]. Additionally, large dose immunosuppressive therapy employed post-HT, an important part of the recovery and anti-rejection strategy, causes *de novo* deleterious effects on skeletal muscle[7,9,12]. Therefore, immunosuppressive therapy may further complicate recovery from the exercise intolerance experienced by CHF patients who become HTR.

Endurance exercise has gained acceptance as a possible therapeutic method for increasing the activity levels and reducing the level of fatigue in CHF patients, and

HTR[4,8,20-23]. Cardiovascular exercise may lead to increases in oxygen delivery, number of cardiac myocytes, levels of antioxidant enzymes, and levels antioxidant defenses in general[24-29]. Some investigators have studied the effects of resistance training on acute levels of blood and muscle inflammatory parameters, oxidative stress, and antioxidant parameters[30-36]. A recent study by Ramel et al.[36] demonstrated that a single bout of submaximal resistance exercise triggered an increase of fat soluble antioxidants in the plasma of healthy males. However, the chronic effects of a progressive resistance training regimen on resting levels of inflammatory and antioxidant parameters has yet to be investigated in HTR.

Resistance training has been widely documented to increase muscle and bone mass, as well strength in healthy populations. More recently, Braith et al.[37] showed that resistance exercise restored bone mineral density in HTR. Additionally, Braith et al.[11,38] demonstrated that resistance training could possibly serve to attenuate the muscle fiber loss observed in CHF and HTR, as well as initiate a shift toward the highly oxidative, fatigue resistant myosin heavy-chain Type 1 muscle fibers (MHCI) from glycolytic, less fatigue resistant and more injury prone MHC Type IIa and MHC Type IIx muscle fibers.

Research Question

The purpose of this investigation was to determine the effects of a progressive, 6-month resistance-training program on inflammatory and antioxidative parameters in HTR. With recent investigations into resistance training and its positive effects on muscle morphology and osteoporosis in HTR, an investigation of resistance training as a viable therapeutic intervention for decreasing levels of inflammatory markers and upregulating levels of antioxidant defenses was warranted.

Study Design

This investigation was a prospective, randomized, controlled study. Prior to transplant, end-stage heart failure patients listed for transplant at Shands Hospital at the University of Florida were randomized to either a control group receiving standard care, or to an intervention group that received a supervised resistance-training protocol in addition to receiving standard care.

This investigation measured resting levels of markers of inflammation (C-Reactive Protein {CRP}, Tumor Necrosis Factor Alpha {TNF- α }, soluble Tumor Necrosis Factor Alpha Receptors {sTNFR}, Interleukin-6 {IL-6}), 8-iso-prostaglandin F_{2 α} (8-iso-PGF), a marker of oxidative stress, and the antioxidant enzyme superoxide dismutase (SOD).

Specific Aims and Hypotheses

Specific Aim I

To investigate differences in resting levels of inflammatory markers (CRP, TNF- α , sTNFR, and IL-6) before and after exercise intervention, in HTR.

Hypothesis I

HTR who participate in a six-month, progressive resistance-training protocol will show decreased resting levels of CRP, TNF- α , sTNFR, and IL-6 compared to those HTR receiving only standard care.

Specific Aim II

To investigate differences in resting levels of lipid peroxidation in HTR, specifically 8-iso-PGF, before and after exercise intervention as a marker of oxidative stress.

Hypothesis II

HTR who participate in a six-month, progressive resistance-training protocol will show decreased resting levels of 8-iso-PGF compared to those HTR receiving only standard care.

Specific Aim III

To investigate differences in antioxidant capacity before and after exercise intervention; specifically, resting levels of the antioxidant enzyme SOD, in HTR.

Hypothesis III

HTR who participate in a six-month, progressive resistance-training protocol will show upregulated resting levels of the antioxidant enzyme SOD compared to those HTR receiving only standard care.

CHAPTER 2 LITERATURE REVIEW

Chronic Heart Failure Overview

Chronic heart failure (CHF) is a debilitating illness, which is commonly associated with exercise intolerance due to breathlessness and fatigue, cardiac dysfunction, and a high rate of mortality[19,20,39]. Heart failure can be 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, or to do so only from an elevated filling pressure[18]. The etiology of heart failure and number of CHF patients have changed in recent years due to three main factors: First, pharmacological and technological developments, such as improved thrombolytic agents and improved viability of stents, have led to an increased survival rate after myocardial infarction (MI). At the same time, the elderly population comprises the fastest growing demographic in the world. Due to better health care provision and an overall increase in health awareness, the longevity of the elderly is increasing. Lastly, the population of obese individuals is increasing, which has led to an increase in type II diabetes and thus an increase in the number of CHF patients.

Heart failure is generally characterized by systolic or diastolic impairment, or a combination of both[40]. Most commonly, failure is the result of an ischemic condition, such as coronary artery disease or necrotic myocardium as a result of MI. Cardiomyopathy, a non-ischemic etiology, can be idiopathic (viral in nature) or secondary (thyrotoxicosis—overactivity of the thyroid gland). CHF can also arise from

dysfunctional valves, myocardial stress induced by hypertension, or simple congenital inheritance of disease. Patients who present with CHF typically show reduced cardiac output, low maximal oxygen uptake, increased sodium retention, overly active sympathetic nervous system stimulation, and increased plasma concentrations of vasoconstrictors[3,18-20,39-41]. CHF can be caused by a variety of factors, and usually develops gradually as a result of deteriorating contractility of the viable myocardium[18].

CHF is known as a systemic disease, but may be more appropriately termed a complex syndrome. As previously mentioned, the underlying mechanism in CHF syndrome is deficient pump function that usually begins with one of a variety of cardiac insults. Conraads et al.[41] have outlined four aspects of the central and peripheral manifestations of systemic heart disease, including: the cardiorenal model, the hemodynamic model, the neurohumoral model, and the inflammation model (Figure 2-1). Each model has its own role in CHF propagation; however, all are characterized by their involvement of peripheral organs, tissues, and cells.

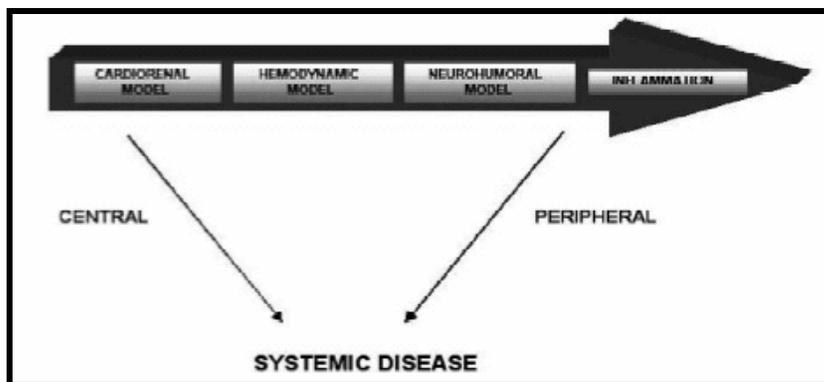


Figure 2-1. Chronic Heart Failure: A Systemic Disease[41].

Explaining the detailed facets of each of the aforementioned models is not within the scope of this review. However, each share common mechanisms suggesting that heart failure progression is the result of compensatory mechanisms overexpressing

biologically active molecules, which initially provide homeostasis, but when persisting have ultimately damaging consequences. The three salient components of the body's acute defense to heart failure are peripheral vasoconstriction, increased cardiac stimulation, and sodium and water retention. Activation of the renin-angiotensin-aldosterone system (RAAS) induces peripheral vasoconstriction, salt and water retention, and upregulation of the sympathetic nervous system (Figure 2-2)[40]. All of these mechanisms serve to increase cardiac output (Q) and to maintain blood pressure (BP), the two main deficiencies of a failing pump.

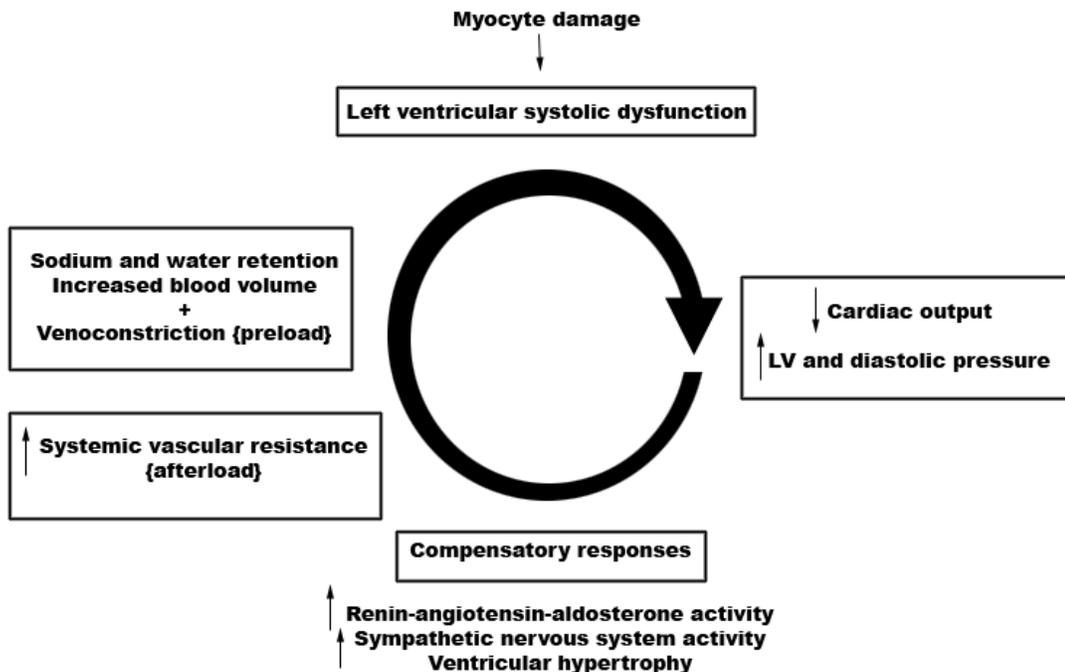


Figure 2-2. The Chronic Heart Failure Cycle[40].

The chronic effects of prolonged upregulation of the acute compensatory mechanisms can lead to various disease-state etiologies, including dilated cardiomyopathy (DC) or hypertrophic cardiomyopathy (HC). A heart with DC will exhibit increased ventricular chamber size, however will not show a commensurate increase in ventricular wall thickness, leading to a diminished ejection fraction (EF) and

Q. A heart exhibiting HC will generally exhibit a marked increase in left ventricular (LV) inner-wall mass, decreased LV chamber size and obstructed aortic outflow, which all cause decreased EF and Q. In each case, the myocardial oxygen demand increases for two main reasons: First, the increased sympathetic tone leads to tachycardia and increased myocardial workload. Second, the increased circulating angiotensin II causes myocardial and vascular growth, increases release of epinephrine and norepinephrine from sympathetic nerve terminals, promotes aldosterone production, fibrosis, and may play a role in endothelial dysfunction and vascular remodeling[40,41].

Many CHF patients experience decreased levels of physical activity and exercise intolerance. These symptoms may be in great part due to the decreased metabolic capacity of the skeletal musculature observed with a failing heart, which leads to weakness, fatigue, and atrophy. It has been shown that there is a strong correlation between the fiber type shift (from type I fatigue resistant fibers, toward type II fatigueable fibers) observed in CHF patients and impaired physical capacity [18,22]. This intolerance only serves to further propagate the symptoms of a failing heart. While the majority of the body's compensatory mechanisms take effect to ensure the core organs and brain are adequately perfused under ischemic conditions, the peripheral blood flow is decreased and this may contribute to the impaired oxidative capacity of CHF patient's musculature. Altered peripheral blood flow may be one of the central factors causing the deleterious changes seen in peripheral tissue, particularly skeletal muscle myopathy.

Skeletal Muscle Myopathy in Heart Transplant Recipients

Since 1974, heart transplantation (HT) has evolved into an accepted life-extending procedure for end-stage heart-failure patients. With 5-year survival rates exceeding

seventy percent, the desired procedural outcome has shifted from patient survival to improving the negative peripheral manifestations common to CHF and HT patients, including skeletal muscle atrophy, weakness, and fatigue. Although cardiovascular function is markedly improved with restoration of heart function, the persistent skeletal muscle myopathy and cardiac cachexia severely limit the activity and functionality of heart transplant recipients (HTR).

There are two distinct stages in the etiology of skeletal muscle myopathy in HTR. Firstly, CHF patients display antecedent skeletal muscle myopathy, which has developed and progressed throughout years of heart failure. Poor pump function leading to decreased cardiac output in CHF patients may be a principal factor in the gross skeletal muscle wasting observed in the periphery. The ‘Muscle Hypothesis’ of CHF, as described by Andrew Coats[3], postulates that a decreased oxygen delivery by peripheral vasculature, as well as a decreased oxygen uptake by skeletal muscle tissue, is not only causal of exercise intolerance, but further serves to propagate loss of skeletal muscle tissue in heart failure patients.

Secondly, HTR experience further post-operative *de novo* skeletal muscle loss that can be directly attributed to the initiation of post-transplant (post-HTR) immunosuppressive therapy. Administration of bolus glucocorticoids, as part of the anti-rejection strategy, has been well documented to assist in skeletal muscle wasting via increasing protein catabolism, amino acid efflux, and decreasing protein synthesis[4-12]. The most commonly used immunosuppressive drugs in HTR include glucocorticoids (prednisone, methylprednisolone) and cyclosporine, and their detrimental effects on skeletal muscle have been well documented[4-9,11,12,42]. Because skeletal muscle

abnormalities are not immediately resolved via heart transplantation, these abnormalities contribute to the exercise intolerance experienced indefinitely by HTR[10,43,44].

Biological Markers of Disease/Inflammation

Cytokines: Interleukin(IL) and Tumor Necrosis Factor (TNF)

Cytokines are soluble glycoproteins that are produced by and mediate communication between and within immune and nonimmune cells, organs and organ systems throughout the body[45,46]. Two classes of cytokines exist: Pro-inflammatory cytokines, and Anti-inflammatory cytokines, which serve to attenuate the actions of the previous. Cytokines merit continuing study in disease populations because they can exert their powerful effects in the nano- to picomolar concentration range, and they have a potential for powerful modulation by stimuli such as infection and exercise[46]. Tumor necrosis factor (TNF) and interleukins (IL) comprise two families of pro-inflammatory cytokines that are upregulated by various stimuli. The systemic and local inflammation associated with these two families have been suggested to play an important role in the progression of many diseases, and circulating levels of cytokines and cytokine receptors are gaining acceptance as prognostic markers[47]. Medical conditions such as diabetes mellitus, cancer, atherosclerosis, CHF, and lifestyle activities such as smoking all have been associated with higher levels of proinflammatory cytokines and may contribute to future disability[48]. The ‘cytokine hypothesis’ of CHF holds that the progression of this disease is, at least partly, the result of the toxic effects exerted by cytokines on the heart, vessel walls, skeletal muscle, and other peripheral organs[45,46].

TNF- α and IL-6

Tumor necrosis factor alpha (TNF- α) is part of a group of peptides originally named cachectins. Cachectins are believed to play significant catabolic roles in many of the

diseases previously mentioned[32]. Plasma levels of TNF- α and other pro-inflammatory cytokines increase with age, and further increase in disease states, which suggests a role of TNF- α in inflammation and skeletal muscle wasting[32]. In the Health ABC (Health, Aging, and Body Composition) study, Visser et al.[48] found that higher levels of IL-6 and TNF- α were associated with lower muscle mass and lower muscle strength in healthy elderly men and women 70-79 years of age. In CHF patients and patients with cachexia, TNF- α and IL-6 have been shown to exist at supraphysiological levels in the plasma with a strong correlation to reduced lean tissue mass[29,33,45,46,49]. Current evidence suggests that TNF- α initiates its inflammatory actions by upregulating the expression of surface adhesion molecules such as, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin; all of which promote the migration of leucocytes to the site of inflammation[46]. TNF- α may also compound these effects by increasing the lifespan of certain leukocytes. Neutrophils normally have a circulatory lifespan of about 6 days, but during sepsis they are rescued from apoptosis by cytokines such as TNF- α and IL-6[46].

There are several proposed mechanisms of TNF- α -induced skeletal muscle wasting, including: directly inducing the loss of skeletal muscle protein via the ubiquitin/proteasome pathway, indirect protein loss via induction of the transcription factor NF- κ B leading to increased iNOS expression and the production of reactive oxygen species (ROS), and lastly, the cytokine may activate cell intracellular death domains expressed on TNFR capable of triggering apoptosis or necrosis[32,50,51]. Other investigators have suggested the potential of TNF- α to inhibit myoblast differentiation, thus limiting the amount of satellite cells that can respond to muscle

injury, as the mechanism responsible for muscle wasting[49]. Additionally, insulin resistance in skeletal muscle has been linked to high levels of TNF- α . TNF- α is expressed in human skeletal muscle and its expression is augmented in the skeletal muscle of patients with type-2 diabetes[52]. Supporting this hypothesis is the observation that TNF- α decreases insulin-stimulated rates of glucose storage in cultured human muscle cells[53]. Animal models analyzing the effects of TNF- α on skeletal muscle have also suggested catabolic effects. Administration of IL-6 or TNF- α in rats leads to increases in skeletal muscle protein loss, a decreased rate of protein synthesis, and skeletal muscle wasting[54-57].

Interleukins, specifically IL-6, can exist in various forms in the body, and each form can have a differing biological activity. Some of the functions of IL-6 are similar to those of TNF- α . However, there are several key differences between IL-6 and TNF- α . TNF- α is mainly produced by mononuclear phagocytes, but it is also produced in T lymphocytes, neural cells, Kupffer cells, and endothelial cells. The majority of cells in the body express TNF receptors (TNFR), and soluble TNFR have been shown to attenuate the powerful inflammatory reactions of TNF. IL-6 is produced by many different cells, but the main sources in vivo are stimulated monocytes/macrophages, fibroblasts, and vascular endothelial cells, which could be indicative of its role in the modulation of the immune system[58]. It can also be produced by non-immune cells such as, smooth muscle cells, chondrocytes, astrocytes, and glial cells[46]. Cells that express receptors of IL-6 include T- and B-cells, bone marrow cells, osteocytes, hepatocytes, and various tumor cell lines[46].

It is believed that IL-6 has pro-inflammatory and anti-inflammatory actions. Muscle contractions induce production and release of IL-6 into the circulation[35]. Tissue damage has been implicated in the induction of blood-borne and tissue IL-6[46]. Many studies have suggested that IL-6 and TNF- α have similar functions, but numerous studies suggest the contrary. IL-6 may serve to induce anti-inflammatory cytokine production in circulating monocytes and suppress the synthesis of pro-inflammatory cytokines such as TNF- α [58]. Other studies suggest one of the most important functions of IL-6 is to activate hepatocytes, B-cells, and the mononuclear phagocytes responsible for the production of TNF- α [33,45,46,49]. Thus, simultaneously elevated levels of both cytokines in diseased states may carry merit, but not suggest that they carry out the same functions[58].

IL-6 has been shown to activate osteoclasts and induce bone resorption, as well as serve as one of the most important mediators of the acute phase response: a conserved cascade of reactions that is stimulated by tissue damage or inflammation, which serves to prevent further damage and initiate tissue repair processes[46]. One acute phase protein that is synthesized by the liver (and thus its levels are possibly mediated by IL-6) is C-Reactive Protein (CRP). Febbraio and Pedersen[58] hypothesize that muscle derived IL-6 may be involved in the regulation of glucose homeostasis either by affecting hepatic glucose production and/or muscle glucose uptake, and thus may aid in maintaining metabolic homeostasis during periods of altered metabolic demand, such as exercise. IL-6 has been shown to inhibit glycogen synthase activity and accelerate glycogen phosphorylase activity[58]. IL-6 is also important in lipolytic processes, as it is secreted by adipose tissue. However, a recent study demonstrated that IL-6 production by adipose

tissue is suppressed during exercise but elevated after exercise[59]. In several studies, plasma concentrations of IL-6 have been shown to predict total and cardiovascular mortality[60,61].

The high levels of TNF- α and IL-6 in CHF patients have been proposed to be a main factor in skeletal muscle loss, specifically a shift from MHC I fibers toward MHCII fibers[18,22]. In the absence of exogenous anabolic stimuli, increased levels of TNF- α could directly stimulate a progressive loss in total muscle protein content and cause an overall decrease in muscle mass[49]. The progressive loss of MHCI oxidative fibers and the shift toward MHCII glycolytic fibers is possibly a result of the decreased oxygen availability to the muscle, as well as increased levels of TNF- α . This shift in fiber type toward the highly fatigueable type II fiber is surely to contribute to the increased fatigue and exercise intolerance seen in CHF patients.

TNF- α Receptors

The responses of muscle cells to TNF- α are mediated by two sarcolemmal receptor populations, type I (TNFRI, 55kDa), and type II (TNFRII, 75kDa)[49]. The wide range of TNF activities is explained by the presence of TNFRs on almost all cell types[62]. Both TNF type I and type II receptors have been implicated as mediators of the NF- κ B signaling pathway. However, it has been indicated that TNFRIs, but not TNFRIIs, stimulate muscle protein loss[41,49,62]. NF- κ B activation is a primary step in the activation of the ubiquitin/proteasome pathway leading to muscle catabolism. The ubiquitin/proteasome pathway is responsible for degradation of the bulk of all intracellular proteins, specifically, but not limited to, the major skeletal muscle contractile proteins actin and myosin. This pathway is also involved in regulation of signal

transduction, cell cycle progression, transcriptional regulation, and antigen presentation[49].

Soluble TNFR (sTNFR) in the circulation during inflammatory states have been reported in several studies[33,35,49,62,63]. Plasma levels of sTNFR and TNF- α are strongly correlated, as sTNFR bind TNF- α with high affinity and may act as inhibitors or carriers of the cytokine. Some investigators indicate that plasma levels of sTNFR are long term predictive markers of local and systemic TNF- α production[35,64,65]. It has been proposed that the mechanism of TNFR shedding in diseased patients (such as CHF) is a protective response against the supraphysiologic levels of circulating TNF- α . By binding to the circulating TNF- α , the catabolic effects could be partially attenuated because the monomerization of TNF- α subunits (required for biological activity) is blocked[62]. Additionally, the shedding leaves a decreased level of active receptors at the cellular level, which could further serve to lessen the toxic effects of the cytokine. However, it has also been proposed that the binding of sTNFR to TNF- α in plasma can only temporarily subdue its catabolic action, while concurrently serving as a slow-release biological store (increasing the half-life) of circulating TNF- α [35]. This could be significant as it is possible that the relevant effects of TNF- α are more related to the persistence of the cytokine, rather than to its peak levels[62].

TNF- α and Oxidative Stress

Recent evidence has accumulated indicating that oxidative stress may play an important role in the progression of heart failure, no matter the etiology. One possible mechanism links the high levels of TNF- α to oxidative stress. TNF- α can induce skeletal muscle catabolism indirectly through the production of reactive oxygen species (ROS) in

the mitochondrial electron transport chain. The release of ROS from the mitochondria post-binding of TNF- α to its receptor (TNFR1), begins a sequence of reactions, ultimately ending in protein loss (Figure 2-3)[49].

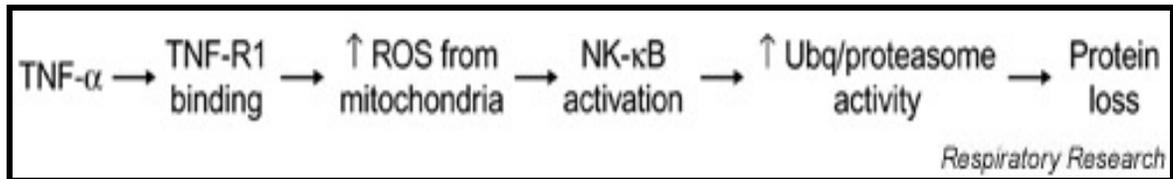


Figure 2-3. Schematic of the Proposed Indirect TNF- α -Induced Muscle Catabolism[49].

Xanthine oxidase and NADPH oxidase are enzymes found in immune cells that are responsible for catalyzing reactions which produce ROS, which are then used to destroy invading microbes and infection. In an upregulated inflammatory state, these enzymes can lead to overproduction of molecules such as superoxide, which can leak unnecessarily into tissues and cause degradation. There is a strong correlation between serum uric acid, an indicator of xanthine oxidase activity, and circulating markers of inflammation (such as TNF- α) in patients with CHF[29].

Exercise Effects on TNF- α and IL-6

Exercise and its effects on muscle and plasma levels of TNF- α and IL-6 is currently a highly investigated area, due to the possibility of exercise as a therapeutic method to reduce systemic inflammation and attenuate the skeletal muscle wasting commonly observed in diseased patients. Many studies have evaluated the effect of endurance exercise on plasma levels of TNF- α [47,66-73]. Most investigations into plasma levels of TNF- α have shown an increase in release immediately after prolonged endurance exercise[66,68,69,71-73], while other studies have shown a decrease or no change[70,74-76]. After 12 weeks of endurance exercise training in a supervised cardiac rehabilitation program, Larsen et al.[47] found significant decreases in plasma TNF- α

levels. However, within the same study exercise training did not induce significant changes in plasma IL-6 levels.

Few studies have analyzed the effects of resistance exercise on plasma TNF- α levels[32,35]. Greiwe et al.[32] showed that both TNF- α mRNA and protein levels decreased along with increases in strength and protein synthesis levels, after 12 weeks of resistance training elderly subjects. Conraads et al.[33] exercised twenty-three patients with stable CHF due to coronary artery disease (CAD), or idiopathic dilated cardiomyopathy (IDCM) for four months using a combined endurance/resistance training regimen. Although plasma concentrations of cytokines and sTNFRs were significantly elevated in patients versus health controls, TNF- α , sTNFRII and IL-6 plasma concentrations were not significantly altered. However, sTNFRI levels before and after the training period showed a significant decrease. The decrease in sTNFRI was found in patients with CHF due to CAD, but no effect was seen in the case of IDCM.

Plasma levels of TNF- α seem to be at least partially influenced by the intensity and duration of the exercise bout. Training may induce alterations in time, quantity, and duration of TNF- α release. The wide range of results in measuring blood levels of TNF- α may be partially explained by inconsistencies in experimental design and exercise protocol. Most studies have evaluated transient fluctuations after a single exercise bout, with few studies investigating the effect of a progressive resistance-training regimen on resting levels of TNF- α . The effect of blood glucocorticoid levels and induction of TNF- α in exercising humans is an area that is understudied.

Nearly all investigations into the effects of endurance exercise on IL-6 have reported that exercise generally causes a strong but transient induction[67,69,77-81], with

levels of IL-6 increasing up to 100-fold during muscular contraction. Plasma elevation of IL-6 seems to be correlated to the intensity and duration of the exercise bout, with increases in plasma IL-6 possibly being correlated to the increasing levels of epinephrine[58]. Febbraio and Pedersen[58] proposes that IL-6 induction in response to exercise of long duration is independent of muscle damage, whereas muscle damage per se is followed by repair mechanisms including invasion of macrophages into the muscle leading to IL-6 production. Consequently, the IL-6 production in relation to muscle damage occurs later and is of smaller magnitude than IL-6 production related to muscle contractions. Once again, the effect of a prolonged training regimen on resting levels of IL-6 has scarcely been studied. The effect of progressive resistance training on skeletal muscle and plasma levels of IL-6 in HTR has not been studied.

C-Reactive Protein in CHF/HTR

C-reactive protein (CRP) was discovered decades ago in Oswald Avery's laboratory during investigations involving patients with *Streptococcus pneumoniae* infection[82]. CRP is an acute phase reactant that markedly increases during an inflammatory response[83,84]. CRP is widely recognized as a marker of atherosclerosis, acute myocardial ischemia, CHF, and other cardiovascular disease states, and has been investigated in many studies[17,23,83-86]. CRP is produced in liver hepatocytes, and its release is believed to be stimulated by increased levels of IL-6 and TNF- α [23,58,84-88]. Several studies indicate that increased levels of IL-6 in the serum are derived from the cytokine's secretion from adipose tissue, and thus, increased CRP could be associated with increased adiposity[23,85]. Elevated serum CRP has been associated with circulating risk factors such as elevated serum fibrinogen and clotting

factors, insulin resistance, elevated blood glucose, and depressed high-density lipoprotein cholesterol[89]. Mendall et al.[84] suggests three possibilities for what these associations represent: Firstly, elevations in CRP may be a non-specific response to any environmental stimulus. Second, elevations may be a response to inflammation occurring in the vessel wall at the site of an atherosclerotic lesion. And lastly, elevated serum CRP may simply be due to other inflammatory mediators such as TNF- α , which may play direct roles in the pathogenesis of heart disease.

Although many patients with high levels of systemic inflammation show increased levels of CRP, it is still unclear whether CRP is merely an indicator of disease, or whether it contributes to the pathogenesis[83,84]. Harris et al.[86] found that serum levels of CRP and IL-6 combined were predictors of all cause mortality in healthy, non-disabled elderly. The Framingham Study found that patients with increased serum levels of CRP experienced over a twofold increased risk of CHF[87]. Supporting the hypothesis that increased levels of CRP are reactionary to the pro-inflammatory response, some studies indicate that CRP may trigger the induction of anti-inflammatory cytokines in circulating monocytes and suppress the synthesis of pro-inflammatory cytokines in tissue macrophages[90,91].

Recent studies into CRP levels in CHF patients and HTR have focused on CRP as a marker of disease progression and transplant rejection. Levels of CRP and pro-inflammatory cytokines may provide an indication of acute and chronic allograft rejection. In a study by Hsu et al.[17], increased levels of P-selectin, ICAM-1, and CRP were found in HTR, early post-transplant. The mechanisms of CRP activation and its regulation have not been completely ascertained at this time.

Exercise Effects on CRP

Exercise and the possibility of its therapeutic role in decreasing serum CRP has been studied by several investigators[23,85]. In a study by Okita et al.[85] performed with healthy Japanese women, an aerobic exercise-training regimen was shown to decrease adiposity and lower serum CRP levels. A similar study by Duncan et al.[23], where healthy adults took part in a 6-month exercise program consisting of walking at moderate (45-55% individual heart rate reserve) or high (75% individual heart rate reserve) intensity, found that exercise training failed to alter serum CRP levels. However, this study did not employ exercise supervision and reported that the levels of exercise completed, in terms of total minutes, fell below the amounts prescribed. The effects of resistance training on serum CRP has been scarcely investigated. However, a recent study by Castaneda et al.[34] showed that a 12 week resistance training program lead to decreased serum levels of CRP and IL-6 in patients with chronic kidney disease.

Reactive Oxygen Species, Antioxidant Defenses and Lipid Peroxidation

The human body employs many endogenous defense mechanisms to help prevent tissue injury and combat challenges to internal homeostasis. Antioxidant defenses, classed as enzymatic or non-enzymatic, comprise a very important protective mechanism against reactive oxygen species (ROS), highly reactive molecules or atoms produced via a variety of processes with damaging effects. Although some level of ROS is important to maintain proper function of various processes (immune system regulation, cell signaling, regulation of calcium release in skeletal and cardiac muscle, blood pressure control), excessive amounts of ROS (commonly seen in a pro-inflammatory state) can lead to a state of oxidative stress and have deleterious effects at subcellular to organ-system levels[92-94].

There are many sources of reactive oxygen species, including, but not limited to, the electron transport chain in the mitochondria[24-28,95,96], oxidoreductases (xanthine oxidase) in endothelial cells of blood vessels and skeletal and cardiac muscle[24,27,31,95], nitric oxide (NO) in the vasculature and skeletal muscle[27], auto-oxidation by catecholamines epinephrine and nor-epinephrine[27,28,31,95], metabolism of arachidonic acid from cell membranes, and immune cell release during host-cell protection[24-28,31,95]. Some of the most debilitating ROS include superoxide radicals, hydroxyl radicals, and hydrogen peroxide. Aerobic organisms produce ROS during normal respiration, as well as during inflammatory conditions such as sepsis, chronic heart and lung diseases, and cachexia[26,27]. Cells continuously produce free radicals and ROS as part of a host of metabolic processes[95], and it is estimated that 1% to 5% of the oxygen consumed during mitochondrial oxidative phosphorylation generates ROS[26,95]. During aerobic exercise, oxygen consumption can increase up to twenty times normal resting levels[26], leading to increased ROS production. Therefore, antioxidant defense systems are constantly attempting to minimize oxidative stress, while maintaining optimal tissue function in resting and non-resting states, in healthy and unhealthy populations.

Three major enzymatic antioxidants are superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). Major non-enzymatic antioxidants include glutathione (GSH), ubiquinone, flavanoids, and vitamins A, E and C. Each of the antioxidant enzymes catalyzes a one-electron reduction of their substrate ROS[27].

Superoxide Dismutase

SOD is the primary cellular defense against superoxide radicals[26,27], as it catalyzes the dismutation of superoxide anions ($O_2^{\bullet-}$) to form H_2O_2 and O_2 .



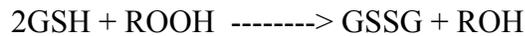
In mammals, two isoenzymes of SOD are known to exist in skeletal muscle. The first is the copper- and zinc-dependent isoform (Cu/Zn-SOD), which can be found in the cytosol. The second is the manganese-dependent isoform (Mn-SOD), which is principally found in the mitochondria.

Glutathione Peroxidase

GPX is primarily responsible for catalyzing the reduction of H₂O₂ and organic hydroperoxides to water and an alcohol, using the reduced form of the antioxidant GSH as an electron donor[26,27].



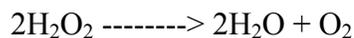
and/or



The glutathione family is thus responsible for reducing a wide variety of organic and non-organic hydroperoxides, and it is an invaluable defense mechanism against ROS-mediated damage to proteins, membrane lipids, and nucleic acids. The major sites of GPX activity are the same as those for SOD, with slightly greater activity being found in the mitochondria versus the cytosol[26,27].

Catalase

The primary function of catalase is to catalyze the decomposition of H₂O₂ to water and molecular oxygen.



Catalase is widely distributed in the cell, with highest concentrations being found in peroxisomes and mitochondria[26-28,96]. In comparison to GPX, CAT has a rather low affinity for H₂O₂, suggesting that GPX may play a greater role during periods of low-to-

moderate levels of H_2O_2 , with activity of CAT becoming more important during states of injury or disease where higher levels of ROS are seen[27].

Lipid Peroxidation

It has been well established that ROS can damage membrane lipids and circulating lipoproteins, leading to decreased membrane fluidity at the cellular level[26,27,30,31] as well as becoming an underlying contributor to various disease states, including atherosclerosis, diabetes, and stroke[27,30]. Although there is no gold standard for measurement of lipid peroxidation biomarkers, conjugated dienes such as 8-iso-prostaglandin- $F_{2\alpha}$ (8-iso-PGF) have been commonly used.

Exercise Modulation of ROS and Antioxidants

The effects of endurance exercise on oxidative stress and antioxidant defenses have been well documented[24-28,31,95-99], while the effects of resistance training have received little attention[30]. It is well known that muscular activity, through increased activity of metabolic processes, results in increased production of radicals and other forms of reactive oxygen species[24-28,31,95-99]. It is believed that exercise-induced oxidative stress is a primary mediator in the disturbance of muscle homeostasis through damage to enzymes, protein receptors, lipid membranes, and DNA[26]. It is also believed that ROS may also contribute to the late phase of exercise-induced muscle injury[98]. Although ROS are acutely increased with muscular activity, many studies have reported decreases in resting levels of oxidants due to an upregulation of antioxidant enzymes[24,26,27,96,99], decreased adiposity, altered lipid profiles, receptor and transport-protein adaptations, and improved mitochondrial coupling with exercise[26]. This effect has not been documented in long-term resistance training studies.

Antioxidant enzyme activity is found at greater levels in type I MHC, oxidative skeletal muscle fibers in comparison to type IIa/x MHC, fatigueable fibers[27]. Thus, in disease, where skeletal muscle wasting is prevalent and a marked shift toward type II MHC muscle fibers is seen, antioxidant capacity is diminished. In a study by Braith et al.[38], a progressive resistance exercise training protocol in heart transplant recipients caused a significant shift toward type I MHC fibers, producing an increase in oxidative enzymes. Ramel et al.[36] recently demonstrated that an acute bout of submaximal resistance exercise in healthy male subjects upregulated plasma levels of fat soluble antioxidants, but failed to significantly decrease markers of lipid peroxidation[31]. Although not yet investigated, the possibility exists that a progressive resistance exercise-training regimen may produce an increase in resting levels of plasma antioxidants and antioxidant enzymes in HTR.

Exercise Training in CHF and HTR

Endurance exercise has been widely accepted as a therapeutic method for increasing the activity levels and reducing the level of fatigue in CHF patients[20,22,33]. Cardiovascular exercise leads to increases in oxygen delivery, number of cardiac myocytes, levels of antioxidant enzymes and antioxidant defenses in general. However, the effects of resistance training and its ability to alter inflammatory and antioxidant profiles in HTR has not been extensively studied. Progressive resistance training is universally recognized as the most effective method of inducing skeletal muscle hypertrophy and strength increases. Although resistance training was initially discouraged by physicians for recovering HTR, the Center for Exercise Science at the University of Florida has pioneered the use of resistance training among HTR. The resistance-training protocol was implemented initially in an attempt to reverse

glucocorticoid-induced osteoporosis in HTRs[37]. Resistance training has since been shown to be efficacious in protecting and restoring bone mineral density in HTR[37,100] and appears to have protective and restorative properties in skeletal muscle as well[8,11,21,100].

In studies by Braith et al.[11,38], a resistance-training intervention initiated 2-months post transplant was successful in restoring muscle mass and strength to pre-transplant levels in HTR. HTR control subjects, who did not participate in resistance training post-transplant, experienced continued loss of muscle mass and increases in fat mass[11]. Although strength improvements were observed in the control group (significant vs. early HT baseline), gains were significantly less than those experienced by the resistance-trained group[11].

In a study by Vaquero et al.[101], utilization of electrical stimulation to strengthen the quadriceps muscle of HTR resulted in a significant increase in maximal VO_2 over an eight-week period. Unfortunately, the investigators did not measure changes in muscle strength or morphology as a result of the electrical stimulation protocol.

While the initial research investigating the protective and restorative nature of resistance training on skeletal muscle of HTRs has been encouraging, outcome variables were limited to functional parameters[11,101]. Other studies that have investigated ultrastructural adaptations, via muscle biopsy in HTR, used either a cardiovascular exercise protocol or an extremely conservative manual resistance-training protocol that did not include weight lifting[8,21,102]. A complete understanding of the morphologic and biochemical changes that are occurring in the blood and skeletal muscle as a result of a comprehensive exercise protocol, initiated early post transplant, is still lacking.

CHAPTER 3 METHODOLOGY

Subjects and Experimental Design

Twenty candidates for orthotopic heart transplantation were recruited from the United Network for Organ Sharing (UNOS) waiting list at Shands Hospital at the University of Florida. Prior to transplant, the patients were randomized to either a training group that participated in a 6-month program of resistance training after transplantation or to a control group that did not participate in a resistance exercise program after transplantation. All of the HTRs participated in a post-transplant self-monitored walking program, but only the training group performed resistance exercise. Dosages of immunosuppressive agents were recorded for each patient.

Venous blood samples were collected at two specific time points. The first collection occurred at 2 months post-HT and prior to the exercise intervention. Resistance exercise programs cannot be initiated until the median sternotomy has time to heal. The second collection occurred at 8 months post-HT, after either a 6-month training or control period. Venous blood samples were assayed for resting levels of the inflammatory markers C-Reactive Protein (CRP), Tumor Necrosis Factor Alpha (TNF- α), soluble Tumor Necrosis Factor Alpha Receptors (sTNFR), Interleukin-6 (IL-6), as well as 8-iso-prostaglandin F_{2 α} (8-iso-PGF), a marker of oxidative stress, and the antioxidant enzyme superoxide dismutase (SOD).

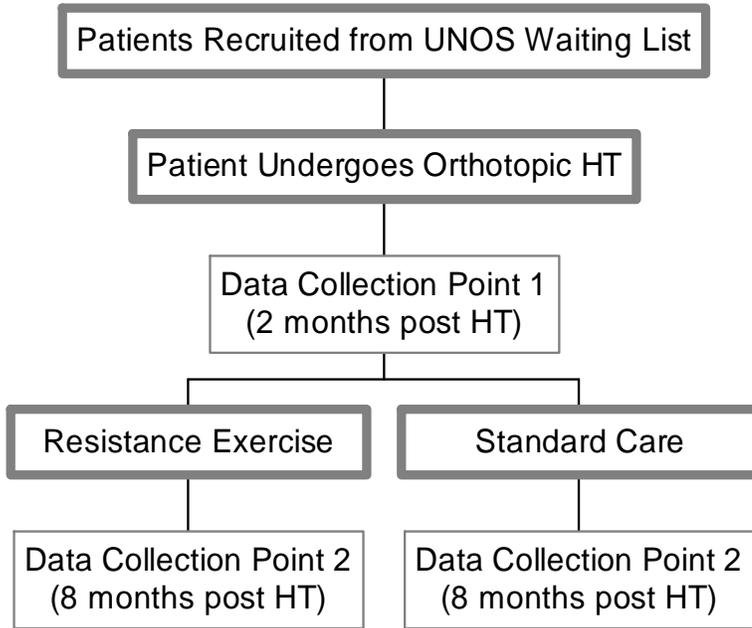


Figure 3-1. Experimental Design

Resistance Exercise Training Protocol

The intervention protocol was initiated 2 months after HT. This time frame permitted sufficient time for surgical wound healing before beginning upper body resistance exercise. The 6-month training regimen consisted of upper- and lower-body resistance training 2 days/week using MedX variable resistance machines (MedX, Ocala, FL). All training sessions involved one transplant recipient being supervised by at least one exercise specialist. Before each resistance exercise session, seated blood pressure and pulse rate measurements were recorded. Before beginning the resistance training session, subjects completed 5 minutes of warm-up with low-intensity treadmill walking and 5-10 minutes of static stretching. The training sessions consisted of one set of 10-15 repetitions performed on 8 machines. The initial training weight represented 50% of the subject's one repetition maximum (1-RM). The subjects were not permitted to exceed 15 repetitions. Rather, when 15 repetitions were performed successfully through a full range

of motion (ROM), the weight was increased by 5% to 10 % at the next training session. This exercise prescription was intended to have subjects use the greatest resistance possible to complete 15 repetitions while avoiding a low-repetition and high-resistance regimen that could cause musculoskeletal injury in subjects at risk for steroid induced osteoporosis.

The following exercises were performed in order: chest press, knee extension, pull-down, knee flexion, shoulder press, triceps extension, biceps flexion, and low-back extension. Since the denervated, transplanted heart is preload dependent, special precautions were taken to assure adequate maintenance of blood pressure. Upper-body exercises were alternated with lower-body exercises in an attempt to prevent blood pooling. Symptomatic subjects walked 2 minutes between exercises or performed standing calf raises to augment venous-blood return to the heart. All subjects concluded each training session with a 5-minute cool-down period where they walked at low intensity on the treadmill.

Collection of Blood Samples

Blood samples were collected from all subjects at 2 months post-HT and after 6 months of a resistance exercise or control period. Venous blood was collected from a vein of the left or right forearm using a butterfly catheter. Blood was collected in tubes containing EDTA and samples immediately underwent centrifugation at 3,000 rpm for approximately 15 minutes. All samples were stored at -80°C and analyzed in a single batch at the end of the study.

Measurement of Inflammatory Markers

CRP

Serum levels of CRP were quantified using a highly sensitive enzyme-linked immunoassay from Alpha Diagnostic International, Inc. (San Antonio, TX). All standards and samples were assayed in duplicate. Samples were pipetted directly into wells of an antibody pre-coated microplate. An enzyme conjugate was then added prior to a short incubation. After the addition of a substrate solution and a second incubation, the reactions were stopped with a stop solution. Absorbances were measured using a microplate reader at 450nm. Absorbances were proportional to the amount of CRP bound in the wells.

TNF- α

Serum concentrations of TNF- α were measured using a quantitative sandwich enzyme immunoassay technique from R&D Systems (Minneapolis, MN). All standards and samples were assayed in duplicate. A monoclonal antibody specific for TNF- α was pre-coated onto a microplate. Standards and samples were pipetted into the wells, and any TNF- α present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TNF- α was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. After an incubation period, an amplifier solution was added to the well and absorbances were measured on a microplate reader at 490nm. Absorbances were proportional to the amount of TNF- α bound in the wells.

TNFR-I and -II

Plasma concentrations of TNFR I and TNFR II were determined using the quantitative sandwich enzyme immunoassay technique from R&D Systems (Minneapolis, MN). All standards and samples were assayed in duplicate. A monoclonal antibody specific for sTNFR II was pre-coated onto a microplate. Standards and samples were pipetted into the wells and any sTNFR II present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for sTNFR II was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. After an incubation period, an amplifier solution was added to the wells and the absorbances were measured on a microplate reader at 490nm. Absorbances were proportional to the amount of TNFR II bound in the wells.

IL-6

Plasma concentrations of IL-6 were measured using a quantitative sandwich enzyme immunoassay technique from R&D Systems (Minneapolis, MN). All standards and samples were assayed in duplicate. A monoclonal antibody specific for IL-6 was pre-coated onto a microplate. Standards and samples were pipetted into the wells, and any IL-6 present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-6 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. After an incubation period, an amplifier solution was added to the wells and absorbances were measured on a microplate reader at 490nm. Absorbances were proportional to the amount of IL-6 bound in the wells.

Measurement of Oxidative Stress

Plasma levels of lipid peroxidation were quantified using the StressXpress 8-iso-PGF_{2α} (direct) ELISA Kit from Stressgen Bioreagents (Victoria, BC Canada). All standards and samples were assayed in duplicate. This assay used a rabbit polyclonal antibody specific for 8-iso-PGF to bind competitively to either 8-iso-PGF in the sample or to 8-iso-PGF covalently attached to alkaline phosphatase. After a simultaneous incubation at room temperature, the excess reagents were washed away and p-nitrophenyl phosphate substrate was added. The enzyme reaction was stopped with an acid stop solution, which converted the end point color to yellow. The intensity of the bound yellow color is inversely proportional to the concentration of 8-iso-PGF in either the standards or samples. The absorbances were read on a microplate reader at 405nm.

Measurement of Antioxidant Defenses

Serum levels of SOD activity were quantified using an assay kit from Cayman Chemical (Ann Arbor, MI). All standards and samples were assayed in duplicate. The kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD activity was defined as the amount of enzyme needed to exhibit a 50% dismutation of the superoxide radical. The assay measured all three types of SOD (Cu/Zn-, Mn-, and Fe-SOD). After the final addition of xanthine oxidase and a short incubation period, the plate was read at 450nm. After obtaining the absorbance reading, a linearized rate was calculated for all samples and standards, and this was plotted as a function of the final SOD activity, to obtain the standard curve. SOD activity for each sample was calculated from the equation for the linear regression of the standard curve.

Statistical Analysis

Patient descriptive characteristics were analyzed using a between groups t-test. A 2 X 2 repeated measures ANOVA (Analysis of Variance) was used to analyze inflammatory marker, oxidative stress, and antioxidant data for significant group by time interactions. All statistical analyses were performed using Microsoft Excel and SPSS statistical programs. The alpha level was set at $P \leq 0.05$ for statistical significance.

CHAPTER 4 RESULTS

Measurement Design

A total of 15 HTR completed the study. Four control and one intervention HTR withdrew from the study due to post-operative clinical complications. Five HTR completed the control protocol consisting of standard care, while ten HTR completed the resistance-training protocol. For the remainder of this chapter, the baseline measurements taken at 2 months post-transplant will be referred to as T1, and the measurements taken at 8 months post-transplant will be referred to as T2.

Subject Characteristics

Descriptive characteristics of subjects were measured at T1 and the results are shown in Table 4-1. There were no statistically significant ($p \geq 0.05$) differences in subjects assigned to the two groups at T1.

Table 4-1. Descriptive Characteristics

	Control (n=5)	Resistance Training (n=10)
Age (yr)	56.4±8.8	50.1±9.9
Gender	5 M, 0 F	10 M, 0 F
Height (cm)	177.8±6.5	176.4±5.6
Weight (kg)	88.3±6.6	80.4±11.1
Body Mass Index (kg/m ²)	27.9±1.4	25.9±3.6

Values are mean ± SD.

Markers of Inflammation

All measurements of inflammatory markers were made in the serum or plasma of subjects in both groups. Specifically, CRP and TNF- α were measured in serum samples,

while IL-6, sTNFRI and sTNFRII measurements were made in plasma samples. There were no statistically significant ($p \geq 0.05$) differences between the Control and Resistance Training groups at T1. Results are shown in Table 4-2. IL-6 was significantly ($p \leq 0.05$) reduced by 6 months of resistance training (Figure 4-5). There were no significant differences ($p \geq 0.05$) between time points T1 and T2 in CRP (Figure 4-1), TNF- α (Figure 4-2), TNFRI (Figure 4-3), or TNFRII (Figure 4-4) for either the Control or Resistance Training groups.

Table 4-2. Markers of Inflammation

	Control (n=5)		Resistance Training (n=10)	
	T1	T2	T1	T2
CRP (mg/L)	6.301 \pm 8.818	5.215 \pm 7.682	2.938 \pm 1.950	3.261 \pm 2.296
TNF- α (pg/mL)	1.826 \pm .584	1.588 \pm .246	1.276 \pm .481	1.458 \pm .466
TNFR1 (pg/mL)	2466 \pm 1199	2356 \pm 588	2127 \pm 807	1835 \pm 706
TNFR2(pg/mL)	3332 \pm 994	3126 \pm 575	2783 \pm 592	2775 \pm 355
IL-6 (pg/mL)	7.149 \pm 11.883	9.641 \pm 10.860	3.227 \pm 1.945	1.963 \pm 1.054*

Values are mean \pm SD. * $p \leq 0.05$ vs. T1. CRP = C-Reactive Protein, TNF- α = Tumor Necrosis Factor-alpha, TNFR1 = Tumor Necrosis Factor Receptor Type 1, TNFR2 = Tumor Necrosis Factor Receptor Type 2, IL-6 = Interleukin-6.

Markers of Oxidative Stress and Antioxidant Enzymes

All measurements of oxidative stress and antioxidant enzymes were made in the serum or plasma of subjects in both groups. Specifically, SOD activity was measured in serum samples, while 8-iso-PGF measurements were taken in plasma samples. There were no statistically significant ($p \geq 0.05$) differences between the Control and Resistance Training groups at T1. Results are listed in Table 4-3. There were no significant differences ($p \geq 0.05$) in 8-iso-PGF (Figure 4-6) or SOD (Figure 4-7) from T1 to T2 for the Control or Resistance Training groups.

Table 4-3. Markers of Oxidative Stress and Antioxidant Enzymes

	Control (n=5)		Resistance Training (n=10)	
	T1	T2	T1	T2
8-iso-P (mg/L)	1207±530	1232±545	1096±531	1129±328
SOD (U/mL)	1.558±.516	1.729±.354	1.958±.607	2.262±.287

Values are mean ± SD. 8-iso-P = 8-iso-Prostaglandin F_{2α}, SOD = Superoxide Dismutase.

NS = Not Significant

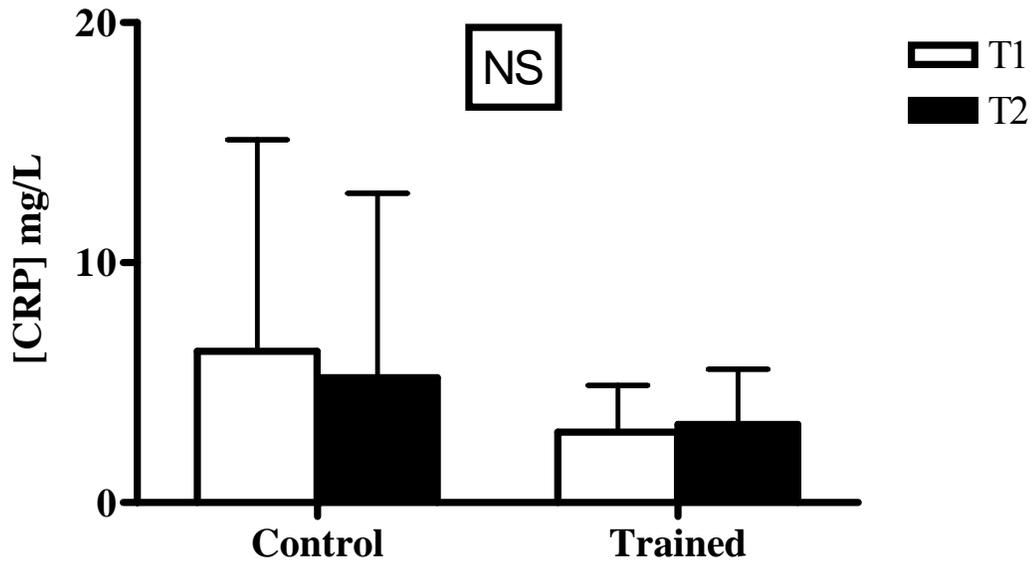


Figure 4-1. C-Reactive Protein. Values are means ± SD.

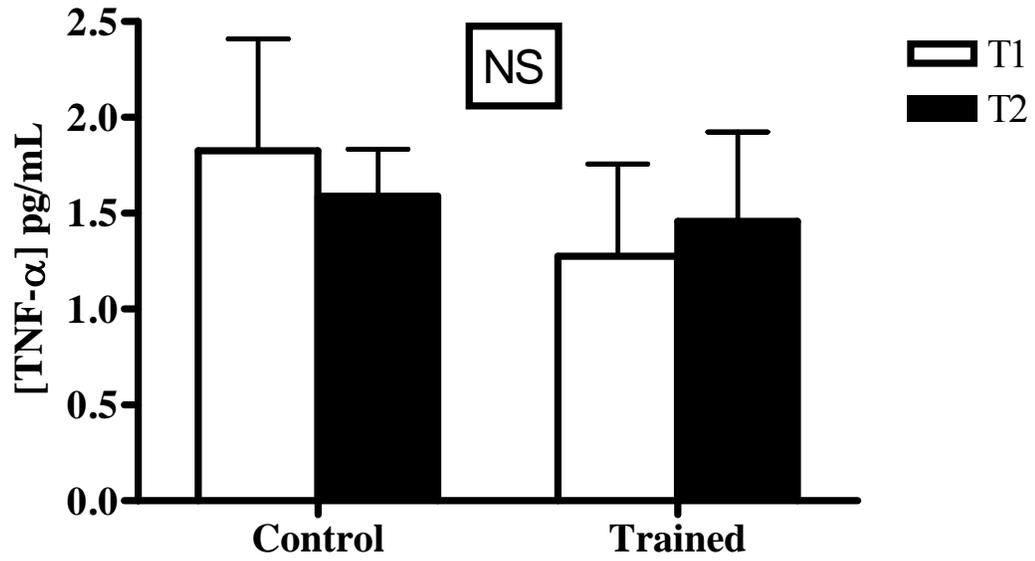


Figure 4-2. Tumor Necrosis Factor- α . Values are means \pm SD.

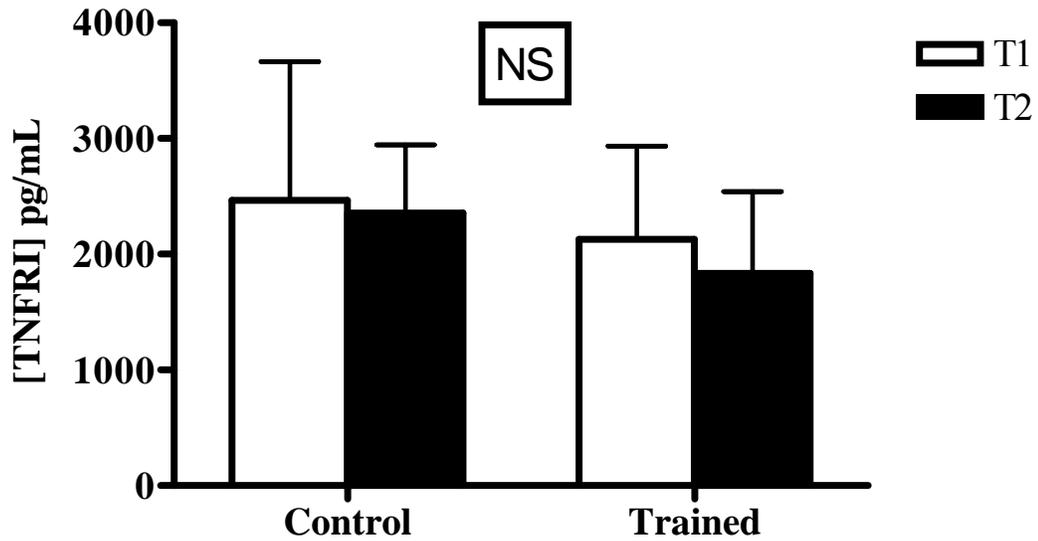


Figure 4-3. Tumor Necrosis Factor Receptor Type I. Values are means \pm SD.

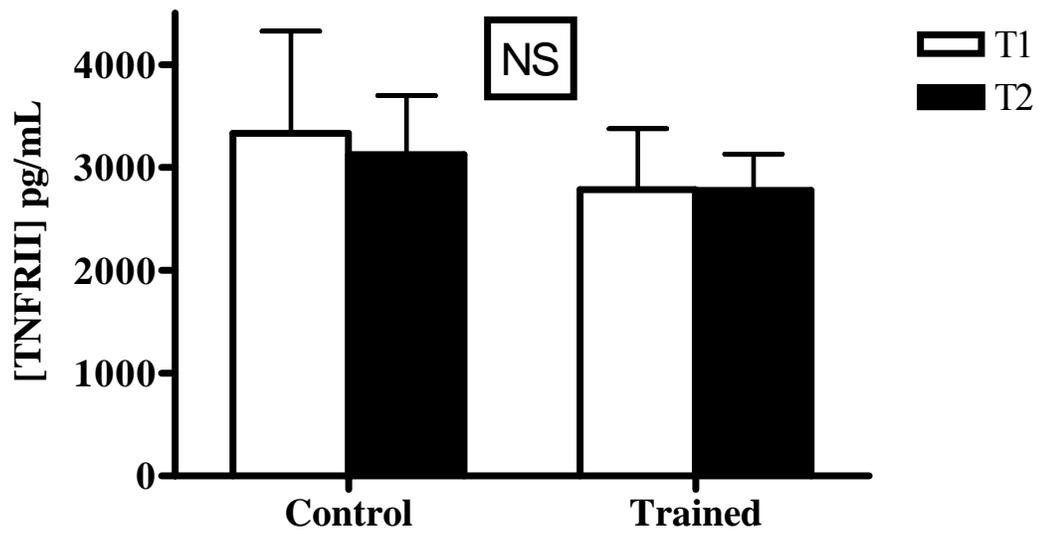


Figure 4-4. Tumor Necrosis Factor Receptor Type II. Values are means \pm SD.

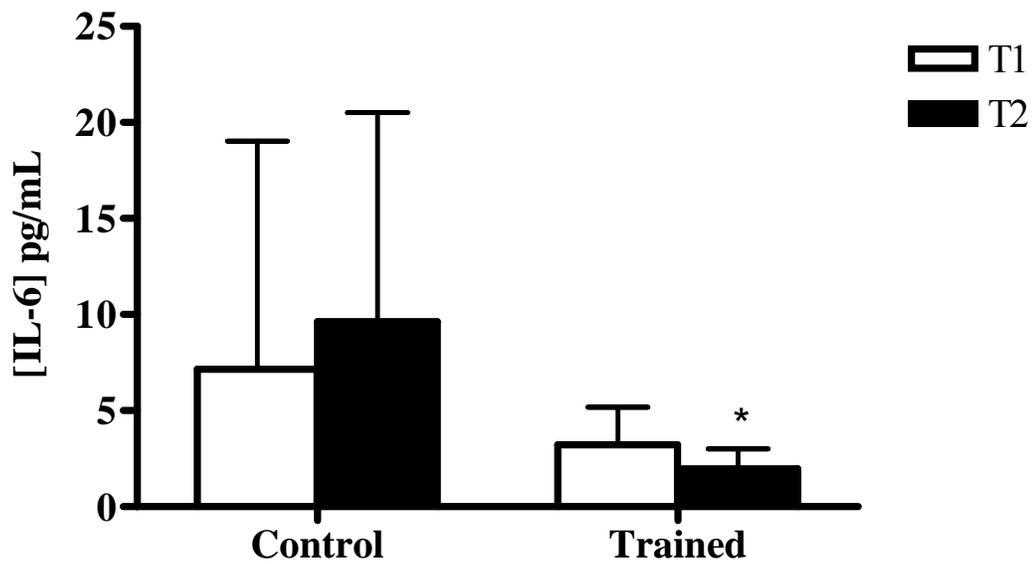


Figure 4-5. Interleukin-6. Values are means \pm SD. * $p < 0.05$ vs. T1.

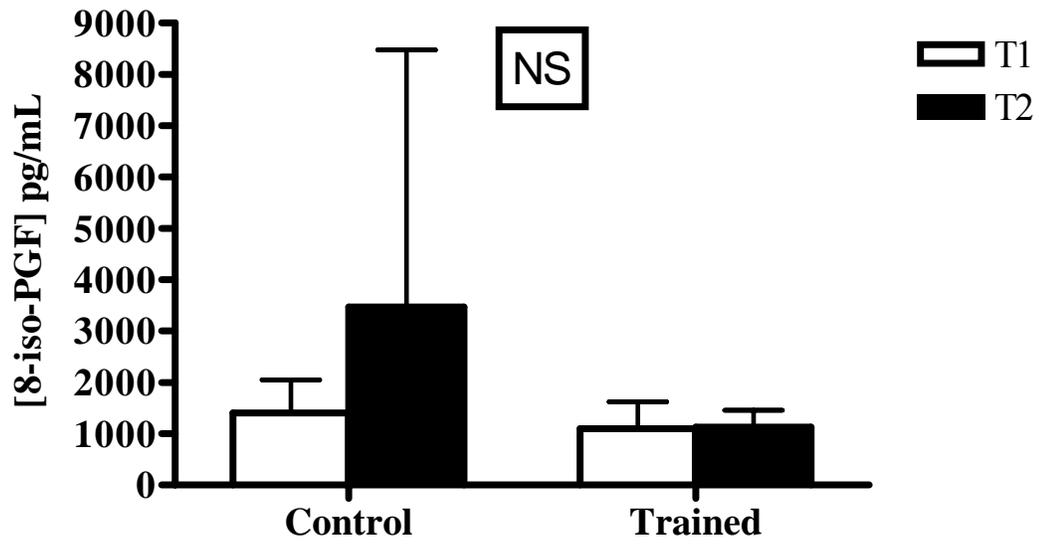


Figure 4-6. 8-iso-Prostaglandin- $F_{2\alpha}$. Values are means \pm SD.

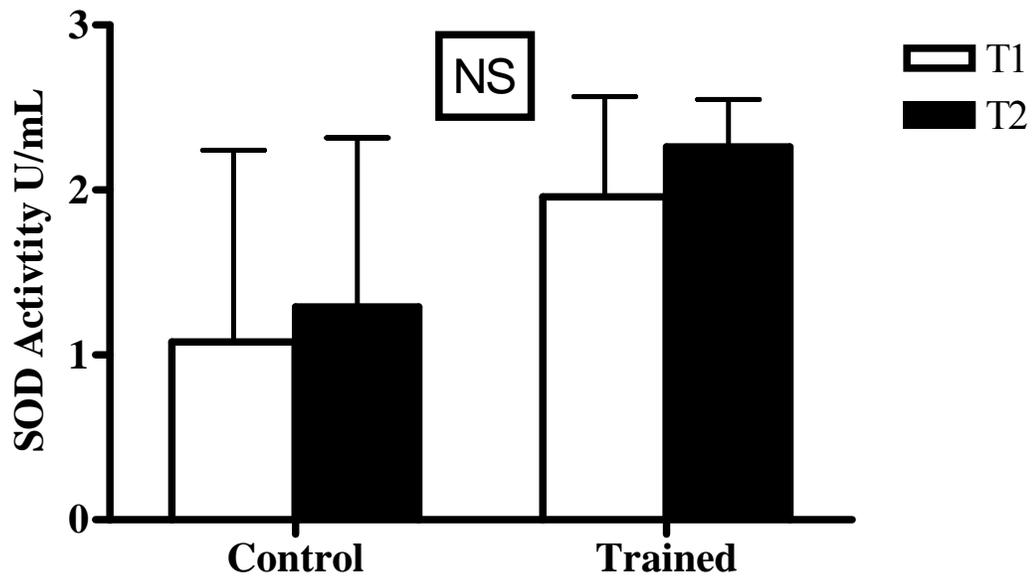


Figure 4-7. Superoxide Dismutase Activity. Values are means \pm SD.

CHAPTER 5 DISCUSSION

Overview and Principal Findings

This was the first prospective study to evaluate the effects of a supervised, 6-month progressive resistance-exercise program on markers of inflammation, markers of oxidative stress, and antioxidant defenses in HTR. Given the positive outcomes of various resistance-training studies on bone loss[37] and skeletal muscle ultrastructure[11,103] in HTR, we decided to test the following three hypotheses:

1. HTR who participate in a six-month, progressive resistance-training protocol will show decreased resting levels of CRP, TNF- α , TNFR, and IL-6 compared to those HTR receiving only standard care.
2. HTR who participate in a six-month, progressive resistance-training protocol will show upregulated resting levels of the antioxidant enzyme SOD compared to those HTR receiving only standard care.
3. HTR who participate in a six-month, progressive resistance-training protocol will show decreased resting levels of 8-iso-prostaglandin-F_{2 α} (8-iso-PGF) compared to those HTR receiving only standard care.

The data from the study incompletely support hypothesis I. Our data do not support either hypothesis II, or III. The major findings of this study are that a 6-month, 2-day/week resistance-training intervention did not alter basal levels of the inflammatory markers CRP, TNF- α , and TNFR in a small cohort of HTR. Also, the resting levels of SOD and 8-iso-prostaglandin-F_{2 α} were unaltered. However, the resistance training intervention did succeed in decreasing resting levels of IL-6.

Markers of Inflammation

It is well established that inflammation plays a key role in the progression of disease states. Evidence exists that suggests resistance training can lead to alterations in inflammatory markers in subject populations other than HTR, such as: the elderly[32], CHF[33], CAD patients[33], and chronic kidney disease patients[34]. Our data regarding resistance exercise and its effects on inflammatory markers is semi-congruent with the current literature. Bruunsgaard et al.[35] showed that resistance training for a 12 week period in the elderly produced no change in plasma levels of TNF- α , sTNFR1, or IL-6. Conraads et al.[33] reported that plasma concentrations of TNF- α and IL-6 were unchanged by four months of combined endurance/resistance exercise, but showed that sTNFR1 were lowered in CHF patients and sTNFR1 and sTNFR2 were lowered in CAD patients. Casteneda et al.[34] found that a 12-week resistance training program decreased levels of CRP and IL-6 in patients with chronic kidney disease. In the present study, resistance training in HTR decreased levels of IL-6, but failed to alter TNF- α , sTNFR1, sTNFR2, or CRP.

IL-6 serves as one of the most important mediators of the acute phase response: a conserved cascade of reactions that is stimulated by tissue damage or inflammation, which serves to prevent further damage and initiate tissue repair processes[46]. The acute phase response leads to the production of immuno-regulating cytokines, which trigger CRP release from liver hepatocytes in attempt to neutralize infection. However, toxic concentrations of these cytokines on a systemic level, as seen with many etiologies of heart disease, can lead to further progression of clinical symptoms. Thus, it is relevant to investigate non-pharmacological adjunctive therapies, such as endurance and/or

resistance exercise, that may reduce the global, toxic concentrations of pro-inflammatory cytokines.

It is unclear why CRP levels in the present study were not reduced concurrent with the observed reduction in IL-6. Increased hepatic production of CRP is believed to be regulated by pro-inflammatory cytokines such as TNF- α and IL-6, as mentioned previously. Although only IL-6 was altered significantly in this study, there are a few mitigating circumstances that may have influenced the data. First and most importantly, the HTR were immunosuppressed with cyclosporine A (CsA) and tacrolimus (TAC), which are the most commonly used immunosuppressive agents used to block T-cell proliferation by inhibiting the phosphatase activity of a calcium-activated enzyme called calcineurin at nanomolar concentrations[104]. Both drugs reduce expression of several cytokine genes that are normally induced following T cell activation, including IL-2, whose synthesis by T lymphocytes is an important growth signal for T-cells[104]. Therefore, it is possible that the training effect on CRP, TNF- α , and TNFRII may have been masked by the effects of calcineurin inhibitors on pro-inflammatory cytokines. Secondly, blood volume in HTR is highly expanded (range = 5-15%)[105,106]. Thus, some subjects' serum and plasma samples could have been more highly diluted in comparison to others, leading to errors in analysis of true circulating levels of the measured markers. The present study was not designed to measure plasma volume. In support of these theories, several studies have reported higher resting levels of pro-inflammatory cytokines than those reported in this study. Resting levels of TNF- α reported by Conraads et al.[33] in patients with CHF (3.5 pg/mL; 1.33-7.2), CAD (3.7 pg/mL; 1.8-7.2), and IDCM (3.1 pg/mL; 1.3-5.5), were higher than those reported in the

present study. Resting values of TNF- α reported by Larsen et al.[47] in CHF(28.7 ± 18.5 pg/mL) were higher than those reported in the present study. Resting levels of TNF- α (2.72 ± 1.5 pg/mL) and IL-6 (31 ± 37 pg/mL) reported by Niebauer et al.[107] in CHF patients were higher than those in the present study.

Oxidative Stress and Antioxidants

This is the first study to evaluate the effects of progressive resistance-training on oxidative stress and antioxidant parameters, in HTR. The data in this study suggest that a 6-month resistance-training program consisting of a single set of eight exercises performed twice weekly does not alter resting levels of 8-iso-PGF, or resting levels of the antioxidant enzyme SOD in HTR. Although many studies have reported the ability of endurance exercise to decrease basal levels of oxidative stress[25-28] and increase basal levels of antioxidant enzymes[24,28,96,99,108], few have analyzed the effects of resistance training on oxidative and antioxidative parameters. Vincent et al.[30] reported that a 6-month resistance-training regimen in healthy elderly, aged 60-83 years, reduced resting levels of lipid peroxidation in light- (50% 1RM) and heavy-resistance (80% 1RM) groups. However, exercise volume in this study was higher (12 exercises, 3 days/week) than the volume in the present study. Ramel et al.[31] reported transient increases in plasma antioxidants and lipid peroxidation in healthy, young males after an acute bout of resistance training. However, because this study did not evaluate the chronic effects of a resistance-training regimen, the results are not pertinent to the current study.

We speculate that resistance training twice a week may not provide sufficient stimulus to induce a change in these parameters. It is possible that the mechanisms

leading to SOD production (and therefore decreased oxidative stress) require a higher training frequency and/or training volume for adaptation to occur.

Conclusions

Our data demonstrate that a progressive, 6-month resistance-training regimen may serve as a viable method to reduce some markers of inflammation in the HTR population. However, the regimen utilized did not alter the oxidative stress/antioxidant balance. Antagonistic treatment of inflammatory markers in HTR, whether through exercise or pharmacologic inhibitors, may augment patient functionality and help prevent episodes of rejection. When drawing conclusions about the effects of exercise on immune system parameters, it is important to be specific as to the type, intensity, and duration of exercise, the timing of sample collections, sample preparation, and the study population involved. Failure to do so can lead to conflicting results, and confusion in the literature regarding the impact of exercise on immunity.

Limitations

A limitation of this study was that the number of patients investigated was small. Prospective and longitudinal studies involving adherence to a supervised exercise regimen involve considerable patient burden and limit enrollment. Another limitation is that time of sample collection was not standardized. This could have affected data concerning circulating levels of cytokines. Lastly, samples were not stored in an antioxidant. Thus, measured markers of inflammation and oxidative stress could have become oxidized, changed conformation, and rendered altered data as to their true levels.

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BIOGRAPHICAL SKETCH

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