ENDOTHELIAL DYSFUNCTION AND ARTERIAL STIFFNESS IN HEART TRANSPLANT RECIPIENTS

By

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This dissertation is dedicated to my wife Cathy. Her unwavering support, patience, and unconditional love for the past 4 years has made this accomplishment the most rewarding of my life. I am happy I could share it with the woman I love. I want to thank her for being a remarkable friend, spouse, career woman, and caring and dedicated mother to our daughter Carolyn.

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Heart transplantation (HT) has become a life-extending intervention for patients with end-stage heart failure (HF). However, frequent complications after HT, such as hypertension and coronary artery vasculopathy (CAV), have been linked to vascular endothelial dysfunction (VED) and arterial stiffness (AS), and jeopardize the long-term survival of heart transplant recipients (HTR). Recent studies suggest that endurance exercise training modifies VED and AS in chronic HF and hypertensive individuals. Therefore, the purpose of this study was to investigate the effects of HT on VED and AS in end-stage HF patients, and the effects of endurance exercise training early after HT.

Twelve (n=12) end-stage HF patients awaiting orthotopic HT at Shands Hospital at the University of Florida were recruited in the observational part of the study. Peripheral endothelial function, AS, and plasma vasoactive balance, lipid peroxidation, antioxidant enzyme activity, inflammation and nitric oxide (NO) inhibition were measured before and 8 weeks after HT. HTR were randomly assigned to an exercise

training group (TRAINED; n=9) who performed 12 weeks of supervised endurance exercise training beginning at 8 weeks post-HT, or to a non-exercise control group (CONTROL; n=7). All vascular and plasma measurements were performed before and after 12 weeks.

Brachial artery flow-mediated dilation (+49.5%) and calf (+34.8%) hyperemic blood flow (BF) improved after HT. Superoxide dismutase activity (SOD), C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), and soluble intercellular adhesion molecule (sICAM-1) decreased significantly ($p<0.05$) 17, 23, 63, and 35% after HT, respectively. In the exercise training study, brachial artery FMD did not change in TRAINED, however there was a significant decrease in FMD (-29%) in CONTROL after 12 weeks. Peak forearm (+34%) and calf (+17%) hyperemic blood flow increased significantly in TRAINED, but did not change in CONTROL. Furthermore, there was a significant increase in TNF-α (+53%) in CONTROL, but no change in TRAINED after 12 weeks.

We found that HT improved peripheral endothelial function and partially reduced the hyperinflammatory state in end-stage HF subjects. Furthermore, endurance exercise training attenuated a progressive decline in brachial artery endothelial function, improved endothelial function of limb resistance vasculature, and attenuated a progressive increase in TNF-α in HTR.

CHAPTER 1
INTRODUCTION

Heart transplantation (HT) has become a life-extending intervention for patients with end-stage heart failure (HF). Although HT reverses many of the primary symptomatic and physiological derangements of the chronic HF syndrome, lifelong immunosuppressive therapy and cardiac denervation result in *de novo* physiological and clinical sequelae that jeopardize long-term survival of the heart-transplant recipient (HTR). Post-transplant hypertension and coronary artery vasculopathy (CAV) may be the most frequent complications in HTR surviving greater than one year (Ventura et al. 1997; Davis et al. 1996; Hollenberg et al. 2001), and have been linked to vascular endothelial dysfunction (VED) and arterial stiffness (Davis et al 1996; Hollenberg et al. 2001; Schofield et al. 2003).

One of the earliest events in the pathophysiology of cardiovascular disease is VED. VED is associated with traditional risk factors for cardiovascular disease before manifestations of clinical atherosclerosis, and is a key contributor in the progression of advanced symptomatic disease. VED refers to a pathological phenotype of the vascular endothelium including pro-inflammatory and thrombotic properties, enhanced platelet aggregation, impaired inhibition of vascular smooth-muscle growth, and impaired endothelial-dependent vasodilation (EDV) (Cai and Harrison 2000). Clinically, impaired EDV of the coronary (Halcox et al. 2002; Suwaidi et al. 2000) and peripheral (Gokce et al. 2003; Heitzer et al. 2001) circulation are independent predictors of adverse cardiovascular events in individuals with (or at risk for) cardiovascular disease.

Physiologically, impaired EDV of coronary arteries may contribute to impaired myocardial perfusion and myocardial ischemia (Halcox et al. 2002), whereas impaired EDV of peripheral conduit and resistance arteries may contribute to increased vascular resistance (Hambrecht et al. 2000), large-conduit artery stiffness (Wilkinson et al. 2004), and reduced exercise muscle blood flow during exercise (Kao 1994). Thus, pharmacological or non-pharmacological interventions that preserve endothelial function should be a major therapeutic goal for individuals at risk for cardiovascular disease.

EDV occurs from the release of endothelial-derived vasodilators including nitric oxide (NO), prostacyclin (PGI_2), and endothelial-derived hyperpolarizing factor (EDHF) (Mombouli and Vanhoutte 1999). Of the three vasodilators, endothelial-derived NO has been the most widely studied because impaired synthesis or enhanced degradation of NO plays a key role in VED. NO is synthesized by a 5-electron oxidation of the amino acid L-arginine by the endothelial isoform of nitric oxide synthase (eNOS) enzyme. NO induces EDV via agonist stimulation of a muscarinic receptor on the endothelial membrane, or by a mechanical shear stress-mediated mechanism from increased laminar blood flow along the endothelial wall. One mechanism responsible for impaired EDV is decreased bioavailability of endothelial-derived NO (Vallance and Chan 2001). Cai and Harrison (2000) hypothesized that reduced bioavailability of endothelial-derived NO occurs due to several potential mechanisms. First, decreased synthesis of NO may be due to decreased eNOS gene transcription, increased post-transcriptional degradation of eNOS mRNA, or post-translational modification eNOS enzyme activity. Moreover, increased asymmetric dimethylarginine (ADMA), an endogenous intracellular

competitive inhibitor of eNOS, may also contribute to decreased NO synthesis (Tran et al. 2003).

A second potential mechanism of impaired EDV is increased inactivation of NO by reactive oxygen species (ROS), such as superoxide anion (Cai and Harrison 2000). In states of vascular homeostasis, superoxide is rapidly dismutated to hydrogen peroxide (H_20_2) and water by intracellular Cu/Zn superoxide dismutase (SOD) in the endothelial cytosol (Cai and Harrison 2000). In the vascular wall, superoxide produced is quickly dismutated by the primary extracellular isoform of SOD (ecSOD), which is strategically located on the endothelial membrane between the endothelial and smooth muscle layer (Fukai et al. 2002). However, when excess superoxide overwhelms the primary antioxidant defenses, superoxide reacts quickly with NO to generate the potent oxidant, peroxynitrate (ONOO-), and the metabolite nitrate, both of which have minimal vasodilating properties (Cai and Harrison 2000). In addition, ROS (such as superoxide and ONOO-), inactivate dimethylarginine dimethylaminohydrolase (DDAH), the enzyme that degrades intracellular ADMA, thus increasing ADMA and further inhibiting NO synthesis (Sydow and Munzel 2003).

A third mechanism that contributes to VED and impaired EDV is increased vasoconstrictor peptides endothelin (ET-1) and angiotensin II (ANG-II), which oppose the vasodilator action of NO and promote vasoconstriction (Nickenbig and Harrison 2002). ET-1 is produced by the endothelial cells and other tissues when exposed to cytokines such as TNF-α and the immunosuppressive agent cyclosporine (Bunchman and Brookshire 1991). ANG II is increased in plasma and vascular tissues in diseased

conditions such as acute and chronic HF due to overactivation of the renin-angiotensin system and sympathetic activity to the kidney (Nickenbig and Harrison 2002).

In HTR receiving immunosuppressive therapy, EDV is impaired in the coronary arteries early after HT (Fish et al. 1988; Mills et al. 1992; Davis et al. 1996; Hollenberg et al. 2001), and is an independent predictor of the development of CAV (Davis et al. 1996; Hollenberg et al. 2001) and cardiac death (Hollenberg et al. 2001). In the peripheral vasculature, impaired EDV is present in conduit brachial artery of HTR (Patel et al. 2002; Lim et al. 2003; Saxonhouse et al. 2000), particularly in HTR with antecedant ischemic HF etiology (Patel et al. 2001). EDV of forearm resistance arteries is improved after HT in end-stage HF patients (Sinoway et al. 1988; Kubo et al. 1993; Cavero et al. 1994), however it is currently unknown whether it returns to that of age-matched controls. Physiologically, impaired EDV contributes to increased vascular resistance (Kao et al. 1994), arterial stiffness (Schofield et al. 2002), decreased exercise capacity (Kao et al. 1994), and decreased exercise muscle blood flow in HTR (Kao et al. 1994). Clinically, impaired peripheral EDV may partly contribute to the development of *de-novo* hypertension after HT, which jeopardizes the long-term success of the cardiac allograft (Lim et al. 2002; Caveo et al. 1994).

Impaired peripheral EDV may also contribute to increased stiffness (reduced compliance) of large elastic and muscular conduit arteries (Wilkinson et al. 2004). Increased arterial stiffness of large elastic and muscular conduit arteries increases the amplitude of the forward (incident) pressure wave during LV ejection, and increases pulse wave velocity of reflected pressure waves returning to the ascending aorta. This change in amplitude and timing of forward and reflected pressure waves causes the

reflected waves to return early to the ascending aorta during systole instead of diastole, augmenting aortic systolic pressure. Thus, augmented aortic systolic and pulse pressure due to arterial stiffness of large conduit arteries contributes to increased LV afterload, myocardial oxygen demand, and wasted energy by the heart (Nichols and Singh 2002).

Large-artery stiffness has been reported in primary aging (Tanaka et al. 2000), hypertension (Nichols and Singh 2002), renal failure (Laurent et al. 2003), and chronic HF (Nichols and Pepine 1992; Lage et al. 1994; Mitchell et al. 2001) population. Arterial stiffness is also an independent predictor of adverse cardiovascular events in patients with renal failure (Laurent et al. 2003) and coronary artery disease (Weber et al. 2003). To date, only one cross-sectional study has evaluated arterial stiffness in HTR. Schofield et al. (2002) reported that that 82% of 53 HTR had an elevated aortic augmentation index, elevated aortic pulse pressure, and a decreased time of reflected wave (inverse of pulse wave velocity), compared to age-matched controls, despite normal brachial systolic and mean blood pressure controlled by antihypertensive medications. Although there are currently no data on the prognostic implications of increased arterial stiffness in HTR, Schofield and colleagues show that HTR may be at increased cardiovascular risk despite having optimally managed brachial blood pressure.

Chronic cyclosporine therapy in HTR results in increased ROS in the vascular wall (Dietrich et al. 1994) and elevated circulating ET-1 and ANG II (Lerman et al. 1992; Haas et al. 1993;Grief et al.1993; Perez-Villa et al.2004). ET-1 has been implicated in the development of post-transplant hypertension and vascular remodeling of smooth muscle. ANG II is elevated secondary to chronic cardiac denervation (Braith et al. 1996; Perez-Villa et al 2004) and cyclosporine-induced overactivation of the renin-angiotensin

system (Julien et al. 1993). ANG II is a potent vasoconstrictor and stimulates production of ROS in the vascular wall via activation of membrane-bound NADPH oxidase. As such, ANG II contributes to impaired EDV and hypertension in HTR (Nickenbig and Harrison 2002), and has been implicated in the development of coronary artery vasculopathy in HTR (Yousufuddinn and Yamani 2004). Thus, interventions that reduce ANG II and ET-1 levels may have important physiological and clinical benefit in HTR.

Cardiovascular disease has recently gained widespread acceptance as an inflammatory disease. As such, inflammatory mediators C-reactive protein (CRP) and soluble intercellular adhesion molecule (sICAM-1) have been reported to be elevated in HTR (Laberrere et al. 2000) and are strong predictors of coronary artery vasculopathy (Pethig et al. 2000), allograft failure (Eisenburg et al. 2000), and mortality in HTR (Laberrere et al. 2002). Besides being a marker of future cardiovascular outcomes in HTR, in vitro studies have recently confirmed that elevated CRP actively contributes to VED by activating expression of the endothelial adhesion molecule sICAM-1 (Pasceri et al. 2000), and decreasing eNOS mRNA, protein, and bioactivity (Venupogal et al. 2002). Proinflammatory cytokines IL-6 and TNF-α (known hepatic stimulants of CRP) are also elevated in HTR and may be involved in VED in HTR (Katz et al. 1994; Holm et al. 2000; Weis et al. 2001). Thus, interventions that attenuate basal levels of inflammatory mediators in HTR may improve endothelial function.

Rationale for the Study

Alterations in endothelial function in humans were first described by Ludmer et al. (1986) who observed paradoxical vasoconstriction of the left coronary artery in response to acetylcholine in patients with CAD. Hambrecht et al. (2000b) reported that lower

body-dynamic endurance-exercise training improved this paradoxical vasoconstriction of the coronary arteries in response to acetylcholine in patients with CAD, suggesting that lower body exercise training can alter endothelial function systemically. In addition, other studies report that lower body exercise training improves peripheral VED in the upper limbs in individuals with essential hypertension (Higashi et al. 1999), CAD (Edwards 2004a et al.; Walsh et al. 2003), and chronic HF (Linke et al. 2001). This supports the theory of a systemic improvement in endothelial function, since lower body exercise improved upper-body limb vasculature. This theory was further supported by Anderson et al. (1995) who reported that brachial artery flow-mediated EDV is correlated with acetlycholine-induced coronary EDV in patients with CAD (r=0.36). Furthermore, Takase et al. (1998) reported a strong positive correlation between brachial artery flow-mediated EDV and coronary artery flow-mediated EDV (r=0.79) in CAD patients. Such studies suggest that endothelial function testing in the peripheral vasculature of the upper limbs may be a good adjunct method to detect changes in systemic endothelial function in response to a therapeutic intervention.

As mentioned, impaired coronary artery EDV is a strong predictor of coronary artery vasculopathy (Davis et al. 1996; Hollenberg et al. 2001) and cardiac death in HTR (Hollenberg et al. 2001). Therefore, restorating endothelial function (such as with exercise training) should be a major therapeutic goal to potentially decrease long-term cardiovascular risk in HTR. However, there have been no prospective reports in the literature on the modulating effects of an endurance-exercise training intervention on endothelial function in HTR.

Specific Aims and Hypotheses

Our study is the first prospective, randomized, controlled study to investigate the effects of endurance exercise training on endothelial function, arterial stiffness, oxidative stress, and inflammation in HTR. Our study is also the first longitudinal, prospective study on the effects of HT on endothelial function and arterial stiffness in end-stage HF patients, and comparing them to an age-matched healthy control group. Thus, our experiments are novel and will further the understanding of the mechanisms contributing to VED in HTR.

Specific Aim 1: To measure EDV of limb conduit and resistance arteries, systemic arterial stiffness, plasma vasoactive balance, oxidative stress, antioxidant enzyme activity, and endogenous NO inhibition before and 2 months after HT in patients with end-stage HF, and in an age-matched healthy control group.

Hypothesis 1: Brachial artery flow-mediated dilation (FMD), forearm/calf flow mediated vasodilation (peak FBF/CBF), aortic augmentation index (AI_a), plasma nitrate/nitrite (NOx), ET-1, 8-iso-prostaglandin-$F_{2\alpha}$, ($PGF_{2\alpha}$), ecSOD activity, inflammatory cytokines, and ADMA will improve 2 months after HT, but will remain abnormal compared to age-matched healthy controls.

Rationale: Although it is accepted that coronary artery VED exists in HTR (Fish et al. 1988; Mills et al. 1992), data are conflicting on whether endothelial function of peripheral limb vasculature improves in end-stage HF patients after HT. Several studies suggest that endothelial function of brachial artery does not improve after HT in HTR patients of ischemic HF etiology, and that it does improve in those HTR of non-ischemic HF etiology (Patel et al. 2001). Other studies report reduced brachial artery FMD in

HTR compared to controls irrespective of etiology of HF (Saxonhouse et al. 2000; Lim et al. 2002; Schmidt et al. 2002; Cuppoletti et al. 2003). However, there have been no longitudinal studies of brachial artery FMD before and after HT in the same cohort compared to age-matched, healthy controls.

Three studies have evaluated endothelial function of forearm resistance arteries before and after HT (Sinoway et al. 1988; Kubo et al. 1993; Cavero et al. 1994). In a prospective study before and after HT, Sinoway et al. (1988) reported that forearm EDV in response to reactive hyperemia did not improve within several weeks of HT, but improved by 4 months. Kubo et al. (1993) reported that EDV in response to reactive hyperemia in forearm resistance arteries improved 4 months after HT. Cavero et al. (1994) reported an increase in forearm EDV 24-36 hours after HT, and then no change 1 week and 6 weeks after starting cyclosporine therapy. However, these studies did not have an age-matched, healthy control group for comparison, so it is unclear whether endothelial function in peripheral resistance arteries HTR was restored to normal. Furthermore, no studies have investigated the effects of HT on systemic arterial stiffness in end-stage HF patients, or on the potential mechanisms involved in VED such as oxidative stress, enzymatic antioxidant capacity, NO inhibition, and inflammation.

Specific Aim 2: To measure EDV of limb conduit and resistance arteries, systemic arterial stiffness, plasma vasoactive balance, oxidative stress, antioxidant enzyme activity, and endogenous NO inhibition in HTR before, and after 12 weeks of supervised endurance-exercise training or a 12-week control period.

Hypothesis 2: In HTR, 12 weeks of supervised endurance exercise training will increase brachial-artery FMD, peak forearm and calf BF, plasma NOx, and ecSOD activity; and decrease aortic AI, plasma ET-1, 8-iso-$PGF_{2\alpha}$, and ADMA.

Rationale: Several studies have reported that exercise training improves EDV in both conduit (Hambrect et al. 1998; Linke et al. 2001) and resistance arteries (Katz et al. 1997) in chronic HF patients, and that the improvement is NO-mediated. In a design similar to ours, subjects with essential hypertension, Higashi et al. (1999) reported a 22% increase in peak FBF during reactive hyperemia using plethysmography, after a 12 week exercise training intervention. The increase in FBF was abolished by the NO inhibitor, L-NMMA, suggesting that the increase in EDV of forearm resistance arteries was NO mediated. In HTR, one cross-sectional study has investigated the effects of exercise on endothelial function. Schmidt et al. (2002) reported higher brachial artery FMD in exercise-trained HTR than sedentary HTR. No prospective studies have yet reported the effects of endurance-exercise training on either peripheral-conduit or resistance-artery endothelial function in HTR.

Cross-sectional studies suggest that exercise capacity is positively associated with increased arterial compliance of large arteries in healthy older individuals (Vaitkevicius et al. 1993) and persons with dilated cardiomyopathy (Bonapace et al. 2003). Several prospective studies report that exercise training decreased arterial stiffness in sedentary young (Cameron et al. 1994), sedentary aged (Tanaka et al. 2000), individuals with CAD (Edwards et al. 2004), and chronic HF (Parnell et al. 2002). However, no prospective studies have reported the effects of endurance-exercise training on arterial stiffness in HTR.

Endurance-exercise training increased aortic expression of eNOS mRNA and eNOS protein in both animal (Sessa et al. 1994, Fukai et al. 2000; Woodman et al. 1999) and human models (Hambrecht et al. 2003). Concominant increases in agonist-mediated EDV suggest an increased NO synthesis after chronic exercise training. Plasma NOx, the stable end product of NO metabolism in plasma, also is increases after 8 weeks of exercise training in healthy humans (Jungersten et al. 1997; Maeda et al. 2001) and in CAD patients (Edwards et al. 2004a). Together, these data support the hypothesis of increased systemic NO synthesis after chronic exercise training.

ET-1 is elevated in HTR due to chronic exposure of the endothelium to cyclosporine and may contribute to hypertension and impaired EDV in HTR (Haas et al. 1993; Greiff et al. 1993). Endurance exercise training reduces reduce plasma ET-1 levels in young, healthy subjects (Maeda et al. 2001), and in older women (Maeda et al. 2003), but not in chronic HF patients (Callaerts-Vegh et al. 1998). Plasma ET-1 levels are also inversely correlated to increased plasma NOx after exercise training in young healthy subjects, suggesting that NOx has a modulating effect on ET-1 levels (Maeda et al. 2001). However, the effects of endurance exercise training on circulating ET-1 levels in HTR has not been investigated.

The F_2 isoprostane isomer, 8-iso-prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$), a stable and specific marker for *in vivo* oxidative stress-induced lipid peroxidation, can be measured in plasma or as its metabolite in urine (Roberts and Morrow 2000). Plasma or urinary levels of 8-iso-$PGF_{2\alpha}$), are elevated in patients with cardiovascular risk factors such as smoking, hypercholesterolemia, diabetes (Pratico 1999; Patrono and Fitzgerald 1997), coronary artery disease (Vassalle et al. 2003), and chronic HF (Polidori et al. 2004). Also, 8-iso-

$PGF_{2\alpha}$ has biological vasoconstrictor action (Roberts and Morrow 2000) and is an independent predictor for development of cardiovascular disease (Schwedhelm et al. 2004). Furthermore, Edwards et al. (2004a) found that 12 weeks of endurance exercise training reduced plasma levels of 8-iso-$PGF_{2\alpha}$, and increased plasma NOx and ecSOD activity in patients with CAD. However, no prospective study has yet reported the effects of endurance exercise training on plasma levels of 8-iso-$PGF_{2\alpha}$, NOx, and ecSOD activity in HTR. Moreover, no human studies have tested the effects of endurance exercise training on ADMA. We hypothesize that by attenuating production of ROS and preventing inactivation of DDAH, exercise training may lower ADMA levels and thus preserve NO synthesis.

Specific Aim 3: To measure plasma levels of inflammatory cytokines in HTR before and after 12 weeks of supervised endurance-exercise training or 12 week control period.

Hypothesis 3: In HTR**,** 12 weeks of supervised endurance-exercise training will decrease plasma levels of CRP, TNF-α, IL-6, and sICAM-1.

Rationale: In addition to being markers of future cardiovascular risk, experimental evidence shows that CRP, TNF-α, and sICAM-1 actively contribute to VED (Blake and Ridker 2003). Several prospective exercise-training studies have tested the effects of endurance-exercise training on inflammatory mediators in patients with CAD (Milani et al. 2004; Edwards 2002) and chronic HF (Larsen et al. 2001; Adamopoulos et al. 2003). Milani et al. (2004) reported a 41% decrease in CRP in a cohort of 277 CAD patients who completed 12 weeks of exercise training as part of cardiac rehabilitation. Edwards et al. (2002) reported that 12 weeks of exercise training as part of cardiac rehabilitation

(in patients with CAD) lowered CRP by 45% and IL-6 by 32%. In 28 patients with chronic HF, Larsen et al. (2001) reported a 12.5% decrease in TNF-α but no change in IL-6. Furthermore, in 24 chronic HF patients, Adamopoulos et al. (2003) reported that 12 weeks of endurance exercise training significantly lowered IL-6, TNF-α, sVCAM-1 and sICAM-1. However, no prospective studies have tested the effects of endurance-exercise training on inflammatory mediators in HTR.

CHAPTER 2
REVIEW OF LITERATURE

Before the 1980s, the endothelial layer of the vasculature was believed to be a physiological inert layer of epithelial cells acting as a barrier between the blood and medial layer of the vascular wall. This changed with Furchgott and Zawadzki's (1980) discovery that vasorelaxation of vascular smooth muscle cells in response to acetylcholine is dependent on an endothelial-derived relaxing factor released from endothelial cells. They reported that if the endothelial layer was removed from rabbit aorta, the vessel vasoconstricted in response to acetylcholine, but its vasodilatory response to nitrates was preserved. In the late 1980s, this endothelial-relaxing compound was discovered to be the free radical gas, NO (Palmer et al. 1987; Ignarro et al. 1987). Furthermore, over the last decade it has been discovered that the endothelial layer is not inactive, but is intimately involved in regulating vascular tone and homeostasis.

NO has proven to be a critical component of vascular health such that the decreased bioavailability of NO results in the phenomenon of ED (Cai and Harrison 2000). ED is present in individuals with primary cardiovascular risk factors such as hypercholesterolemia, hypertension, diabetes, and obesity; and with primary aging and in those with documented cardiovascular disease (Drexler 1997). As mentioned, VED develops years before clinical evidence of atherosclerosis develops and remains evident in individuals with occult cardiovascular disease (Drexler 1997). In particular, VED is evident in individuals with both ischemic and non-ischemic HF (Kubo et al. 1991; Patel et al. 2001), and is believed to partly contribute to increased peripheral vascular

resistance (Hambrecht et al. 2000), arterial stiffness (Arnold et al. 1991), and impaired muscle-blood flow and exercise capacity in HF patients (Hambrecht et al. 1998; Linke et al. 2001). Furthermore, both coronary and peripheral ED persists in end-stage HF patients who undergo orthotopic HT (Fish et al. 1988; Mills et al. 1992; Patel et al. 2001; Schmidt et al. 2003), however, the mechanisms have not been elucidated.

Normal Endothelial Function

Nitric Oxide

Endothelial-derived NO is synthesized from the amino acid L-arginine which undergoes a five-electron oxidation to NO and L-citrulline by the endothelial isoform of the nitric oxide synthase (eNOS) enzyme (Moncada and Higgs 1993). Since NO has a short biological half-life (3-10 seconds) at physiological pH, and is rapidly oxidized to nitrate (NO_2^-) and then nitrite (NO_3^-) by oxygenated hemoglobin (Moncada and Higgs 1993), the primary biological signaling activity of endothelial NO occurs a short diffusion distance across the endothelial wall into the smooth muscle layer. NO binds to the heme moiety of the enzyme guanylate cyclase activating it to catalyze the conversion of GTP to the second messenger cyclic guanosine 3', 5-monophosphate (cGMP). cGMP mediates vascular smooth muscle relaxation via increase Ca^{+2} extrusion from the smooth muscle cells (Moncada and Higgs 1993).

Nitric Oxide Synthase

There are three isoforms of nitric oxide synthase (NOS): constitutively expressed neuronal NOS (nNOS) and endothelial NOS (eNOS), and the inducible NOS isoform (iNOS). nNOS and eNOS activation are Ca^{+2}-dependent and are located in neurons and vascular endothelial cells, respectively (Mayer and Hemmens 1997). Endothelial cells constitutively express eNOS which is an NADPH-dependent oxygenase that requires the

cofactors tetrahydrobiopterin (BH_4), FAD, and FMN (Mayer and Hemmens 1997). In endothelial cells, eNOS, is located in special invaginations in the cell membrane called caveolae, and is associated with a specialized protein, caveolin, which interacts with signaling proteins, such as eNOS, and inhibits its activity (Feron et al. 1998). Stimulation of endothelial cells by agonists such as acetylcholine or bradykinin, dissociates the caveolin/NOS complex and allows Ca^{+2}/calmdulin complex to bind to NOS and activate NO synthesis (Feron et al. 1998). This compartmentalization of eNOS allows rapid conversion of L-arginine to NO since the y+ transporter for L-arginine is also located in the cell membrane near the caveolae (Harrison 1997).

Mechanism of NO Release

Basal release of NO

There is a continuous basal release of NO from vascular endothelium to maintain resting vascular tone. The first evidence of this was in 1989 by Vallance et al. (1989) who demonstrated that by infusing an inhibitor of eNOS, N^G-monomethyl-L-arginine (L-NMMA), into the brachial artery of the human forearm, there was a dose-dependent reduction in resting blood flow. L-NMMA is a methylated analogue of L-arginine which prevents the synthesis of NO and when systemically infused into experimental animals and humans (Vallance et al. 1989), results in an increase in mean arterial pressure. Thus, these data demonstrate the importance of NO in maintaining tonic peripheral arterial vasodilation and blood pressure in vivo.

Agonist-mediated release of NO

Several substances can stimulate muscarinic receptors on the endothelial membrane and activate eNOS to increase NO synthesis via a Ca^{+2}-dependent mechanism. In particular, acetylcholine, bradykinin, and substance P, can stimulate EDV of resistance

and conduit arteries in humans, which can be partially inhibited by L-NMMA. In addition, other endothelial vasodilators, such as prostacyclin and EDHF may also be involved in EDV but to a lesser extent.

Shear stress-mediated release of NO

Laminar pulsatile flow of blood along the endothelial wall causes a mechanical shear stress which provides the stimulus for both short-term and long-term regulation of eNOS and NO synthesis. *In vitro* studies suggest that specific potassium ion channels respond immediately to increase shear stress and induce increase intracellular calcium within one minute and increase short-term eNOS acitivity and NO synthesis (Cooke et al. 1991). After one hour exposure of endothelial cells to increased shear stress, serine-threonine protein kinase B (Akt) phosphorylates eNOS activating the enzyme and increasing NO synthesis six-fold independent of increase in intracellular calcium (Dimmeler et al.1999). Prolonged exposure of increased shear stress for 24 hours, induces increased eNOS mRNA expression in a dose dependent manner in bovine and human endothelial cells (Uematso et al. 1995), and in isolated soleus feed arteries from rats exposed to increased luminal shear stress (Woodman et al. 2004). Furthermore, human studies suggest that flow-mediated dilation of forearm brachial (Lieberman et al. 1996) and resistance arteries (Meredith et al. 1996) is partially attenuated by the eNOS inhibitor, L-NMMA, suggesting that shear-stress mediated vasodilation is NO dependent.

Pleiotropic Actions of NO

Endothelial-derived NO not only modulates vascular tone, but also has antiatherosclerotic, antithrombotic, and anti-inflammatory functions on the endothelial wall (Vallance and Chan 2001). Specifically, NO suppresses platelet aggregation, leukocyte migration and adhesion to the endothelial wall, and prevents vascular smooth

muscle migration and proliferation into the subendothelial space (Mombouli and Vanhoutte 1999). Thus, the decrease in bioavailability of NO not only promotes an endothelial phenotype of vasoconstriction, but also of platelet aggregation, leukocyte migration and adhesion to the endothelial layer, and smooth muscle migration and proliferation into the subendothelial layer (Mombouli and Vanhoutte 1999).

Other Endothelial Vasodilators

The endothelium also was discovered to release several other vasodilating compounds including prostacyclin and an unknown endothelial derived hyperpolarizing factor (EDHF). The production of prostaglandins is regulated by the availability of membrane-bound arachadonic acid (AA) and the activity of the enzyme cyclooxygenase (COX). AA is derived from the phospholipid membrane which is enzymatically released via action of the enzyme phospholipase A_2 and is converted to prostaglandin H_2 by COX and peroxidase (Savidge 2001). Prostacyclin is the major endothelial metabolite derived from arachadonic acid (AA) and diffuses into vascular smooth muscle and activates the enzyme adneylate cyclase. Adenylate cyclase converts ATP to 3', 5 cyclic adenosine monophosphate (cAMP) and induces vasorelaxation of vascular smooth muscle (Savidge 2001).

Endothelial hyperpolarizing factor (EDHF) is less well characterized but is believed to act through activation of calcium-activated K+ channels on the smooth muscle membrane resulting in hyperpolarization and vasorelaxation (Mombouli and Vanhoutte 1999; Triggle et al. 2004). Although identification of EDHF is still unclear, possible EDHF's include hydrogen peroxide, isoprostanes, potassium, or the AA metabolite, epoxyeicosatrienoic acid (Triggle et al. 2004)

Endothelial Vasoconstrictors

The endothelial wall also secretes vasoconstrictors ET-1, ANG II, and vasoconstrictor prostaglandins which compete with NO for the vasoactive balance.

Endothelin-1

ET-1, the major endothelin isoform, is produced by the endothelium and is derived from the precursor big ET-1 by the enzyme endothelin converting enzyme (ECE). ET-1 can act in a paracrine or autocrine manner via ET type A or ET type B receptors on adjacent endothelial and smooth muscle cells. ET_A and ET_B receptors are on smooth muscle cells and both mediate vasoconstriction, cell proliferation, and hypertrophy. ET_B exist on endothelial cells as well, and mediate vasodilation via release of NO and prostacyclin (Taddei et al. 2001). Although ET-1 is generally considered to be a potent vasoconstrictor, this activity can be attenuated by the increased expression or activity of ET_B receptor mediated release of NO by the endothelium (Taddei et al. 2001). However, systemic infusion of ET-1 into animals results in a decrease in glomerlular filtration rate, renal blood flow, and an increase in mean arterial blood pressure (Goetz et al. 1988). In humans, infusion of an ET-1 receptor antagonist significantly decreased peripheral vascular resistance and blood pressure (Haynes et al. 1996). Taken together, these data support the idea that ET-1's vasoconstrictor activity on the smooth muscle predominate and strongly contributes to basal vascular tone and blood pressure.

Angiotensin II

ANG II is a strong vasoconstrictor peptide that also has direct and indirect salt and water regulatory actions on the kidney (Nickenig and Harrison 2002). ANG II is formed in the circulation when angiotensinogen production is increased via increased adrenergic stimulation release of renin from juxtaglomerular cells in the afferent arteriole of the

kidney. Angiotensinogen is converted to angiotensin I, which is quickly converted to ANG II by angiotensin converting enzyme (ACE). Besides being a potent vasoconstrictor, ANG II stimulates kidney tubule absorption of salt and water directly, as well as indirectly via stimulation of aldosterone from the adrenal cortex. In addition, the endothelial and smooth muscle wall contain a vascular form of ACE which is responsible for production of ANG II in the vascular wall (Nickenig and Harrison 2002). Furthermore, ANG II is major stimulus of NADPH oxidase production of superoxide anion in the vascular wall (Nickenig and Harrison 2002; Cai and Harrison 2000), thus, in conditions of elevated levels of ANG II such as chronic HF, ANG II may be partially responsible for increased vascular oxidative stress.

Vasoconstrictor prostaglandins

As mentioned earlier, the production of prostaglandins is regulated by the availability of AA and the activity of the enzyme COX (Savidge 2001). Under certain pathophysiological conditions, increased thromboxane A_2 is formed from its precursor prostaglandin H_2, both of which bind to endoperoxide/thromboxane receptors on vascular smooth muscle and induce vasoconstriction (Mombouli and Vanhoutte 1999). However, in states of vascular homeostasis, NO, EDHF, and prostacyclin, override any influence of endothelial vasoconstrictors, promote vasodilation and an antithrombotic phenotype of the endothelium. (Mombouliand Vanohoutte 1999). However, when the endothelial-NO pathway is disrupted, the vasoconstrictor, pro-thromobotic, and proinflammatory phenotype of the endothelium prevails.

Vascular Endothelial Dysfunction

Several mechanisms have been implicated in VED including: 1) decreased NO synthesis due to decreased expression or activity of nitric oxide synthase (eNOS); 2)

decreased NO synthesis due to increased competitive inhibition by ADMA; 3) post-translational inactivation of NO by ROS such as superoxide radical; and 4) enhanced production of vasoconstrictor substances such as ET-1, ANG II, and vasoconstricting prostaglandins, which oppose vasodilatory effects of NO.

Decreased NO Synthesis by eNOS

Decreased synthesis of NO can occur via several distinct mechanisms. Pathophysiological factors such as TNF-α, hypoxia, oxidized LDL (Harrison 1999), and reduced blood flow and shear-stress in heart failure (Smith et al. 1996), have been shown to decrease eNOS levels through both transcriptional regulation and post-transcriptional modifications in half-life of eNOS mRNA (Harrison 1999). In contrast, shear stress increases gene transcription of eNOS mostly by transcriptional regulation (Uematsu et al. 1995; Harrison 1999). Thus, although eNOS is constitutively expressed, eNOS undergoes various degrees of expression under different physiological and pathophysiological conditions (Harrison 1999).

NO synthesis can be also can be decreased when eNOS becomes uncoupled due to reduction of the essential eNOS cofactor, BH_4 (Cai and Harrison 2000). Decreased BH_4 results in eNOS transferring electrons to molecular oxygen instead of L-arginine, resulting in increased production of superoxide radical. As such, pathophysiological conditions such as insulin resistance, cigarette smoking, and hypercholesterolemia can cause impaired EDV due to BH_4 depletion, and supplementation with BH_4 restores EDV in these clinical conditions (Vallance and Chan 2001).

Lastly, ADMA, an endogenous inhibitor of eNOS, can also decrease NO synthesis. ADMA is derived from methylation of the side chain nitrogen of arginine residues in

nuclear proteins by enzymes called protein-arginine methyltransferases (PRMTs) (Tran et al. 2003). Specific PRMTs methylate L-arginine residues on intracellular RNA binding proteins in endothelial cells, which are released into the cytoplasm upon normal cellular protein turnover (Tran et al. 2003). DDAH, the enzyme responsible for degradation of ADMA into L-citrulline and dimethylamine (Tran et al. 2003), is responsible for 85-90% of ADMA degradation with only a small amount of ADMA excreted in the urine (Tran et al. 2003). As such, impairment of this DDAH may be a key mechanism for ADMA accumulation under certain pathophysiological conditions (Tran et al. 2003).

Vallance et al. (1992) first described the *in vivo* effects of ADMA on EDV in healthy humans. They infused ADMA into brachial artery of healthy subjects and caused a dose-dependent vasoconstriction that was completely reversed with infusion of L-arginine. In the same study, ADMA was reported to be elevated in chronic renal failure patients which was attributed to reduced renal excretion. However, ADMA has been reported to be elevated in clinical populations with normal renal function including, CAD, peripheral artery disease (PAD), hyperhomocysteinemia, hypercholesterolemia, primary aging (Tran et al. 2003), chronic HF (Usui et al. 1998), and HT (Fearon et al. 2004), suggesting that elevated ADMA must be due to some other mechanism than poor renal function. As such, *in vitro* studies report that DDAH contains a reactive cysteine residue in the active site which can be reversibly inhibited by s-nitrosylation by NO-derived oxidants or via oxidation by superoxide radical (Sydow and Munzel 2003). Additionally, there is some evidence that suggests that ADMA may be a direct mediator of oxidative stress by causing uncoupling of eNOS from oxidation of the eNOS cofactor, BH_4 (Sydow and Munzel 2003). Hence, ROS may contribute to decreased NO

bioavailibility indirectly by inhibiting DDAH activity, thus promoting the accumulation of ADMA, or directly, by enhanced degradation of synthesized NO (Sydow and Munzel 2003).

NO Degradation by Reactive Oxygen Species

Vascular redox homeostasis involving superoxide levels are normally controlled by intracellular superoxide dismutase (SOD) isoforms copper/zinc (Cu/Zn) SOD and manganese (Mn) SOD; and the extracellular isoform of SOD (ecSOD), which is located on the endothelial membrane and in extracellular space between endothelial and smooth muscle cells (Fukai et al. 2002). However, during states of increased production of superoxide, excess superoxide reacts rapidly with NO because this reaction has a rate constant three times faster than with SOD. This results in loss of bioactivity and forming of peroxynitrite anion (ONOO-) which is a potent oxidant and has minimal vasodilating properties (Cai and Harrison 2000).

There are several potential sources of superoxide production in the vascular wall. Membrane-bound NADH/NADPH oxidase is postulated to be the major source of ROS in endothelial and smooth muscle vasculature (Fukai et al. 2002; Cai and Harrison 2000). NADH/NADPH oxidase uses NADH and NADPH as substrates for electron transfer to molecular oxygen. Experimental evidence shows that NADH/NAPDH oxidase activation can occur via stimulation by ANG II and TNF-α, both of which are elevated in HF and HTR's. In vitro studies show that ANG II is a primary stimulus of NADH/NADPH oxidase activity, and in vivo studies report that chronic infusion of ANG II in rats results in increased superoxide production and impaired EDV (Harrison 1999).

Another potential enzymatic source of superoxide production in the vascular wall is xanthine oxidase, which catalyzes conversion of hypoxanthine to xanthine in endothelial and smooth muscle cells (Cai and Harrison 2000). Xanthine oxidase is synthesized as xanthine dehydrogenase and under normal conditions uses NAD^+ as an electron acceptor. However, under pathophysiological conditions such as exposure to TNF-α or ONOO- in endothelial cells, xanthine dehydrogenase is converted to xanthine oxidase (Landmesser et al. 2002). Thus, increased xanthine oxidase activity transfers electrons to molecular oxygen instead of NAD^+, resulting in excess superoxide production and impaired EDV (Harrison 1999). Moreover, supplementation with the xanthine oxidase inhibitor, allopurinol, results in improved EDV in smokers (Guthikonda et al. 2003), diabetics (Butler et al. 2000), and chronic HF patients (Farquharson et al. 2002), suggesting that xanthine oxidase contributes at least, in part, to impaired EDV in these conditions.

Vascular Endothelial Dysfunction before Heart Transplantation

Impaired EDV of peripheral conduit and resistance arteries exists before HT in chronic HF patients (Kubo et al. 1991; Katz et al. 1992; Hornig et al. 1996; Hambrecht et al. 1998; Linke et al. 2001). Kubo et al. (1991) was the first to demonstrate that EDV of forearm resistance vasculature was attenuated in patients with chronic HF in response to a muscarinic agonist, methacholine, compared to healthy controls. The mean increase in forearm blood flow to three dosages of methylcholine using strain gauge plethysmography was significantly attenuated in HF patients. Katz et al (1992) then demonstrated that endothelium-dependent blood flow velocity of conduit femoral artery was attenuated in HF patients compared to healthy controls in response to infusion of

acetylcholine. Taken together, these early studies confirmed that EDV was impaired in both resistance and conduit arteries of HF patients.

The mechanisms for impaired EDV in HF likely include several pathophysiological mechanisms. First, Smith et al. (1996) reported a significant decreased expression of eNOS and COX-1 mRNA and a 70% reduction in eNOS protein in aortas of dogs after 1 month left ventricular pacing-induced HF suggesting regulation at the transcriptional or post-transcriptional level. Second, HF is associated with increased levels of circulating TNF-α (Levine et al. 1990) which, in vitro, post-transcriptionally degrades eNOS mRNA (Yoshizumi et al. 1993). In vivo evidence to support this, Katz et al. (1994) found that elevated TNF-α levels to be highly correlated with impaired forearm EDV in response to acetylcholine. Thirdly, in humans, administration of vitamin C, a known scavenger of superoxide anion radical, reverses impaired radial artery EDV in HF, suggesting that superoxide plays a significant role in endothelial dysfunction in HF patients (Hornig et al. 1999). Excess superoxide production may be due to hyperactivity of the RAAS in HF, which results in increased levels of ANG II via the enzyme angiotensin-converting enzyme (ACE) (Nickenbig and Harrison 2002). However, not only does ANG II and TNF-α stimulate NADPH oxidase production of superoxide anion, but vascular ACE also degrades bradykinin, which stimulates release of NO and endothelial-derived hyperpolarizing factor (EDHF) from the endothelium (Drexler 1997). TNF-α also upregulates ET-1 production, which is also elevated in HF, and therefore competes with endogenous vasodilators and promote systemic vasoconstriction and impairesd EDV. Indeed, ET_A receptor blockade improves EDV of the brachial artery in chronic HF patients (Berger et al. 2001). Taken together, decreased eNOS and COX-1 gene

expression, increased ANG II-stimulated superoxide degradation of NO, and increased vasoconstrictors ET-1 and ANG II likely all contribute to impaired EDV in chronic HF.

ADMA levels are also elevated in chronic HF patients (Usui et al. 1998). Usui et al. (1998) found that chronic HF patients had elevated plasma levels of ADMA and NOx compared to healthy controls. In addition, ADMA and NOx were significantly associated with NYHA functional HF class and NOx inversely associated with ejection fraction ($r=-0.33$, $p=0.004$) (Usui et al. 1998). Moreover, there was a significant positive relationship between plasma ADMA and NOx in only the moderate and severe HF patients ($r=0.41$, $p=0.01$). Thus, the authors suggested that increased NOx may be due to inflammatory cytokine-induced excessive NO production in severe HF, which may have negative inotropic effects on the myocardium. Therefore, increased ADMA may be a compensatory mechanism against hyperactive systemic or myocardial NO synthase activity and NO production.

Mechanisms for increased ADMA in HF are unclear, but may include decreased renal excretion of ADMA due to renal failure, since renal plasma flow and excretion decline as HF progresses. However, Usui et al. (1998) excluded all HF patients with renal dysfunction making this hypothesis unlikely. Finally, as mentioned earlier, it is postulated that increased ROS in endothelial cells in severe HF may contribute to increased ADMA by oxidatively inactivating DDAH, the enzyme that degrades intracellular ADMA. However, the effects of ROS on ADMA in HF patients has not been investigated.

Vascular Endothelial Dysfunction after Heart Transplantation

Fish et al. (1988) were the first to demonstrate that HTR had impaired coronary EDV early after HT. They demonstrated paradoxical vasoconstriction to acetylcholine in

12 of 13 HTR who were 12 months post-HT. Using intravascular ultrasound, Mills et al. (1992) also described vasoconstriction of conduit coronary arteries in response to acetylcholine in HTR who had no evidence of coronary vasculopathy one year after HT. Davis et al. (1996) reported that impaired coronary artery EDV predicts development of coronary allograft arteriosclerosis by one year. Furthermore, Hollenburg et al. (2002) not only reported that impaired coronary EDV is an independent predictor of development of coronary allograft arteriosclerosis but also of cardiac death in HTR. Thus, coronary ED develops early after HT, and provides valuable prognostic information on long-term risk of the cardiac allograft in HTR.

In contrast, similar prognostic data using peripheral endothelial function testing in HTR is not available at this time. However, Anderson et al. (1995) reported that peripheral endothelial function and coronary endothelial function correlate, albeit modestly, and therefore may provide some valuable clinical information in HTR if coronary endothelial function testing is not available. As such, peripheral endothelial dysfunction is present after HT and persists indefinitely (Saxonhouse et al. 2000; Patel et al. 2001; Lim et al. 2002; Schmidt et al. 2002; Cuppoletti et al. 2003). Several cross-sectional studies report that brachial artery FMD is impaired in HTR compared to healthy controls (Saxonhouse et al. 2000; Patel et al. 2001; Lim et al. 2002; Schmidt et al. 2002; Cuppoletti et al. 2003). Saxonhouse et al. (2000) reported that brachial artery FMD in HTR one to seven years post-transplant, was similar to stable class IV HF patients, and was decreased compared to age-matched healthy controls. Patel et al. (2001) compared brachial FMD in a group of ischemic vs. non-ischemic HF patients, to two groups of HTR with antecedent ischemic and non-ischemic HF etiology. Ischemic and non-

ischemic HF patients did not differ in ejection fraction, duration of heart failure illness, total cholesterol, or ACE inhibitor use. Brachial artery FMD was the same in both HF groups (3.6% vs. 5.1%, p=NS), but significantly less than controls (13.9%, $p<0.001$). Ischemic vs. non-ischemic HTR did not differ in time since transplant, duration of pre-transplant heart failure, ejection fraction, cyclosporine levels, lipid lowering therapy, and cardiac risk factors. HTR with ischemic HF etiology had significantly decreased brachial FMD compared to non-ischemic HTR (5.5% vs. 13.0%, p=0.002). FMD in non-ischemic HTR did not differ from healthy, age-matched controls (13.0% vs. 13.9%, p=NS). Thus, this data suggests that EDV of conduit brachial artery is restored after HT in non-ischemic HTR, but not in HTR with ischemic etiology. Although this was not a prospective follow-up of the same patients before and after transplant, this study illustrates that etiology of heart failure can influence endothelial function after HT.

Lim et al. (2002) reported reduced brachial artery FMD in14 young HTR (mean age 18 years) with non-ischemic HF, etiology compared to age- and gender-matched healthy controls (3.0% vs. 15.5%, $p<0.05$). Interestingly, there was no relationship in HTR between impairment in brachial artery FMD and gender, time since transplantation, number of rejection episodes, cyclosporine levels, or presence of hypertension. Thus, this suggests that other factors may contribute to impairment of brachial FMD, however, the small size of the study limits the generalizability of the results.

Schmidt et al. (2002) reported a reduced brachial FMD in sedentary HTR (age 60 ± 6 years) six years post-transplant compared to age-matched sedentary, healthy control group (1.4% vs. 8.4%, $p<0.05$). Lastly, Cuppoletti et al. (2003) measured brachial FMD at one and six months after heart transplant in the same 12 HTR. They reported that 10

of HTR (83%) had a brachial FMD < 4% at one month (0.4% vs. 9.9%, p=0.01), and at six months, brachial FMD remained < 4% in all 10 HTR and as well as the remaining two HTR suggesting that VED persists early after HT.

In a longitudinal study, Sinoway et al. (1988) measured EDV of resistance arteries of forearm using strain-gauge plethysmography during reactive hyperemia following 5 minutes of upper arm occlusion with a blood pressure cuff. They measured EDV before, 18 days after HT, and four months after HT in 10 HTR who had severe HF. Basal forearm blood flow did not significantly increase immediately after transplantation, but increased four months after-transplant. Similarly, peak hyperemic forearm blood flow following arterial occlusion did not increase immediately after transplant, but increased significantly at four months. This suggests that impaired forearm blood flow is not directly related to normalizing cardiac output, but that it is increased after several months probably as a result of resumption of daily physical activities. However, because there was no age- and weight-matched healthy group, it was unknown whether the four month peak blood flow was completely normalized.

In a cross-sectional study and longitudinal design, Kubo and associates (1993) investigated EDV of forearm resistance arteries using measurement of forearm blood flow by strain gauge plethysmography. In the cross-sectional design, forearm blood flow was measured during infusion of the muscarinic agonist metacholine in three doses in a group of HF and HTR. Forearm blood flow at three doses of methacholine was higher in HTR than CHF patients. In addition, they measured EDV in the forearm during reactive hyperemia in both groups as well. Reactive hyperemia forearm blood flow following upper arm occlusion was not statistically different in HTR than HF patients. This data

suggests that the EDV following brief ischemia of forearm resistance vasculature is similar, but agonist-mediated vasodilation is impaired in HF compared to HTR. Furthermore, endothelium-independent vasodilation (EIV) using an NO donor, nitroprusside, showed that smooth muscle vasculature was not different between groups.

In the longitudinal design, forearm blood flow was measured after methacholine and reactive hyperemia in the same six patients before and 4 months after HT. Patient characteristics before and after HT had similar resting forearm blood flow (3.3 ± 1.1 vs. 3.7 ± 1.5 ml/min/100 ml) and forearm vascular resistance (30.5 ± 11 vs. 37 ± 16 U). After HT, HTR had higher blood pressure, cholesterol, and cyclosporine levels, but a lower pulmonary wedge pressure and norepinphrine. Forearm blood flow increased significantly at four months after HTX at each doses of methacholine. Also, reactive hyperemia forearm blood flow increased significantly after HTX from 19.0± 3.7 to 44.8± 6.4 ml/min/100 ml. This data suggests that both agonist-mediated (methacholine) and flow-mediated (reactive hyperemia) forearm vasodilation is increased after HT. However, subjects did not have nitroprusside EIV mediated forearm blood flow measured, so it is not known if this increase in blood flow after transplant was partially mediated by improvement in forearm vascular smooth muscle. Furthermore, it is not known whether this increase in blood flow is normalized since there was no age-matched healthy control group. The discordant results in forearm EDV to reactive hyperemia may be due to the inherent limitations in cross-sectional study and small number of subjects studied.

In a longitudinal study, Cavero et al. (1994) investigated the effects cyclosporine on peripheral vascular EDV in end-stage HF patients before and after HT. Peak FBF

during reactive hyperemia was measured in HF patients 1) before HT; 2) 24-36 hours after HT but before initiation of cyclosporine therapy; 3) 6-8 days after HT in presence of therapeutic cyclosporine levels; and 4) 4-6 weeks post-transplantation on cyclosporine. Forearm blood flow to reactive hyperemia after 10 minutes of upper arm cuff occlusion, increased significantly after HT (11.2 vs. 21.2 ml/min/100 ml, $p<0.05$), but did not change significantly after 6-8 days (22.3 ml/min/100ml), or after 6 weeks (22.7 ml/min/100ml) on cyclosporine therapy. Taken together, this suggests that HT results in an immediate increase in peak forearm blood flow during reactive hyperemia which does not change in the ensuing several weeks. However, there was no age-matched healthy control group to compare to so it is unknown if peak FBF is completely normalized.

Cyclosporine and vascular endothelial dysfunction in heart transplant recipients

Cultured endothelial cells exposed to cyclosporine increase expression of eNOS mRNA (Navarro-Antolin et al. 2000), but also increase production of ET-1 (Bunchman et al. 1991) and superoxide anion levels (Navarro-Antolin et al. 2001). Diederich et al. (1994) reported that pretreatment of mesenteric arteries with SOD, normalized acetylcholine-induced impaired vasodilation in cyclosporine treated rats, suggesting that superoxide was a contributing mechanism for impaired NO mediated vasodilation. Sudhir et al. (1994) showed attenuated acetylcholine-induced vasodilation of epicardial and conductance coronary arteries of dogs treated with cyclosporine. Vasoconstriction induced with L-NAME (NO inhibitor) was exacerbated by cyclosporine suggesting that cyclosporine attenuates release of, or increases degradation of, NO in coronary arteries. Furthermore, human studies show that cyclosporine is associated with increased production of ET-1 (Grief et al. 1993; Lerman et al. 1992), and sympathetic nervous hyperactivity and hypertension in HTR (Scherrer et al. 1990). Thus, taken together these

data suggest that despite increased eNOS expression in endothelial cells, cyclosporine therapy may partly contribute to impaired EDV via increased superoxide anion release, ET-1 production, and increased sympathetic hyperactivity in HTR.

Inflammation and vascular endothelial dysfunction in heart transplant recipients

CRP is an acute phase protein released by liver cells in response to inflammatory cytokines IL-6 and TNF-α. CRP is a strong predictor of cardiovascular events in previously healthy men and women, and in individuals with existing cardiovascular disease (Blake and Ridker 2003). However, recently inflammatory proteins have been suggested as a contributing mechanism to VED by *in vitro* studies which suggest that CRP is directly involved in the development of VED (Pasceri et al. 2000; Venupogal et al. 2002). Venupogal et al. (2002) demonstrated that endothelial cells incubated with CRP decrease expression of eNOS mRNA, eNOS protein, and eNOS bioactivity, and increase expression of vascular adhesion molecules VCAM-1, ICAM-1, and P-selectin (Pasceri et al. 2000). Decreased NO and increased ROS also activate expression of endothelial VCAM-1 and sICAM-1 which initiates an inflammatory response and activate T-lymphocytes, monocytes, and macrophages into the endothelial wall to release proinflammatory cytokines such as IL-6 and TNF-α (Blake and Ridker 2003). Furthermore, Fichtlscherer et al. (2000) reported that elevated CRP correlated inversely with impaired EDV in a cohort of CAD patients, and a reduction in CRP after 3 months was associated with improved EDV.

In HTR, CRP and has been reported to be a strong independent predictor of coronary artery vasculopathy, cardiac allograft failure, and mortality (Pethig et al. 2000; Eisenberg et al. 2000; Labarrare et al. 2002). Holm et al. (2001) found that elevated

levels of IL-6 and TNF-α correlated negatively with acetylcholine-induced peripheral EDV. Moreover, Weis et al. (2001) reported that treatment with simvastatin lowered IL-6 and TNF-α and was associated with improved acetylcholine-induced coronary EDV. Taken together, these in vitro and clinical data suggest that CRP may not solely a marker of future cardiovascular risk in HTR, but may be intimately involved in the development of VED.

Asymmetric dimethylarginine and vascular endothelial dysfunction in heart transplant recipients

Lastly, recent accumulating evidence suggests that a contributing mechanism for VED in HTR may due to elevated intracellular and plasma ADMA. A recent study reported a 200% increase in plasma ADMA levels in HTR compared to healthy controls (Weis et al. 2004) and was slightly more elevated in CMV-positive HTR's (Weis et al. 2004). In addition, in vitro, human endothelial cells infected with CMV, had decreased DDAH activity and produced more ADMA (Weis et al. 2004). Furthermore, a recent study demonstrated that the degree of impaired EDV of coronary arteries of HTR was more profound in those with elevated ADMA levels (Fearon et al. 2004). Therefore, ADMA may also be a key contributor of decreased NO bioavailability and impaired EDV in HTR, particularly in those who are CMV-positive.

Arterial Stiffness

The arterial system can be divided into three anatomic regions with distinct physiological functions. Large elastic arteries of the central circulation, such as the aorta and carotids, act as a "buffering" or "cushioning" function to absorb pressure and flow pulsations from LV ejection. This "Windkessel" effect, allows blood (and potential energy) to be stored in the large arteries during systole, and then expelled to the

peripheral circulation during diastole (Nichols and Singh 2002). This allows for continuous blood flow in the capillaries throughout the cardiac cycle, and dampening of pressure oscillations from intermittent ejection from the LV (Safar et al. 2003). Second, large muscular conduit arteries, such as femoral, brachial, or radial, have a thicker layer of smooth muscle (Wilkinson et al. 2004) and are about twice as long as the elastic arteries (Nichols and Singh 2002). The muscular conduit arteries can alter smooth muscle tone and therefore, modify the speed of forward and reflected pressure waves, also known as pulse wave velocity. Third, the small arterioles, or resistance vessels, control blood flow into tissues and can affect mean arterial pressure by altering their diameter. Additionally, the state of arteriolar tone can affect the distance of reflecting sites, whereby increased tone can result in reflecting sites "closer" to the ascending aorta and an early return of reflected waves from the periphery to the heart (Nichols and Singh 2002).

Arterial compliance (inverse of stiffness) is a function of structural elements of the vessel wall (elastin/collagen/smooth muscle) and distending pressure or mean arterial pressure (Wilkinson et al. 2004). During aging, decreased arterial compliance (increased arterial stiffness) in large elastic central arteries can occur due to 'passive" alterations in elastin/collagen matrix resulting in the inability to absorb pulsations from LV ejection and increased pulse wave velocity of forward and reflected traveling pressure waves. Increased arterial stiffness in the central elastic arteries results in increased central systolic pressure and pulse pressure because they are dependent on LV stroke volume and compliance of the proximal ascending aorta. In contrast, "active" increases in peripheral muscular conduit artery and arteriolar tone, contribute to arterial stiffness by increasing

pulse wave velocity of forward and reflected traveling pressure waves, and by decreasing the distance to peripheral reflecting sites, respectively (Nichols and Singh 2002). Increased pulse wave velocity results in early return of reflected pressure waves to the ascending aorta during systole instead of diastole. Reflected waves merge with forward (incident) pressure waves resulting in augmentation of systolic pressure. This alteration in arterial reflected wave properties creates an undesirable "mismatch" between the LV and the arterial system (ventricular/vascular coupling), thus increasing LV afterload and myocardial oxygen demand. Moreover, increased LV afterload or 'wasted energy," increases the risk of LV hypertrophy and myocardial ischemia (Nichols and Singh 2002).

In 1980, Murgo et al. published a detailed description of invasively recorded ascending aortic pressure and flow waves in humans. Murgo et al. (1980) characterized the aortic pressure and flow wave reflection in young and older individuals. In young individuals, they reported that the aortic pressure augmentation occurs in diastole after LV ejection and closing of the aortic valve. This aortic pressure waveform was designated a "type c" wave, in which augmentation occurs in late systole or diastole after peak pressure and flow ejection. Therefore, augmentation is negative and the augmented pressure wave corresponded to the peak flow wave. In middle-aged individuals, augmented pressure occurred earlier in the cardiac cycle during mid-late systole resulting in a "type b' wave. Type b wave had an augmentation between 0 and 12% of pulse pressure, and also did not affect aortic flow. Lastly, older individuals had a "type a" wave which showed augmented pressure in early systole, resulting in augmentation >12% of pulse pressure (O'Rourke and Pauca 2004). Calculation of augmentation, also known as AI, is the ratio of the reflected wave amplitude or augmented pressure divided by pulse

pressure. Thus, AI is a measure of amplitude and timing of reflected pressure waves arriving at the large central arteries, therefore, is an indirect measure of pulse wave velocity and arterial stiffness of the entire arterial tree (Nichols and Singh 2002). Furthermore, AI of the carotid or aorta can be measured noninvasively using a high fidelity transducer (e.g., applanation tonometry) to record the pressure waveform of the carotid directly, or the aorta indirectly by recording the radial pressure wave and using a generalized transfer function to obtain an aortic pressure waveform (O'Rourke et al. 2001).

A primary mechanism for a change in arterial stiffness in both large elastic and muscular arteries is acute changes in distending or mean arterial pressure (Wilkinson et al. 2004). However, smooth muscle tone of muscular conduit arteries, are also influenced by circulating and local vasoactive substances, and sympathetic nervous tone. In particular, endothelial-derived NO can influence arterial stiffness through its vasodilatory properties. Systemic studies to stimulate or inhibit release of NO can be confounded by changes in mean arterial pressure (Wilkinson et al. 2004). However, recent *in vivo* studies in sheep and in humans, show that NO released by local intraarterial infusion of acetylcholine, indicate that NO release decreases arterial stiffness (Wilkinson et al. 2004). Furthermore, exogenous NO donors (e.g., nitroglycerin) and phosphodiesterase inhibitors (e.g., sildenifil), reduce arterial reflected pressure waves and arterial stiffness, independent of any change in mean arterial pressure (Wilkinson et al. 2004). Thus, arterial stiffness may be present in clinical conditions in which endothelial dysfunction and reduced NO bioavailability are present.

Arterial Stiffness and Cardiovascular Risk

Several recent studies investigated the effects of arterial stiffness on cardiovascular risk. Weber et al. (2003) recently reported that aortic AI was an independent predictor for developing premature coronary artery disease in men undergoing coronary angiography. London et al (2001) reported that increased carotid AI to be an independent predictor of all cause and cardiovascular mortality in renal failure patients. Furthermore, Laurent et al. (2001) reported that aortic pulse wave velocity was an independent predictor of all-cause and cardiovascular mortality in patients with essential hypertension. Thus, measures of arterial stiffness may provide important prognostic in populations at risk for cardiovascular disease. However, the long-term prognostic implication of arterial stiffness in HTR has not been determined.

Arterial Stiffness before Heart Transplantation

Systemic measures of arterial stiffness such as aortic input impedance are increased are increased in chronic HF patients (Nichols and Pepine 1992; Mitchell et al. 2001). Regional measures of arterial stiffness, such as in the carotid (Lage et al. 1994), iliac (Ramsey et al. 1995), and brachial (Arnold et al. 1991; Ramsey et al. 1995; Nakamura et al. 2004) conduit arteries are also elevated in HF patients (Nichols and Pepine 1992; Ramsey et al. 1995; Nakamura et al. 2004). Using invasive simultaneous measures of ascending aortic pressure and flow, Nichols and Pepine (1992) reported an increased aortic impedance and resistance, the pulsatile and nonpulsatile component of LV afterload, respectively, in HF patients compared to age- and pressure- matched controls. Elevation of aortic input impedance, consisting of aortic elastance (stiffness) and wave reflection at various oscillatory frequencies, suggests that arterial stiffness of the arterial tree is increased in HF patients (Nichols and Pepine 1992).

Ramsey et al. (1995) found decreased pulse wave velocity in the common iliac artery in response to intraarterial infusion of acetylcholine in healthy subjects but not patients with HF. This suggests that distensibility of the iliac artery in healthy subjects is influenced by stimulated release of NO, and that decreased release of NO may be contributing to increased stiffness of large muscular conduit artery in HF patients. This was further supported by noninvasive measures of brachial artery flow-mediated dilation and distensibility during reactive hyperemia, which both were significantly decreased in HF patients compared to healthy controls. Using high resolution ultrasound, Nakamura et al. (2004) and Arnold et al. (1991) also found decreased compliance of the brachial artery in HF patients, and Lage et al. (1994) reported reduced carotid artery compliance and increased wall thickness in non-ischemic HF patients. Taken together, these data support the hypothesis that regional muscular conduit artery stiffness is present in chronic HF patients.

However, systemic noninvasive measures of arterial stiffness such as aortic-femoral pulse wave velocity, total arterial compliance, and AI have yielded conflicting results (Mitchell et al. 2001). Using noninvasive applanation tonometry and high-resolution ultrasound, Mitchell et al. (2001) reported that pulse wave velocity and total arterial compliance was not different than age-matched controls who had coronary artery disease or risk factors. However, they reported a lower carotid AI in the HF patients (8 vs. 21%, $p=0.001$) than in controls, but a higher central pulse pressure, characteristic impedance, and lower proximal aortic compliance. This discordant results do suggest that proximal aortic stiffness is elevated, but that systemic measures of AI and pulse wave velocity may not be able to detect these changes. As such, during chronic HF, reduced cardiac output

and reduced left ventricular ejection duration lead to reduced mean arterial pressure, pulse pressure of incident pressure wave, and decreased perfusion pressure of organs (kidney, skeletal muscle, etc). Thus, return of reflected waves to the ascending aorta from the periphery reduces the aortic blood flow wave during deceleration phase, rather than add to second systolic peak of the aortic pressure wave (Nichols and O'Rourke 1998). Moreover, augmented pressure and AI may appear normal in HF patients, but ascending aortic flow is reduced due to the increased arterial stiffness.

Arterial Stiffness and Hypertension after Heart Transplantation

Post-transplant hypertension is a common complication in HTR occurring in 72% of HTR by one year, and 95% HTR by five years (Hertz et al. 2002). Post-transplant hypertension jeopardizes the long-term survival of the allograft by increasing left LV afterload and LV mass, and increases the risk of the of coronary artery vasculopathy, the leading cause of death in HTR surviving one year (Hertz et al. 2002). Mechanisms proposed for this *de novo* hypertension in HTR include 1) cyclosporine-induced sympathetic system hyperactivity and nephrotoxicity (Scherrer et al. 1990); 2) failure of the renin-angiotensin-aldosterone axis to reflexly suppress volume expansion-induced hypertension due to cardiac denervation (Braith et al. 1996); and 3) arterial stiffness due to structural changes in large elastic arteries and increased peripheral conduit arterial tone due to endothelial dysfunction. However, it is currently unknown the relative contribution of each of the above mechanisms on post-transplant hypertension.

Schofield et al. (2002) recently reported that 82% of 53 HTR had elevated aortic AI and decreased time of the reflected wave (ΔT_p), despite being on optimal hypertensive therapy as indicated by brachial blood pressure measured by standard cuff

sphygmomanometry. In addition, when HTR were stratified by Murgo aortic wave types, this revealed significantly different aortic pulse pressure, reflected wave amplitude, and aortic AI between the three groups, despite having similar mean arterial blood pressure. This data suggests that a subgroup of HTR have increased aortic augmented systolic and pulse pressure and arterial stiffness which cannot be identified by standard brachial sphygmomanomtry assessment. Although it is currently unknown whether elevated aortic augmented pressure and AI is a predictor of future cardiovascular risk in HTR, it is conceivable that any intervention that can attenuate these physiological parameters of arterial stiffness may have beneficial prognostic implications.

There may be several mechanisms for increased arterial stiffness in HTR. Structural changes in the large proximal elastic arteries (e.g. aorta, carotids) during chronic HF prior to HT may occur due to chronic neurohormonal and sympathetic hyperactivity. Increased chronic salt and water retention in the vascular wall, and the hypertrophic effects of elevated ANG II and ET-1 on the vascular smooth muscle layer may contribute to increased proximal arterial stiffness. As such, increased stroke volume, cardiac output, and mean arterial pressure from HT in this setting, may lead to increased pulse pressure and amplitude of the forward traveling pressure wave and increased pulse wave velocity (Pierce et al. 2004).

ED and cyclosporine-induced sympathetic hyperactivity (Scherrer et al. 1990) may also contribute to increase stiffness in HTR due to elevated vascular resistance via increased tone of peripheral muscular conduit and resistance arteries. Increased vascular tone leads to increased pulse wave velocity of forward and reflected pressure waves, while increased tone of small resistance vessels decreases distance to reflecting sites.

The cumulative effect contributes to increased timing and amplitude of forward and reflected waves to the peripheral reflecting sites and back to the ascending aorta. Thus, increased LV afterload due to increased vascular resistance and AI due to alterations in wave reflections, may lead to increased myocardial oxygen demand and long-term deleterious effects on the cardiac allograft.

Role of Exercise Training in HTR

Exercise Training and Functional Capacity

Exercise capacity and peak heart rate increase in the first year after transplantation, but remain approximately 60-70% of age-matched normals for the subsequent five years (Givertz et al. 1997). The reasons for this persistent subnormal exercise capacity in HTR is related to several mechanisms. First, cardiac output may be limited during peak exercise due to impaired chronotropic reserve and mild allograft diastolic dysfunction (Kao et al. 1994). Chronic cardiac denervation limits heart rate during submaximal and peak exercise and mild diastolic dysfunction of the cardiac allograft reduces end-diastolic volume and stroke volume during peak exercise (Kao et al. 1988). Thus, at low workloads submaximal cardiac output is maintained by augmenting stroke volume in plasma volume expanded HTR via the Frank Starling mechanism, however peak heart rate and stroke volume contribute to a reduced cardiac output at peak exercise compared to age-matched controls (Braith et al. 1998a). Second, myopathy of peripheral skeletal muscle due to chronic deconditioning and glucocorticoid therapy may also contribute to reduced exercise capacity (Braith et al. 1998b). Reduced muscle girth, muscle strength, and metabolic enzyme activity of muscle (Braith et al. 2005), contribute to decreased oxygen utilization and aerobic ATP production during exercise (Kao et al. 1994). Lastly, impaired EDV of peripheral vasculature persists after transplantation in HTR, and may

contribute to reduced muscle blood flow and a-VO_2 difference during peak exercise (Kao et al. 1994).

There has been only one randomized, controlled study of the effect of endurance exercise training in HTR. Kobashigawa et al. (1999) randomized twenty-seven HTR two weeks after HT to an exercise or control group. They reported that 24 weeks of supervised exercise training as part of a cardiac rehabilitation program increased peak VO_2 by 49% and exercise duration 59%, compared to 18% and 18% in the non-supervised control HTR, respectively. To date there is no evidence that exercise training improves central hemodynamic parameters such as LV ejection fraction or stroke volume at rest or during exercise in HTR, so it reasons that improvement in exercise capacity in HTR following chronic exercise training likely involves peripheral mechanisms. As such, peripheral improvements in metabolic capacity of muscle or increased muscle blood flow due to improvement in shear-stress mediated EDV of peripheral conduit and resistance arteries, likely play a significant role improved exercise capacity in HTR.

Exercise Training and Endothelial Dysfunction

Several recent randomized, controlled studies in chronic HF patients report that chronic lower-body endurance training improves EDV of peripheral conduit arteries (Hambrecht et al.1998; Linke et al. 2001). Linke et al. (2001) reported that 4 weeks of lower body cycle training improved radial artery EDV in response to acetylcholine. In addition, they reported that change in EDV of the radial artery after the exercise intervention correlated positively with the change in peak VO_2 ($r=0.63$, $p<0.05$). Hambrecht et al. (1998) also reported a high positive correlation ($r=0.64$) between the increase in EDV of conduit femoral artery blood flow velocity and the increase in peak oxygen uptake (VO_2) following an exercise training program in HF patients. The results

of these studies suggest that lower body exercise training results in a systemic improvement in endothelial function in chronic HF, since EDV was improved in both an upper limb conduit artery (Linke et al. 2001), and lower body conduit artery (Hambrecht et al. 2000). Moreover, the studies suggest that peripheral endothelial dysfunction contributes significantly to impaired exercise capacity in HF because improvement in EDV correlated highly with improvement in peak VO_2.

Brachial artery FMD has become an accepted non-invasive test of EDV of upper limb conduit artery function (Corretti et al. 2002). Edwards et al. (2004a) showed that 12 weeks of treadmill walking as part of a cardiac rehabilitation program resulted in an improvement in brachial artery FMD (7.9 vs. 11.2%, $p<0.05$). In contrast, Goyce et al. (2002) showed a trend but no statistical difference in brachial FMD after 12 weeks of cardiac rehabilitation in patients with coronary artery disease (6.4% vs. 8.3%, $p>0.05$), but they did report a significant increase in posterior tibial artery FMD after the training period (9.7% vs. 11.7%, $P<0.05$). Furthermore, Walsh et al. (2003) recently reported that 8 weeks of cross-training (aerobic/resistance training) in CAD patients resulted in improved brachial artery FMD (3.0% to 5.7%, $p<0.05$).

In a similar design to our study, Higashi et al. (1999) reported a 24% increase in peak FBF during reactive hyperemia using plethysmography in 20 patients with essential hypertension after a 12-week exercise training intervention. In eight of the 20 exercise patients who showed an improvement in peak FBF, the increased FBF was abolished by the NO inhibitor, L-NMMA, suggesting that the increase in EDV during reactive hyperemia of forearm resistance arteries was NO mediated.

In HTR, there is a paucity of data on the effects of exercise training on endothelial function. Only, one cross-sectional study has evaluated brachial artery FMD in trained and untrained HTR six years after HT (Schmidt et al. 2002). Trained HTR participated in 6 months of cycling for 40 minutes 2 to 3 times per week. Brachial artery FMD was significantly higher in trained HTR compared to sedentary HTR (7.1% vs. 1.4%, $p<0.05$).

Patel et al. (2003) reported that brachial artery FMD was significantly correlated to maximal exercise treadmill time and to duration of exercise after ventilatory threshold, but not with time to threshold in HTR four years after transplantation. Thus, these observational data suggest that peripheral artery conduit function may be a valuable therapeutic target for improving exercise capacity in HTR. However, there have been no prospective, controlled studies on the effects of lower body dynamic exercise training (e.g., walking, cycling) on brachial artery FMD or resistance vessel EDV in HTR.

Exercise Training and Arterial Stiffness

Cross-sectional studies indicate that elevated large artery stiffness is associated with reduced exercise capacity in healthy, sedentary individuals (Vaitkevicius et al. 1993), middle-aged athletes (Kingwell et al. 1995), and individuals with chronic HF (Bonapace et al. 2003). In a large cohort of sedentary, healthy individuals (mean age 55 years) in the Baltimore Longitudinal Study of Aging, Vaitkevisius et al. (1993) reported that aortic AI (men: r=-0.34; women: r=-0.49) and aortic pulse wave velocity (men: r=-0.54; women: r=-0.74) were inversely correlated to peak VO_2 even after controlling for age. In another study, Kingwell et al. (1995) found an inverse correlation between aortic β-stiffness index and peak VO_2 (r=-0.44) in aerobically trained middle-aged athletes (age 30-59). Lastly, in a study of 78 patients with stable, chronic HF and dilated

cardiomyopathy, Bonepace et al. (2003) reported an inverse correlation between peak VO_2 and aortic pulse wave velocity (r=-0.39). Thus, these data suggest that large artery stiffness, at least partially, affects exercise capacity in various populations.

Several prospective, controlled exercise training studies have reported an improved arterial stiffness in healthy sedentary, young men (Cameron and Dart 1994), healthy, sedentary middle- aged and older men (Tanaka et al. 2000), men with coronary artery disease (Edwards et al. 2004), and chronic HF patients (Parnell et al. 2002). Cameron and Dart (1994) reported increased systemic arterial compliance and aortic β-stiffness index in previously, healthy young men after 30 minutes of cycling 3 days per week for 4 weeks. Tanaka et al. (2000) studied twenty middle-aged and older men who exercise trained for three months of walking 3 to 4 days per week at 60% of maximal heart rate, and progressed to 4 to 6 days per week at 70-75% of maximal heart rate. They reported a 5% increase peak VO_2 and a 25% increase in dynamic carotid arterial compliance and 20% decrease in carotid β-stiffness index after the exercise intervention. Lastly, Edwards et al. (2004) investigated the effects of 12 weeks of endurance exercise training (walking) in men with CAD. Twenty patients with previous myocardial infarction or documented CAD via coronary angiography were assigned to supervised exercise as part of cardiac rehabilitation program or a non-exercise control group. The 12-week training intervention resulted in a decrease in aortic AI and an increase in duration of the reflected wave (inverse of pulse wave velocity) in the exercise group, but with no change in brachial systolic or mean arterial blood pressure. Furthermore, there was no change in the time-control group, thus suggesting that regular exercise training decreased wave

reflection and systemic arterial stiffness of large arteries in patients with CAD and reduced the dynamic components of LV afterload.

In a prospective, controlled study using simultaneous noninvasive measures of aortic blood flow velocity and right carotid arterial pressure, Parnell et al. (2002) reported that 8 weeks of endurance exercise training in chronic HF patients improved systemic arterial compliance. However, other indices of arterial stiffness, such as aortic pulse pressure, aortic AI, and aortic pulse wave velocity, did not change following the exercise intervention. The authors explained the discordant results by suggesting that the total arterial compliance measurement was specific for changes in the ascending aorta independent of any changes in pulse wave reflection, and thus explaining the lack of change in pulse wave velocity or AI. Taken together, these studies indicate that regular endurance exercise training may be a valuable adjunct to attenuate arterial stiffness and thus may be one mechanism by which exercise reduces long-term cardiovascular risk. Furthermore, the mechanism for improved large artery stiffness with regular exercise training are currently unknown, but may include reduced vascular smooth muscle hypertrophy, reduced connective tissue cross linking, reduced sympathetic nervous tone, or improvement in endothelial function (Joyner 2000).

Exercise Training and Nitric Oxide Synthesis

Regular endurance exercise training results in increased aortic expression of eNOS and EDV of aorta (Sessa et al. 1994; Fukai et al. 2000), coronary (Woodman et al. 1997), and peripheral resistance vessels in animal models (Spier et al. 2004), and in human models using the left internal mammary coronary artery (LIMA) (Hambrecht et al. 2003). Sessa et al. (1994) reported increased eNOS mRNA and eNOS protein content in aortas of dogs after 10 days of treadmill training. Woodman et al. (1997) reported that 6 weeks

of exercise training in miniature swine resulted in increased eNOS mRNA in coronary resistance arteries. Fukai et al. (2000) reported a 3-fold increase in aortic eNOS protein expression in mice after 3 weeks of treadmill exercise. Furthermore, Spier et al. (2004) recently reported increased eNOS mRNA and eNOS protein were increased rat skeletal muscle soleus arterioles after 10-12 weeks of treadmill exercise in aged rats. Thus, in animals, there is clear evidence in the aorta, coronary arteries, and the peripheral resistance arteries, that chronic exercise training increases expression of eNOS, the enzyme responsible for synthesis of vascular NO.

In humans, a recent elegant study by Hambrecht et al. (2003), stable coronary artery disease patients undergoing elective coronary artery bypass surgery were randomly assigned to a 4 week exercise training program or control period. EDV and average peak velocity in response to acetylcholine (agonist-mediated EDV) and adenosine (flow-mediated EDV) were measured invasively before and after the 4-week period in both groups. Additionally, during bypass surgery, part of the LIMA tissue was harvested and measured eNOS mRNA, eNOS protein, and phosphorlayed eNOS at serine 1177. After the exercise intervention, LIMA average peak velocity and EDV in response to acetylcholine and adenosine was significantly increased. Additionally, the trained group had 96% higher eNOS mRNA, 200% higher eNOS protein expression, and 300% higher phosphorlated eNOS in the explanted LIMA compared to controls. Furthermore, phosporylated eNOS levels was significantly correlated to change in LIMA average peak velocity. Thus, this was the first study in humans to demonstrate increased eNOS mRNA, eNOS protein content, and phosphorylated eNOS and its relationship to impaired agonist-mediated EDV after exercise training in humans.

Finally, several studies in humans have reported an increase in plasma nitrate, the major metabolite of NO in vivo (Jungersten et al. 1996), in response to acute exercise and after chronic exercise training (Jungersten et al. 1997; Edwards et al. 2004). Jungersten (1997) reported higher resting plasma nitrate in athletes compared to sedentary controls (45 vs. 34 uM, $p<0.01$), and an 18% and 16% increase in plasma nitrate following two hours of cycling in athletes and controls, respectively. Edwards et al. (2004) found a 22% increase in plasma nitrate (28.2 vs. 34.4 uM, $p<0.05$) after 12 weeks of endurance exercise training (walking) in patients with CAD. Together, these animal and human studies suggest that acute and chronic exercise result in increased NO availability in part due to increased NO synthesis.

Exercise Training and Oxidative Stress

CuZn SOD is the major intracellular isoform of SOD in the cytosol that responds to increased shear stress and likely plays an important role in preventing intracellular superoxide accumulation and NO degredation. As such, laminar shear stress in cultured endothelial cells increased Cu/Zn SOD mRNA in a time and dose-dependent manner, and increased Cu/Zn SOD protein and enzyme activity as well (Inoue et al. 1996). In isolated soleus feed arteries, exposure to intraluminal shear stress resulted in an increase Cu/Zn SOD protein, Cu/Zn SOD mRNA, and Cu/Zn SOD activity in coronary arterioles of pigs. (Woodman et al. 1999).

However, the major isoform of SOD in the vessel wall is the extracellular form (ecSOD). ecSOD is believed to be the principal regulator of endothelial-derived NO bioactivity in the vascular wall (Fukai et al. 2002). ecSOD is produced by smooth muscle and is bound to heparin sulfate proteoglycans on the endothelial wall between endothelial and smooth muscle, and is in equilibrium with plasma ecSOD (Faraci et al.

2004). Fukai et al. (2000) reported that three weeks of exercise training in mice increased ecNOS protein in aortas, but aortic Cu/Zn SOD was not changed. Additionally, they studied the effects of exercise training in eNOS knockout (eNOS -/-) mice, and its potential role in modulating ecSOD expression. At baseline, ecSOD expression was decreased in eNOS -/- mice, suggesting that basal NO modulates ecSOD. Interestingly, in control eNOS +/+ mice, aortic eNOS protein was increased 3 fold after exercise training, which was paralleled by a 3-fold increase in aortic ecSOD. However, this increased ecSOD protein expression was not observed in aortas from eNOS -/- mice. Thus, this study strongly suggest that endogenous NO production modulates ecSOD expression in the vascular wall, both under basal conditions and in response to exercise training. The authors speculated that enhanced NO formation serves as a feed-forward mechanism by increasing ecSOD expression, therefore increasing its own biological effects.

To date there has been only one human study that has investigated the effects of exercise training on ecSOD activity in plasma. Edwards et al. (2004) observed an 8.3% increased in ecSOD activity in CAD patients who completed 12 weeks of endurance training. In addition, they found an increase in plasma nitrate, a reduction in lipid peroxidation, and an improvement in brachial artery FMD, but no change in non-exercise controls. This data, along with the studies by Fukai et al. (2000), suggests that ecSOD may be an important modulator of oxidative stress in vivo, however more studies in humans are needed to confirm this hypothesis.

Exercise Training and Vasoconstrictors

Several studies have evaluated the effects of exercise training on ET-1 and ANG II. Endurance exercise training has been reported to reduced plasma ET-1 levels in young,

healthy subjects (Maeda et al. 2001), and older women (Maeda et al. 2003). However, Callaerts-Vegh et al. (1998) reported that 12 weeks of exercise training did not alter ET-1 in chronic HF patients. Vanhees et al. (1984) reported that 3 months of endurance exercise training reduced plasma renin activity, but did not change ANG II levels in patients with ischemic heart disease. In chronic HF patients, Braith et al. (1998) reported that 16 weeks of exercise training lowered basal levels of ANG II and aldosterone levels, suggesting that exercise training can modify renin-angiotensin-aldosterone activation in HF patients. However, the effects of endurance exercise training on circulating ET-1 and ANG II levels in HTR has not been investigated.

Exercise Training and Inflammation

Several cross-sectional studies report that higher exercise capacity is associated with lower CRP levels in men and women at risk for cardiovascular disease (LaMonte et al. 2002; Church et al. 2003). In addition, there have only been two prospective studies on effects of exercise training on CRP in patients with CAD (Milani et al. 2004;Edwards et al. 2004). Milani et al. (2004) reported a 41% decrease in CRP in a cohort of 277 CAD patients who completed 12 weeks of exercise training as part of cardiac rehabilitation. Additionally, Edwards et al. (2003) reported that 12 weeks of cardiac rehab lowered CRP by 45% and IL-6 by 32% in CAD patients. Thus, these studies suggest that endurance exercise training has a modulating effect on CRP levels in patients with documented CAD.

There have been two prospective studies on the effects of exercise training on inflammatory cytokines in chronic HF patients. Adamopoulos and colleagues conducted two 12-week, randomized, controlled, cross-over design studies in 24 chronic HF patients and 20 healthy controls (Adamopoulos et al. 2001; Adamopoulos et al. 2002). They

reported a significant decrease in plasma levels of IL-6 (-29%), TNF-α (-39%), apoptosis inducer sFasL (-28%) (Adamopolous et al. 2002), and a 12% decrease in sVCAM-1 (Adamapolous et al. 2001). They found a significant negative correlation between the decrease in TNF-α and the increase in peak VO_2 (Adamopolous et al. 2002). Furthermore, Larsen et al. (2001) reported a 12.5% decrease in TNF-α but no change in IL-6 in 28 patients with chronic HF. Taken together, these data suggest that exercise training has a modulating effect on inflammatory mediators in patients with cardiovascular disease. However, the effect of exercise training on inflammatory mediators in HTR has not been investigated.

CHAPTER 3
METHODS

The experiments in this proposal were designed to investigate the effects of orthotopic HT on endothelial function of peripheral limb vasculature and arterial stiffness in end-stage HF patients. Additionally, this study investigated the effects of 12 weeks of supervised endurance exercise training on endothelial function of limb conduit and resistance arteries and arterial stiffness in HTR. A total of twenty end-stage HF patients listed for transplantation at Shands Hospital were recruited and studied prospectively. Before HT, ten HTR were randomly assigned to a program consisting of 12 weeks of supervised endurance exercise training after HT, and ten HTR were assigned to a control group consisting of usual post-HT medical care but did not participate in a supervised exercise program. In addition, ten age-matched, healthy control subjects were recruited to compare with HTR. The study was approved by the University of Florida Health Science Center Institutional Review Board and all subjects signed written informed consent to participate in the study.

Subjects

All HTR were recruited from the Heart Transplantation Program at Shands Hospital at the University of Florida. Patients were enrolled while inpatient at Shands Hospital and listed as status 1B on United Network of Organ Sharing (UNOS) awaiting HT. The selection of subjects was notbased on gender or racial/ethnic status.

Inclusion Criteria

1. Age 18 to 65

2. Listed status 1B inpatient on UNOS for HT

Exclusion Criteria

1. UNOS status 1A in the intensive care unit
2. Major orthopedic problems that would limit exercise
3. Claudication pain from peripheral artery disease
4. Chronic obstructive lung disease
5. Renal failure

Group Assignments

Twenty patients (n=20) were randomly assigned to 12 weeks of supervised endurance exercise training (n=10) (e.g. treadmill walking or cycling) or a non-exercise control group (n=10) before HT. The control group consisted of HTR who did not perform supervised exercise training but continued to receive their usual post-HT medical care. Ten age-matched, healthy controls (n=10) were also recruited for the study.

Exercise Training Protocol

Exercise training was performed at the "Living Well Center," College of Health and Human Performance, University of Florida, Gainesville, FL. For subjects who did not reside in the Gainesville area, participation in 12 weeks of supervised endurance exercise training occurred in a hospital in their community with an American Association of Cardiovascular and Pulmonary Rehabilitation (AACVPR) certified cardiac rehabilitation program. Exercise prescription guidelines were provided to each program, and progression and compliance updates were sent every 4 weeks and at the end the study to the primary investigator. HTR in the exercise group participated in 12 weeks of supervised endurance exercise training beginning at 8 weeks after HT. Exercise training will began with 30 minutes of continuous treadmill walking or stationary cycling 3 days

per week not including warm-up and cool-down periods, and progressed to 35-40 minutes as tolerated after the initial 4 weeks.

Cardiac denervation in HTR prevents heart rate from being used as an accurate measure of exercise intensity (Braith et al. 1998a). Therefore, intensity begin at 50-60% of VO_2 peak determined from a graded exercise test, and the "Borg" rating of perceived exertion (RPE) scale was used to maintain intensity in the 11 to 13, or "moderate" to "somewhat hard" range, in accordance with ACSM guidelines (ACSM 2000). Exercise intensity was progressed to 60-70% VO_2 peak, or RPE in the 12 to 14 Borg scale range "as tolerated" by each subject. Each session begin with five minute warm-up period with RPE range of 7 to 9 ("very light to light"), and a five minute cool down in the same range. Exercise sessions were under the direct supervision of an ACSM exercise specialist or registered nurse certified in basic life support (BLS) and advanced life support (ACLS) and overseen by a physician. Blood pressure, symptoms, and ECG rhythm via three-lead telemetry system were monitored throughout each exercise session. Criteria to terminate an exercise session was be based on ACSM guidelines (ACSM 2000). All exercise facilities were equipped with automated external defibrillators and resuscitation equipment and access to emergency medical services (EMS). The control group received standard of medical care for HTR from their transplant physician, but did not participate in supervised endurance exercise training.

Specific Measurements

Subjects visited the laboratory 3 times for testing. Details of the study protocol are outlined in Figure 3-1.

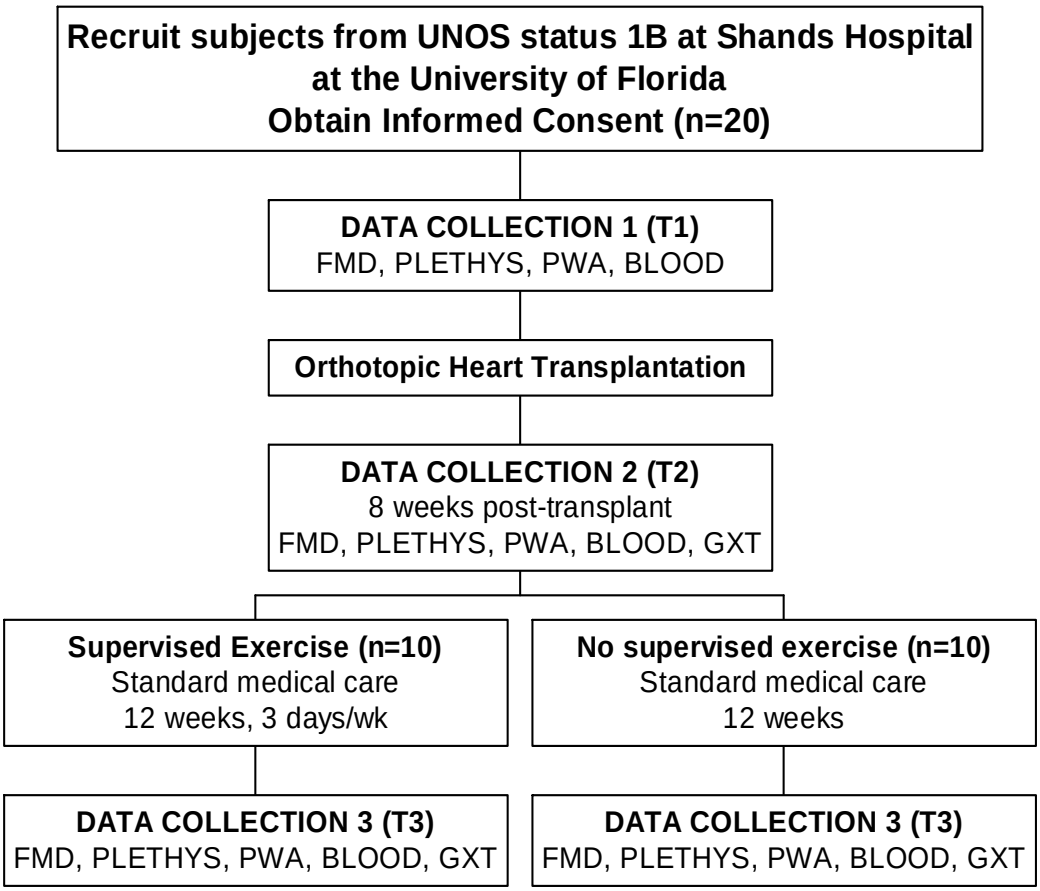

Figure 3-1. Study design. UNOS=United Network of Organ Sharing; FMD=flow-mediated dilation of brachial artery; PLETHYS= venous occlusion plethysmography blood flow; PWA=pulse wave analysis; BLOOD= venous blood sample; GXT=graded exercise test

Arterial Stiffness Testing

Measurement of arterial stiffness were made using pulse wave analysis. Subjects will remained quietly supine for 10 minutes and then blood pressure (BP) was determined in brachial artery in non-dominant arm three times by automated non-invasive BP cuff (Omron, Inc.) and mean was taken as BP value. Next, high-fidelity radial artery pressure waveforms was recorded by applanation tonometry of the radial pulse using a "pencil-type" micro-tip pressure transducer (Millar Instruments, Inc.). Optimal recording of the radial pressure waveform will be obtained by applying perpendicular hold-down force generating a stable baseline for at least 10 seconds. The radial pressure waveform and

the brachial artery BP was entered into a Sphygmocor™ (AtCor Medical, Inc., Sydney, Australia) PWA system which synthesizes an aortic pressure waveform using a mathematical generalized transfer function which has been validated (Chen et al. 1997; Cameron et al. 1998) and is reproducible (Wilkinson et al. 1998).

As shown in Figure 3-2, the aortic pressure wave (P_s-P_d) is the sum of a forward traveling wave with amplitude (P_i-P_d) generated by left ventricular ejection, and reflected pressure wave with amplitude (P_s-P_i) from the periphery arriving at the ascending aorta (Nichols and Singh 2002). The two pressure waves travel along the artery at the same velocity in opposite directions, whereby the reflected traveling pressure wave augments the forward traveling wave. The amplitude of the reflected traveling pressure was estimated by the aortic augmentation index (AI_a), which was obtained from the aortic pressure waveform. AI_a is calculated as the ratio of reflected wave amplitude to the pulse pressure expressed as a percentage, $(P_s-P_i)/(P_s-P_d) \times 100$, where P_s is the aortic systolic pressure; P_i is the inflection point of the beginning of upstroke of reflected pressure wave; P_d is minimum diastolic pressure. The roundtrip travel time (Δt_p) of the forward traveling pressure wave from the ascending aorta to the major reflection site and back was measured from the foot of the forward traveling wave to P_i. Round trip travel time (Δt_p) is inversely related to arterial pulse wave velocity and arterial stiffness, and directly related to the distance to reflecting point (Lo). Furthermore, LV ejection duration (LVED) is equal to duration from P_d to the incisura notch (closure of aortic valve). Aortic systolic tension time index (TTI), an indicator of LV myocardial oxygen demand, is equal to the area under the LVED x aortic P_s curve. Aortic diastolic TTI is equal to

area under the diastolic duration x aortic P_d curve, an indirect indicator of diastolic coronary perfusion (Nichols and Singh 2002).

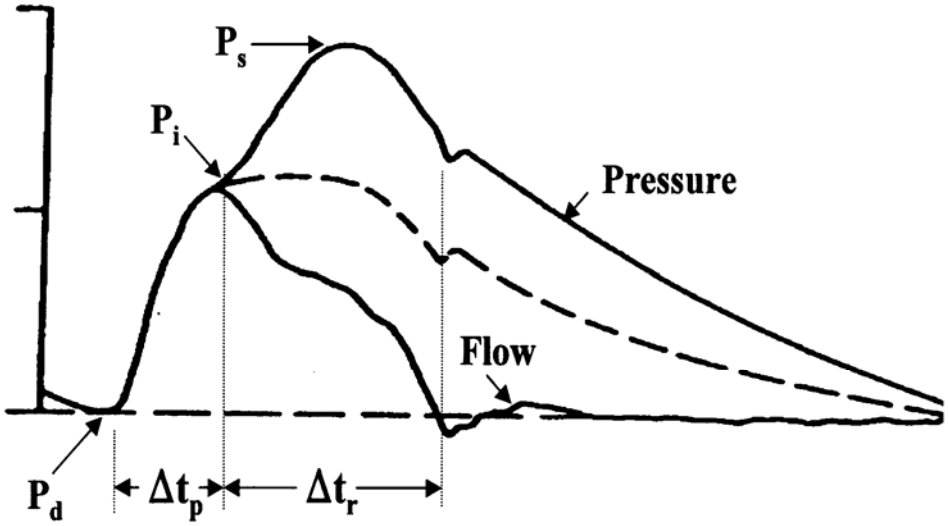

Figure 3-2. Ascending aortic pressure waveform. P_s= aortic systolic pressure; P_d=aortic diastolic pressure; P_i= inflection point of reflected wave; Δt_p= duration of reflected wave from the heart to the periphery and back; Δt_r= systolic duration of reflected wave

Endothelial Function Testing

Brachial artery flow-mediated dilation

Brachial artery reactivity testing was performed using high-resolution ultrasound (ATL, Inc.). Brachial artery reactivity tests were performed when the subject was fasted for at least 4 hours, abstained from caffeine for 12 hours and exercise for 24 hours, and the subject was asked to eat a low fat meal on the day of testing (Plotnick et al. 1997; Gudmundsson et al. 2000). After lying quietly for 15 minutes, a 10.5 MHz linear array ultrasound transducer was used to image the right brachial artery longitudinally and record on a super VHS recorder. After baseline artery diameter was obtained, a blood pressure cuff was inflated to 200 mmHg for 5 minutes on the upper arm proximal to the location brachial artery measurement. The transducer was held in the same location for

the duration of the cuff inflation to ensure the same section of the brachial artery was measured before and after cuff inflation. The proximal cuff position elicits a greater increase in blood flow and dilation during reactive hyperemia, compared to distal (e.g., forearm) cuff inflation (Corretti et al. 2002). Upon release of the cuff, brachial artery diameter was imaged and recorded for three minutes during reactive hyperemia blood flow. Reactive hyperemia blood flow results in flow-mediated dilation (FMD) of the brachial artery due to increased shear stress-induced nitric oxide release from the endothelial wall. This EDV of the brachial artery has been reported to peak between 60 to 90 seconds after cuff deflation, and is a valid measure endothelial-mediated arterial reactivity (Corretti et al. 2002). Images of the brachial artery were transferred to computer by a frame grabber (DT-4152, Data Translation, Inc.) and brachial artery diameter was made during end-diastole by measuring the distance between anterior and posteriors wall of the intima using image analysis software (Image Pro, Data Translation, Inc.). Five anterior to posterior point measures within a 3 cm segment were made and the average distance was recorded as diameter. Reliability of brachial artery testing was confirmed by a pilot study of four young healthy adults who had brachial artery FMD performed during three visits separated by one-week which yielded coefficient of variation (CV%) of 14.5%.

NO donors, such as nitroglycerin or sodium nitroprusside, are commonly used to test endothelial-independent vasodilation (EIV). NO donors act directly on vascular smooth muscle, resulting in normal vasodilation in subjects with CAD, HF, and HT. EIV using an NO donor was not performed in this study in order to reduce the risk of a hypotensive episode in patients with diminished baroreflex sensitivity.

Forearm and calf flow-mediated vasodilation

Forearm blood flow (FBF) and calf blood flow (CBF) responses were determined separately by venous occlusion plethysmography (EC-6, D.E. Hokanson, Inc.) using calibrated mercury strain-gauges as previously described (Hokanson et al. 1975; Wilkinson and Webb 2001). Patients were tested in a quiet, temperature-controlled room approximately 21-22°C and relative humidity approximately 40-50%. Strain-gauges were applied to the widest part of the non-dominant forearm (~5 cm below anticubital fossa) or calf (~10 cm below patella). Patients remained quietly supine for 10 minutes with arms or legs elevated above the right atrium n order to achieve stable baseline measurements of FBF and CBF. To measure FBF, an upper arm cuff (EC-20, Hokenson, Inc) was inflated to 40 mmHg for 5 seconds every 15 seconds using a rapid cuff inflator to prevent venous outflow (Wilkinson and Webb 2001). To measure CBF, an upper thigh cuff was inflated to 40 mmHg for 5 seconds every 15 seconds. One minute before each measurement, a wrist or ankle cuff was inflated to pressure 50 mmHg above systolic pressure to occlude hand or ankle circulation respectively, during FBF or CBF measurements. The FBF or CBF output signal was transmitted to NIVP3 software program (Hokanson, Inc) on a laptop PC computer and expressed as milliliters (mL) per minute per 100 mL of forearm tissue ($mL \cdot min^{-1}$ per 100 mL tissue). Absolute blood flow was determined by the rate of change of limb circumference (e.g., slope) during the five-second venous occlusion, which has been validated to correlate highly to arterial blood inflow into the limb (Greenfield et al. 1963; Hokanson et al. 1975). FBF or CBF for one minute is the average of one plethysmographic measurement every 15 seconds. Mean arterial pressure (MAP) was determined by systolic blood pressure (SBP) and diastolic

blood pressure (DBP) measured by an automatic oscillometric cuff (HEM-739, Omron, Inc) and calculated as DBP + [0.33(SBP-DBP)].

Endothelium-dependent FBF was measured during reactive hyperemia blood flow of the forearm following 5 minutes of upper arm occlusion using a BP cuff inflated at 200 mmHg (Wilkinson and Webb 2001). A blood pressure cuff was placed on the upper arm 5 cm above the anticubital fossa. After baseline FBF was confirmed to be stable for 2 minutes and recorded, the cuff was rapidly inflated to 200 mmHg for 5 minutes and then released. FBF was measured every 15 sec for 4 minutes. Peak FBF was recorded as the highest FBF observed immediately following releases of the cuff, and total FBF for three minutes was recorded as the area under the time x blood flow curve after baseline FBF is subtracted using the trapezium rule (Matthews et al. 1990). Peak FBF during reactive hyperemia has been shown to correlate highly with acetylcholine-induced FBF in patients with essential hypertension (Higashi et al. 2001), therefore it is a good non-invasive measurement of EDV of forearm resistance arteries (Wilkinson and Webb 2001; Higashi et al. 2001). Meredith et al. (1996) reported that peak FBF during reactive hyperemia is NO-dependent, where other studies report that peak FBF is not NO-dependent (Tagawa et al. 1994; Engelke et al. 1996). In fact, these studies suggest that vasodilation of resistance vessels of forearm is prostacyclin-dependent (Engelke et al. 1996), and that total area under the time x blood flow curve is NO-dependent (Tagawa et al. 1994).

Endothelium-dependent CBF was measured following 5 minutes of upper leg (thigh) arterial occlusion. After baseline CBF was stable for 2 minutes, the cuff was rapidly inflated to 200 mmHg for 5 minutes and then released. Peak CBF during reactive hyperemia was recorded as CBF observed immediately following release of the cuff, and

total CBF was recorded as the area under the time x blood flow curve after baseline CBF is subtracted using the trapezium rule (Matthews et al. 1990). A reliability of peak FBF and CBF using venous occlusion plethysmography was confirmed by a pilot study of 9 young healthy adults who had peak FBF and CBF during reactive hyperemia performed during three visits separated by one week (Pierce et al. 2004). Mean CV% of resting and peak FBF was 17% and 6.6%, respectively, and resting and peak CBF was 15.2% and 8.4%, respectively.

Graded Exercise Test

All HTR performed a symptom-limited graded exercise test (GXT) at 8 weeks after HT and again after 12 weeks of the exercise training or the control period. The GXT was be performed on a motorized treadmill (Quinton, Inc.) with collection of respiratory gas analysis using a calibrated metabolic cart (Parvomedics, Inc.) for determination of peak oxygen uptake (VO_2). Subjects performed a Modified Naughton walking protocol, which begins at 1.2 MPH and 0% grade for two minutes, increases to 2.0 MPH for two minutes, and then increases 3.5% grade every 2 minutes thereafter. HTR were monitored continuously during the GXT with a 12-lead electrocardiogram (Quinton, Inc.), blood pressure, and Borg rating of perceived exertion measured once each stage. Criteria for termination of GXT was based upon guidelines published by ACSM (ACSM 2000). All GXT's were performed in the Clinical Exercise Physiology (CEP) Laboratory in the Center for Exercise Science at the University of Florida and were supervised by a cardiologist and a certified ACSM exercise specialist. The CEP laboratory was equipped with a Lifepak 500 automated external defibrillator (Medtronic, Inc.), supplemental oxygen, emergency "crash cart" medications, and telephone.

Blood Collection

Venous blood samples were collected in tubes containing no additive, allowed to clot at room temperature for 15 minutes, and immediately centrifuged at 3,000 rpm for 15 minutes at 4°C. Venous blood for plasma samples were collected in tubes containing EDTA, placed on ice, and centrifuged immediately as noted above. Plasma that was used for measurement of lipid peroxidation was stored with diethylenetriamine pentaacetic acid (DTPA) and butylated hydroxytoluene (BHT) for a final concentration of 0.01 mM to prevent autooxidation during freezing and thawing. All serum and plasma samples were aliquoted into 1.5 ml epindorff tubes and immediately stored at –80°C until analysis at the end of study.

Plasma Biochemical Analysis

Vasoactive balance

Since the vasodilator, NO, is rapidly converted to nitrate and nitrite (NOx) in plasma, NOx will be used to estimate NO production. Plasma NOx has a half-life of 8 hours and can be influenced by dietary nitrate, therefore all subjects will be asked to follow National Institute of Health low nitrate diet guidelines 36 hours prior to each blood draw (Pannala et al. 2003). Plasma NOx was measured using a commercially available kit (Cayman Chemical, Inc.), which converts all nitrate to nitrite using nitrate reductase. Spectrophotometric analysis of total nitrite was performed using Greiss reagent and the absorbance measured at 540 nm. The vasoconstrictor ET-1 was measured using an ELISA kit (Cayman Chemical, Inc.).

Lipid peroxidation

Oxidative stress-induced lipid peroxidation was assessed by measuring plasma levels of 8-iso-PGF_2 using a enzyme-linked immunoassay (ELISA) (Stressgen, Inc.). 8-

iso-$PGF_{2\alpha}$ in plasma competes for binding with 8-isoprostane covalently attached to alkaline phosphatase. The assay plate is then incubated with p-nitrophenyl phosphate and the reaction stopped with the addition of an acid. The plate is read at 405 nm on spectrophotometer and the absorbance is inversely proportional to 8-iso-$PGF_{2\alpha}$ in the plasma sample.

Extracellular antioxidant enzyme activity

SOD activity in plasma was measured using coloremtric assay which uses cytochrome c reduction technique (Cayman Chemical, Inc.). This method utilizes the reduction of cytochrome c by superoxide ions produced by the xanthine oxidase reaction which causes a change in absorbance via spectrophotometry at 450nm. "One unit of SOD activity" is defined as the amount of SOD required for a 50% decrease in cytochrome c reduction rate or absorbance.

Inflammatory markers

Plasma CRP was measured using a sandwich ELISA (Alpha Diagnostics, Inc.). The ELISA is based on simultaneous binding of human CRP from plasma samples to two antibodies, one immobilized on the microtiter well plates, and the other conjugated to the enzyme horseradish peroxidase. The product is read at 450 nm and represents CRP bound to horseradish peroxidase. Plasma levels of IL-6, TNF-α, and sICAM-1 were measured using an ELISA (R&D Systems, Inc) which employ the quantitative sandwich enzyme assay technique.

Endogenous NO inhibition

Plasma levels of the endogenous eNOS competitive inhibitor, ADMA, was measured using an ELISA (Alpco, Inc).

Blood hemoglobin, hematocrit, serum lipids, glucose, creatinine, white blood cell count, cyclosporine, and cytomegliovirus status

Blood hemoglobin, hematocrit, and serum total cholesterol, LDL, HDL cholesterol, triglycerides, glucose, cyclosporine trough levels, and cytomegliovirus (CMV) status were measured by the Clinical Chemistry Laboratory at Shands Hospital at the University of Florida using standard blood lipid chemistry analyzer.

Endocardial biopsy rejection history

The number of allograft rejection episodes identified from endocardial heart biopsies were obtained during the study period in all subjects from the Shands Hospital Transplant Program database.

Statistical Considerations

Data is presented in table format as mean ± standard deviation (SD) for continuous variables and as percent frequencies (%) for categorical variables. Continuous variables were be analyzed by analysis of variance (ANOVA) with repeated measures of brachial FMD, peak and total FBF, peak and total CBF, AI_a, plasma NOx, ET-1, 8-iso-$PGF_{2\alpha}$, SOD activity, ADMA, CRP, IL-6, TNF-α, sICAM-1, demographics, and serum metabolic parameters before and after HT. ANOVA was performed between the exercise and control HTR groups at baseline before the exercise intervention to analyze for baseline group differences. ANOVA with repeated measures was used to compare the above vascular and blood parameters before and after 12 weeks of exercise training or control period. When a significant group-by-time interaction was observed, within-group comparisons between time points and between- group comparisons at each time point were performed using Tukey's post-hoc analysis. Categorical variables were analyzed by

χ^2 analysis. All statistical analysis were performed using Microsoft Excel® and SPSS 10.0 (SPSS, Inc.). An alpha level of $p \leq 0.05$ will be required for statistical significance.

A power analysis was performed to estimate statistical power related to testing the following hypothesis: 12 weeks of exercise training will result in greater peak FBF and brachial artery FMD when compared to a 12 week control period in HTR. Preliminary data on three HTR (n=3) was a peak FBF of 23.1 ± 4.8 ml/min/100ml (mean ± SD) two months after HT. Based on the study by Higashi et al. (1999), a 24% increase in peak FBF was conjectured after 12 weeks of exercise training. As such, the statistical power related to testing the hypothesis that peak FBF is greater in the exercise trained HTR compared to the control HTR is 0.80 for a two-tailed test when the group means were estimated to be 23.1 ml/min/100ml in the control group, and 28.6 ml/min/100ml in the exercise group; the standard deviation was assumed to be 4.8 ml/min/100ml; total sample size was 20 patients; and the alpha level was set at 0.05. Preliminary data on four HTR (n=4) was a brachial FMD of 8.8 ± 2.5% (mean ± SD) two months after HT. Based on the study by Edwards et al. (2004a) a 42% increase in brachial FMD was conjectured after 12 weeks of exercise training. The statistical power related to testing the hypothesis that brachial artery FMD is greater in the exercise trained HTR compared to the control HTR is 0.94 for a two-tailed test when the group means were estimated to be 8.8% in the control group and 12.5% in the exercise group; the standard deviation was assumed to be 2.5%; total sample size was 20 patients; and the alpha level was set at 0.05.

CHAPTER 4
RESULTS

A total of twenty subjects (n=20) were recruited and signed written informed consent for this study. Twelve subjects (n=12) completed all measurements before HT (PREHTX) and after HT (POSTHTX). A total of eight consented subjects (n=8) did not complete the measurements because 24-48 hours from the time of written consent the subject received heart transplantation, increased to status 1A and transferred to intensive care unit, or had left ventricular assist device implanted. Therefore, PREHTX data collection was unobtainable. Seven age-matched healthy controls (n=7) were recruited and completed all measurements.

Before transplantation, ten subjects were randomly assigned to the exercise group (TRAINED; n=10) and ten were assigned to the control group (CONTROL; n=10). One subject in the TRAINED group withdrew for a non-cardiac medical problem not related to exercise. Three subjects in the CONTROL group withdrew from the study, one for a non-cardiac medical reason, and the other two were lost to follow up. Therefore, sixteen subjects (n=16) completed the 12-week intervention part of the study of which seven were assigned to the CONTROL group (n=7) and nine to the TRAINED group (n=9).

Subject Characteristics before and after Heart Transplantation

The characteristics for PREHTX, POSTHTX, and age-matched healthy control subjects are presented in Table 4-1. The PREHTX and POSTHTX groups did not differ with respect to age, weight, body mass index, gender ratio, or number of ischemic etiology of heart failure. PREHTX had more on beta-blocker therapy (12 vs. 0, $p<0.01$)

and nitrate therapy (4 vs. 0, p<0.05) than POSTHTX. All PREHTX and POSTHTX subjects were on ACE inhibitor or angiotensin receptor blocker therapy (12 vs. 12, p=NS), however, there were more POSTHTX subjects on statin therapy (12 vs. 5, p<0.05), and insulin therapy (5 vs. 1, p<0.05). All subjects after HT were receiving standard triple immunosuppressive therapy including cyclosporine (Neoral®), myophenolate mofetil (Cellcept®), and prednisone.

Age-matched healthy controls did not differ significantly from PREHTX and POSTHTX with respect to age, weight, body mass index (BMI), or male/female ratio. Two healthy controls were on statin and ACE inhibitor therapy, but no healthy controls were on beta-blocker, nitrate, or insulin therapy.

Table 4-1 Subject characteristics before and after heart transplantation

	PREHTX (n=12)	POSTHTX (n=12)	Healthy Controls (n=7)
Age (years)	56.8±8.0	57.3±8.0	61.7±8.5
Weight (kg)	85.6±12.6	84.5±11.9	89.0±9.2
Body mass index (kg/m^2)	27.5±3.4	27.1±2.7	28.3±2.4
Male, no. (%)	10 (83)	10 (83)	6 (86)
Female, no. (%)	2 (17)	2 (17)	1 (14)
Ischemic HF etiology, no. (%)	7 (58)	7 (58)	N/a
Days before transplant	76.9±42.3	N/a	N/a
Days after transplant	N/a	66.4±11.6	N/a
IV inotrope therapy, no. (%)	12 (100)	N/a	N/a
ACEI/ARB therapy, no. (%)	12 (100)	7 (58)	2 (29)*
Beta-blocker therapy, no. (%)	12 (100)	0*	0*
Nitrate therapy, no. (%)	4 (33)	0*	0*
Calcium channel blocker therapy, no. (%)	1 (8)	3 (25)	0†
Statin therapy, no. (%)	5 (42)	12 (100)*	2 (29)†
Insulin therapy, no. (%)	1 (8)	5 (42)	0
Cyclosporine therapy, no. (%)	N/a	12	N/a
Prednisone therapy, no. (%)	N/a	12	N/a
Mycophenolate mofetil therapy, no. (%)	N/a	12	N/a

Data are mean ± SD; *P≤0.05 vs. PREHTX; †P≤0.05 vs. POSTHTX; PREHTX= pre-heart transplantation; POSTHTX=post-heart transplantation; HF=heart failure; IV=intravenous; ACEI=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker; N/A=not applicable

Serum Metabolic Parameters before and after Heart Transplantation

Fasting metabolic parameters are presented in Table 4-2. As shown in Table 4-2, hemoglobin (13.1 vs. 11.3 g/L, $p<0.05$) and hematocrit (38.3 vs. 34.6%, $p<0.05$) decreased significantly in POSTHTX compared to PREHTX. Serum lipid analysis showed that total cholesterol (164.9 vs. 193.3, $p<0.05$) and HDL cholesterol (45.8 vs. 64.8 mg/dl, $p<0.05$) increased significantly in POSTHTX, but there was no change in LDL (84.9 vs. 92.0 mg/dl) or triglycerides (171.0 mg/dl vs. 182.6 mg/dl, p=NS) in POSTHTX compared to PREHTX. However, total cholesterol/HDL cholesterol decreased significantly in POSTHTX (3.94 vs. 3.20, $p<0.05$). Finally, fasting glucose (95.0 vs. 113.0 mg/dl, p=NS), creatinine (1.43 vs. 1.26 mg/dl, p=NS), and WBC (8.0 vs. 7.0, $1x10^9$, p=NS) count did not differ in POSTHTX compared to PREHTX, respectively.

Table 4-2 Serum metabolic parameters before and after heart transplantation

	PREHTX (n=12)	POSTHTX (n=12)	Healthy Controls (n=7)
Hemoglobin (g/L)	13.1±1.5	11.3±1.1*	N/a
Hematocrit (%)	38.3±4.0	34.6±2.5*	N/a
Total cholesterol (mg/dl)	164.9±29.5	193.3±26.7*	195.6±54.5
LDL cholesterol (mg/dl)	84.9±28.2	92.0±19.7	117.7±41.5*
HDL cholesterol (mg/dl)	45.8±17.5	64.8±19.2*	54.3±11.2
Total cholesterol/HDL ratio (no.)	3.94±1.20	3.20±0.98*	3.63±0.79
Triglycerides (mg/dl)	171.0±75.0	182.6±60.9	117.1±55.5†
Glucose (mg/dl)	113.0±55.3	95.0±22.3	103.3±10.6
Creatinine (mg/dl)	1.26±0.37	1.43±0.41	0.97±0.13†
White blood cells, $1x\ 10^9$ (no.)	7.0±2.0	8.0±2.1	N/a

Data are mean ± SD. *P≤0.05 vs. PREHTX; †P≤0.05 vs. POST-HTX; PREHTX=pre-heart transplantation; POSTHTX=post-heart transplantation; LDL=low-density lipoprotein; HDL=high density lipoprotein; N/a=not available

Age-matched healthy controls did not differ from PREHTX and POSTHTX subjects with respect to total cholesterol, HDL cholesterol, total cholesterol/HDL ratio, fasting glucose, or white blood cell count. LDL cholesterol was significantly higher in healthy controls compared to PRETX (117.7 vs. 84.9 mg/dl, p=0.05), but not with

POSTHTX (117.7 vs. 92.0 mg/dl, p=NS). Fasting triglycerides were significantly lower in healthy controls than POSTHTX (117.1 vs. 182.6 mg/dl, p<0.05), but not significantly different from PREHTX (117.1 vs. 171.0 mg/dl, p=NS). Healthy controls had significantly lower serum creatinine than POSTHTX (0.97 vs. 1.43 mg/dl, p=0.01), but not different than PREHTX (0.97 vs. 1.26, p=NS).

Brachial Artery Endothelial Function before and after Heart Transplantation

Brachial artery flow-mediated dilation (FMD) and absolute diameter dilation results in PREHTX, POSTHTX, and healthy controls are presented in Table 4-3, Figure 4-1, and Figure 4-2. Brachial artery FMD (9.63 vs. 6.44%, p<0.05) and the absolute diameter dilation (0.45 vs. 0.32 mm, p<0.05) was significantly increased in POSTHTX compared to PREHTX. However, there was no significant change in resting baseline diameter in POSTHTX compared to PREHTX (4.70 vs. 4.93 mm, p=NS).

Table 4-3 Brachial artery flow-mediated dilation before and after heart transplantation

	PREHTX (n=12)	POSTHTX (n=12)	Healthy Controls (n=7)
Baseline diameter (mm)	4.93±0.78	4.70±0.63	4.46±0.78
Absolute diameter dilation (mm)	0.32±0.16	0.45±0.14*	0.30±0.13†
Flow-mediated dilation (%)	6.44±3.30	9.63±3.05*	6.81±2.92

Data are mean ± SD; *P≤0.05 vs. PREHTX; †P≤0.05 vs. POSTHTX; PREHTX=pre-heart transplantation; POSTHTX=post-transplantation

Resting diameter and brachial artery FMD did not differ between age-matched healthy controls and PREHTX or POSTHTX. There was a trend for brachial artery FMD to be significantly greater in POSTHTX than age-matched controls (9.63 vs. 6.81, p=0.06). Lastly, absolute diameter dilation was significantly less in healthy controls compared with POSTHTX (0.30 vs. 0.45 mm, p<0.05), but not significantly different than PREHTX (0.30 vs. 0.32 mm, p=NS).

Blood Pressure and Pulse Wave Analysis before and after Heart Transplantation

Blood pressure components for PREHTX, POSTHTX, and age-matched healthy controls are presented in Table 4-4. POSTHTX had a significant increase in heart rate (95.6 vs. 66.5 b/min, $p<0.01$), peripheral systolic blood pressure (138.8 vs. 109.8 mmHg, $p<0.05$) and peripheral diastolic blood pressure (90.8 vs. 70.7 mmHg, $p<0.01$) compared to PREHTX, but no significant change in peripheral pulse pressure (47.9 vs. 39.1 mmHg, p=NS). There was a significant increase in central systolic blood pressure (122.0 vs. 98.5 mmHg, $p<0.01$), central diastolic blood pressure (92.2 vs. 71.0 mmHg, $p<0.01$), but no significant change in central pulse pressure (29.8 vs. 27.5 mmHg, p=NS) in POSTHTX compared to PREHTX. There was a significant increase in mean blood pressure (105.3 vs. 81.3 mmHg, $p<0.01$).

Arterial pulse wave analysis data are presented in Table 4-4 and Figure 4-3, 4-4, and 4-5. There was no significant change in AI_a normalized for heart rate at 75 b/min (8.9 vs. 13.5 mmHg, p=NS), or augmentation pressure (3.8 vs. 1.8 mmHg, p=NS). In addition, there was no significant change in time duration of the reflected wave to periphery and back (Δt_p) in POSTHTX compared to PREHTX (140.6 vs. 146.0 ms, p=NS), but a significant increase in systolic tension time index (A_sTTI; 3254.3 vs 1826.1 mmHg/sec/min, $p<0.01$), an indicator of systolic LV myocardial oxygen demand, in POSTHTX compared to PREHTX. Lastly, there was no significant difference in diastolic pressure tension index (DPTI), an indicator of diastolic coronary perfusion, in POSTHTX vs. PREHTX.

Age-matched healthy controls had significantly lower heart rate than POSTHTX (58.3 vs. 95.6 b/min, $p<0.01$), and had higher peripheral and central systolic, diastolic,

pulse and mean blood pressure than PREHTX but no significant difference from POSTHTX. In addition, healthy controls had significantly higher augmentation blood pressure than PREHTX (10.4 vs. 3.8 mmHg, $p<0.01$) and POSTHTX (10.4 vs. 1.8 mmHg, $p<0.01$), however, AI_a normalized for heart rate (at 75 b/min) was not significantly different than PREHTX (17.6 vs. 8.9%, $p=0.09$) or POSTHTX (17.6 vs. 13.5%, p=NS). There was no significant difference in Δt_p between healthy controls and PREHTX or POSTHTX, but healthy controls had a significantly higher A_sTTI than PREHTX (2402.3 vs. 1826.1 mmHg/sec/min, $p<0.01$) and significantly lower A_sTTI than POSTHTX (2402.3 vs. 3254.3 mmHg/sec/min, $p<0.01$). However, there was no significant difference in DTPI between healthy controls, PREHTX, or POSTHTX.

Table 4-4 Blood pressure components and pulse wave analysis before and after heart transplantation

	PREHTX (n=12)	POSTHTX (n=12)	Healthy Controls (n=7)
HR (b/min)	66.5±16.7	95.6±11.7*	58.3±9.0†
PSBP (mmHg)	109.8±9.5	138.8±19.2*	133.3±13.6*
PDBP (mmHg)	70.7±7.1	90.8±12.2*	81.8±9.0*
PPBP (mmHg)	39.1±8.6	47.9±14.6	51.4±10.4*
CSBP (mmHg)	98.5±8.2	122.0±17.2*	122.9±13.5*
CDBP (mmHg)	71.0±7.3	92.2±12.1*	82.6±8.9*
CPBP (mmHg)	27.5±6.7	29.8±10.9	40.3±9.6*
MBP (mmHg)	81.3±7.0	105.3±13.1*	98.7±9.3*
AgBP (mmHg)	3.8±2.9	1.8±5.1	10.4±6.8*†
AI_a at HR=75 b/min (%)	8.9±11.6	13.5±15.9	17.6±7.4
Δt_p (ms)	146.0±15.7	140.6±11.7	147.1±4.9
A_sTTI (mmHg/sec/min)	1826.1±392.6	3254.3±617.5*	2402.3±311.3*†
DPTI (mmHg/sec/min)	2843.5±497.4	3079.7±606.1	3719.7±442.5

Data are mean ± SD. *P≤0.05 vs. PREHTX; †P≤0.05 vs. POSTHTX; PREHTX=pre-heart transplantation; POSTHTX=post-transplantation; HR=heart rate; PSBP=peripheral systolic blood pressure; PDBP=peripheral diastolic blood pressure; PPBP=peripheral pulse pressure; CSBP=central systolic blood pressure; CDBP=central diastolic blood pressure; CPBP=central pulse blood pressure; MBP=mean blood pressure; AGBP=augmentation blood pressure; AI_a=aortic augmentation index; Δt_p=round triptravel time of reflected pressure wave from ascending aorta to peripheral reflecting sites and back; A_sTTI=aortic systolic tension-time index; DPTI=diastolic perfusion time index

Forearm and Calf Resistance Artery Endothelial Function before and after Heart Transplantation

Forearm and calf resistance artery blood flow during reactive hyperemia results are displayed in Table 4-5 and Figure 4-6 and 4-7. A subgroup of five subjects (n=5) completed FBF and CBF before and after HT. POSTHTX compared to PREHTX. However, there was a significant increase in peak CBF in POSTHTX compared to PREHTX (22.4 vs. 17.4 ml/min/100ml, $p<0.05$), but no significant change in CBF AUC_{3min} (11.2 vs. 9.3 ml/min/100ml, p=NS) in POSTHTX compared to PREHTX.

There was no significant difference in peak FBF (22.9 vs. 20.4 ml/min/100ml, p=NS) and total excess FBF for 3 min (AUC_{3min}) (8.1 vs. 7.2 ml/min/100ml, p=NS) in Lastly, there was a trend for, but no significant increase in resting FBF in POSTHTX compared to PREHTX (2.4 vs. 1.7 ml/min/100ml, p=0.06), and no significant change in resting CBF in POSTHTX vs. PREHTX (2.6 vs. 2.8 ml/min/100ml, p=NS).

Table 4-5 Forearm and calf flow-mediated vasodilation before and after heart transplantation

	PREHTX (n=5)	POSTHTX (n=5)	Healthy Controls (n=7)
Resting FBF	1.7±0.16	2.4±0.5	2.5±1.8
Peak FBF	20.4±4.8	22.9±4.5	26.4±6.5*
Total FBF $AUC_{3\ min}$	7.2±1.1	8.1±2.6	7.7±1.5
Resting CBF	2.8±0.9	2.6±0.7	3.3±1.9
Peak CBF	17.4±0.5	22.4±4.4*	22.4±7.0*
Total CBF AUC_{3min}	9.3±4.1	11.2±7.3	7.1±5.4

Values are mean±SD; units are ml/min/100 ml tissue; *P≤0.05 vs. PREHTX; †P≤0.05 vs. POSTHTX; PREHTX=pre-heart transplantation; POSTHTX=post-heart transplantation BF=blood flow; AUC=area under flow x time curve.

Peak FBF was significantly greater in healthy controls compared to PREHTX (26.4 vs. 20.4 ml/min/100ml, p=0.05), but not significantly different compared to POSTHTX (26.4 vs. 22.9 ml/min/100ml, p=NS). Peak CBF was significantly greater in the healthy controls compared to PREHTX (22.4 vs. 17.4 ml/min/100ml, p=0.05), but not

significantly different than POSTHTX (22.4 vs. 22.4 ml/min/100ml, p=NS). There was no significant difference between healthy controls, PREHTX, and POSTHTX in resting FBF, resting CBF, total FBF AUC3min, and total CBF AUC3min.

Vasoactive Balance before and after Heart Transplantation

Plasma NOx and ET-1 are displayed in Table 4-6 and Figures 4-8 and 4-9. Plasma NOx, the product of NO metabolism, was not significantly different in POSTHTX compared to PREHTX (39.7 vs. 55.5 μmol/L, p=NS, respectively). The endothelial-derived vasoconstrictor ET-1, was not significantly different in POSTHTX compared to PREHTX (4.9 vs. 4.1 pg/ml, p=NS, respectively).

Plasma NOx was significantly lower in age-matched healthy controls compared to PREHTX (24.6 vs. 55.5 μmol/L, $p<0.05$), but not significantly different than POSTHTX (24.6 vs. 39.7 μmol/L, p=NS). Additionally, ET-1 was not significantly different in age-matched healthy controls compared to PREHTX (5.0 vs. 4.1 pg/ml, p=0.15) and vs. POSTHTX (5.0 vs. 4.9 pg/ml, p=NS).

Table 4-6 Vasoactive balance before and after heart transplantation

	PREHTX (n=12)	POSTHTX (n=12)	Healthy Controls (n=7)
NOx (μmol/L)	55.5±37.7	39.7±23.9	24.61±7.6*
ET-1 (pg/ml)	4.1±2.2	4.9±3.0	5.0±4.9

Values are mean±SD; *P≤0.05 vs. PREHTX; PREHTX=pre-heart transplantation; POSTHTX=post-heart transplantation; NOx=nitrate/nitrite; ET-1= endothelin-1

Plasma Lipid Peroxidation, Antioxidant Defense, and Endogenous Nitric Oxide Inhibition before and after Heart Transplantation

Plasma 8-iso-$PGF_{2\alpha}$, SOD activity, and ADMA are presented in Table 4-7 and Figures 4-10, 4-11, and 4-12. There was no significant difference in plasma 8-iso-$PGF_{2\alpha}$ (1597.4 vs.1474.8 pg/ml, p=NS) or ADMA (0.65 vs. 0.65 μmol/L, p=NS) in POSTHTX

vs. PREHTX, but compared to PREHTX, there was a significant decrease in SOD activity in POSTHTX (2.16 vs. 1.79 U/ml, $p<0.05$, respectively).

Plasma 8-iso-$PGF_{2\alpha}$ was significantly greater in the age-matched healthy controls than PREHTX (2089.5 vs. 1474.8 pg/ml, $p<0.05$), but not POSTHTX (2089.5 vs. 1597.4 pg/ml, p=NS). SOD activity was not significantly different than PREHTX (2.56 vs. 2.16, p=NS), but was significantly higher in healthy controls compared to POSTHTX (2.56 vs. 1.79 U/ml, $p<0.01$). Finally, plasma ADMA was not significantly different in healthy controls than PREHTX (0.75 vs. 0.65 μmol/L, p=NS) or POSTHTX (0.75 vs. 0.65 μmol/L, p=NS).

Table 4-7 Lipid peroxidation, antioxidant enzyme activity, and endogenous nitric oxide inhibition before and after heart transplantation

	PREHTX (n=12)	POSTHTX (n=12)	Healthy Controls (n=7)
8-iso-$PGF_{2\alpha}$ (pg/ml)	1474.8±564.9	1597.4±566.2	2167.7±248.2*†
SOD activity (U/ml)	2.16±0.54	1.79±0.34*	2.56±0.43†
ADMA (μmol/L)	0.65±0.18	0.65±0.23	0.75±0.12

Values are mean±SD; *P≤0.05 vs. PREHTX; †P≤0.05 vs. POSTHTX; PREHTX=pre-heart transplantation; POSTHTX=post-heart transplantation; $PGF_{2\alpha}$=prostaglandin F_2 isoprostane; SOD=superoxide dismutase; ADMA= asymmetric dimethylarginine

Inflammatory Markers before and after Heart Transplantation

Plasma markers of CRP, logCRP, IL-6, TNF-α, and sICAM-1 are displayed in Table 4-8 and Figures 4-13, 4-14, 4-15, 4-16, and 4-17. There was no significant difference in PREHTX vs. POSTHTX in CRP (7.0 vs. 6.0 mg/L, p=NS, respectively) and IL-6 (6.2 vs. 6.6 pg/ml, p=NS, respectively). However, CRP is well known to be non-normally distributed in the population and is skewed to the right (Blake and Ridker 2003), therefore, log transformation of CRP was performed which resulted in a significant decrease in log CRP from PREHTX to POSTHTX (0.75 vs. 0.51 mg/L, p=0.05). Furthermore, there was a significant decrease in TNF-α (2.6 vs. 2.0 pg/ml,

p<0.05, respectively) and sICAM-1 (363.4 vs. 237.8 ng/ml, p<0.01, respectively) from PREHTX to POSTHTX.

Age-matched healthy controls had significantly lower CRP (2.6 vs. 7.0 mg/L, p<0.05), logCRP (0.28 vs. 0.75 mg/L, p=0.01), IL-6 (1.4 vs. 6.2 pg/ml, p<0.05), TNF-α (1.8 vs. 2.6 pg/ml, p=0.05), and sICAM-1 (249.2 vs. 363.4 ng/ml, p<0.05) than PREHTX, but only IL-6 was lower compared to POSTHTX (1.4 vs. 6.6 pg/ml, p<0.05).

Table 4-8 Inflammatory markers before and after heart transplantation

	PREHTX (n=12)	POSTHTX (n=12)	Healthy Controls (n=7)
CRP (mg/L)	7.02±4.20	6.00±5.72	2.63±2.04*
logCRP (mg/L)	0.75±0.32	0.51±0.58*	0.28±0.40*
IL-6 (pg/ml)	6.21±5.08	6.58±4.22	1.43±0.60*†
TNF-α (pg/ml)	2.63±0.84	1.97±0.54*	1.88±0.49*
sICAM-1 (ng/ml)	363.4±144.5	237.8±61.6*	249.2±40.6*

Values are mean±SD; *P≤0.05 vs. PREHTX; †P≤0.05 vs. POSTHTX; PREHTX=pre-heart transplantation; POSTHTX=post-heart transplantation; CRP=c-reactive protein; IL-6= interluekin-6; TNF-α=tumor necrosis factor-α; sICAM-1=soluble intercellular adhesion molecule-1

Baseline Subject Characteristics before Exercise Training or Control

Baseline characteristics of the sixteen transplant subjects who completed the exercise intervention (TRAINED; n=9) or control (CONTROL; n=7) period are displayed in Table 4-9. Subjects in the CONTROL group did not differ significantly from the TRAINED group with respect to age, male/female ratio, ischemic etiology, days after transplant, percentage on immunosuppressive therapy, dose of immunosuppressive therapy, percentage on statin therapy, percentage on ACEI/ARB therapy, percentage on insulin therapy, or number of endocardial biopsy rejection episodes. There was a trend, but no significant difference in body weight for the CONTROL vs. TRAINED at baseline (90.4 vs. 78.6 kg, p=0.08), however, CONTROL had a significantly higher BMI than TRAINED at baseline (28.5 vs. 25.5 kg/m^2, p=0.05).

Table 4-9 Baseline patient characteristics before exercise training or control

	CONTROL (n=7)	TRAINED (n=9)
Age (years)	54.3±9.5	54.4±13.1
Body weight (kg)	90.4±11.5	78.6±12.6
Body mass index (kg/m^2)	28.5±1.4	25.5±3.6*
Male, no. (%)	6 (86)	7 (78)
Female, no (%)	1 (14)	2 (22)
Ischemic HF etiology, no. (%)	4 (57)	5 (56)
Days after transplant	73.6±30.6	67.3±11.2
Cyclosporine therapy, no. (%)	6 (86)	7 (78)
Cyclosporine dose (mg/day)	379.2±123.0	328.6±77.0
Serum cyclosporine trough level (ng/dl)	353.8±125.2	446.7±268.7
Tacrolimus therapy, no. (%)	1 (14)	2 (22)
Prednisone therapy, no. (%)	7 (100)	9 (100)
Prednisone dose (mg/day)	24.3±16.7	22.8±6.1
Mycophenolate mofetil therapy, no. (%)	7 (100)	9 (100)
Mycophenolate mofetil dose (mg/day)	2714.3±393	2800.0±632
Statin therapy, no. (%)	7 (100)	9 (100)
ACEI/ARB therapy, no. (%)	3 (43)	5 (56)
Calcium channel blocker therapy, no. (%)	2 (29)	3 (33)
Insulin therapy, no. (%)	2 (29)	5 (56)
Endocardial biopsy rejection episodes (no.)	12	14
• Grade 1A/B mild (no.)	8	10
• Grade 2 mild/moderate (no.)	3	2
• Grade 3 moderate/severe (no.)	1	2
• Grade 4 severe (no.)	0	0

Values are mean±SD. *P<0.05 vs. CONTROL; ACEI=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker

Body Weight, Serum Metabolic Parameters, and Endocardial Rejection History after Exercise Training

Fasting serum metabolic parameters in the CONTROL and the TRAINED group before and after the 12-week control or exercise intervention period are displayed in Table 4-10. Body weight significantly increased in the CONTROL group (90.4 vs. 96.0 kg, p=0.01), but not in the TRAINED group (78.6 vs. 80.9 kg, p=0.06) after 12 weeks. There was no significant change in hemoglobin, hematocrit, total cholesterol LDL cholesterol, HDL cholesterol, total cholesterol/HDL ratio, triglycerides, glucose, or creatinine in the CONTROL or TRAINED group after 12 weeks. There was a significant decrease in white blood cell count in the TRAINED group (p<0.05) after 12 weeks, but

not in the CONTROL group. One subject in each group tested positive for antibodies for CMV infection at baseline but both were negative at the 12 weeks measurement.

Table 4-10 Body weight, serum metabolic parameters, and endocardial rejection episodes at baseline and after exercise training or control

	CONTROL (n=7)		TRAINED (n=9)	
	Baseline	12 weeks	Baseline	12 Weeks
Body weight (kg)	90.4±11.5	96.0±11.0*	78.6±12.6	80.9±12.7†
Hemoglobin (g/L)	11.5±1.3	11.8±1.1	11.1±1.2	11.4±1.3
Hematocrit (%)	35.6±4.2	34.8±3.1	33.9±2.6	34.9±4.8
Total cholesterol (mg/dl)	188.3±38.8	177.7±38.4	193.9±15.1	172.7±20.0
LDL cholesterol (mg/dl)	81.9±18.6	88.4±29.8	97.6±18.5	86.3±18.6
HDL cholesterol (mg/dl)	69.0±21.6	57.0±17.9	65.6±20.3	61.8±18.5
Total cholesterol/HDL ratio	2.84±0.57	3.24±0.80	3.23±1.12	3.03±0.94
Triglycerides (mg/dl)	188.3±57.3	161.3±58.6	153.9±62.2	122.6±79.0
Glucose (mg/dl)	104.1±26.7	114.4±48.8	87.4±17.6	103.7±28.3
Creatinine (mg/dl)	1.26±0.33	1.54±0.85	1.44±0.52	1.32±0.47
WBC, 1x 10^9 (no.)	8.4±1.9	6.8±2.0	7.3±2.2	5.5±1.2*
CMV positive IgG (no.)	1	0	1	0
Endocardial rejection (no.)	12	3	14	5
• Grade 1 very mild (no.)	8	3	10	4
• Grade 2 mild (no.)	3	0	2	1
• Grade 3 moderate (no.)	1	0	2	0
• Grade 4 severe (no.)	0	0	0	0

Data are mean ± SD. *P≤0.05 vs. Baseline within-groups; †P≤0.05 vs. CONTROL at same time-point; LDL=low-density lipoprotein; HDL=high-density lipoprotein; CMV=cytomegliovirus; IgG=immunoglobulin G antibody; WBC=white blood cells

Brachial Artery Endothelial Function after Exercise Training

Brachial artery FMD results in the CONTROL and TRAINED group are displayed in Table 4-11 and Figure 4-18 and 4-19. There was a significant decrease in brachial artery FMD (11.1 vs. 7.9%, $p<0.05$) and the absolute change in diameter (0.51 vs. 0.39 mm, $p<0.05$) in the CONTROL group after 12 weeks, but no significant change in brachial artery FMD (10.1 vs. 9.6%, p=NS) or absolute change in diameter (0.48 vs. 0.42 mm, p=NS) in the TRAINED group after 12 weeks of exercise training. Furthermore, there was no significant change in baseline diameter in the CONTROL or TRAINED group after 12 weeks.

Table 4-11 Brachial artery flow-mediated dilation at baseline and after exercise training or control

	CONTROL (n=7)		TRAINED (n=9)	
	Baseline	12 weeks	Baseline	12 Weeks
Baseline diameter (mm)	4.50±0.60	4.46±0.64	4.74±0.87	4.64±0.82
Absolute change diameter (mm)	0.51±0.16	0.39±0.23*	0.48±0.22	0.42±0.24
Flow-mediated dilation (%)	11.1±2.6	7.9±5.1*	10.0±6.1	9.6±6.2

Data are mean ± SD. *P≤0.05 vs. Baseline within-group.

Blood Pressure and Pulse Wave Analysis after Exercise Training

Blood pressure components and pulse wave analysis results are displayed in Table 4-12 and Figure 4-20, and 4-21. There was no significant change in heart rate or peripheral or central systolic, diastolic, pulse and mean blood pressure in the CONTROL group or TRAINED group after 12 weeks. Pulse wave analysis results showed that there was no significant change in augmentation pressure, AIa corrected for heart rate at 75 b/min, ΔTp, AsTTI, or DTPI in the CONTROL or TRAINED group after 12 weeks.

Table 4-12 Blood pressure components and pulse wave analysis at baseline and after exercise training or control

	CONTROL (n=7)		TRAINED (n=9)	
	Baseline	12 weeks	Baseline	12 Weeks
HR (b/min)	90.7±7.4	95.1±9.7	95.8±13.7	91.1±17.4
PSBP (mmHg)	150.6±18.3	144.9±16.8	132.9 ±18.1	129.4±16.5
PDBP (mmHg)	93.1±14.1	94.0±9.9	89.7±10.3	87.0±11.3
PPBP (mmHg)	57.4±8.9	50.9±9.1	43.5±15.0†	42.4±12.8
CSBP (mmHg)	132.1±17.9	127.6±16.5	116.6±14.1	114.4±14.0
CDBP (mmHg)	94.9±14.1	95.3±10.0	90.7±9.7	88.2±10.9
CPBP (mmHg)	37.3±8.7	32.3±7.5	25.9±8.9†	26.2±7.2
MBP (mmHg)	111.3±15.1	110.0±13.1	102.1±10.6	99.9±12.1
AgBP (mmHg)	4.3±5.4	2.9±3.1	0.3±2.9	1.6±3.2
AI @ HR=75 b/min (%)	17.0±13.3	17.3±10.0	11.4±14.5	14.3±13.4
Δt_p (ms)	141.6±13.5	143.4±10.3	142.9±9.7	145.8±9.7
A_sTTI (mmHg/sec/min)	3389.1±598	3296.0±533	3134.0±524	2925.5±444
DPTI (mmHg/sec/min)	3263.7±462	3253.6±329	3054.4±583	3013.9±429

Data are mean ± SD. †P≤0.05 vs. CONTROL at same time-point; HR=heart rate; PSBP=peripheral systolic blood pressure; PDBP=peripheral diastolic blood pressure; PPBP=peripheral pulse pressure; CSBP=central systolic blood pressure; CDBP=central diastolic blood pressure; CPBP=central pulse blood pressure; MBP=mean blood pressure; AGBP=augmentation blood pressure; AI_a=augmentation index; Δt_p=round trip travel time of reflected pressure wave from ascending aorta to peripheral reflecting sites and back; A_sTTI=aortic systolic tension-time index; DPTI=diastolic perfusion time index

Forearm and Calf Resistance Artery Blood Flow after Exercise Training

Forearm and calf resistance artery blood flow during reactive hyperemia in the CONTROL and TRAINED group are displayed in Table 4-13 and Figure 4-22 and 4-23. Peak FBF increased 34% (21.5 vs. 28.8 ml/min/100ml, $p<0.05$) in the TRAINED group after 12 weeks compared to baseline, but there was no significant increase (+14%) in peak FBF (25.5 vs. 29.2 ml/min/100ml, $p=0.08$) in the CONTROL group. There was no significant change in resting FBF or total FBF $AUC_{3\ min}$ in the CONTROL or TRAINED group after 12 weeks.

There was a significant 17% increase in peak CBF (25.0 vs. 29.3 ml/min/100 ml, $p=0.05$) in the TRAINED group after 12 weeks compared to baseline, and no significant change (-4%) in the CONTROL group (26.3 vs. 25.2 ml/min/100ml, p=NS). There was no significant increase in resting CBF or total CBF $AUC_{3\ min}$ in the CONTROL and TRAINED group after 12 weeks.

Table 4-13 Forearm and calf flow-mediated vasodilation at baseline and after exercise training or control

	CONTROL (n=5)		TRAINED (n=6)	
	Baseline	12 weeks	Baseline	12 Weeks
Resting FBF	2.4±0.5	2.6±0.7	2.9±1.0	3.3±1.1
Peak FBF	25.5±11.6	29.2±8.1	21.5±4.6	28.8±3.7*
Total FBF AUC_{3min}	14.2±13.0	13.9±4.4	9.8±5.2	10.3±3.0
Resting CBF	3.0±1.1	3.0±0.7	3.0±1.0	3.7±2.8
Peak CBF	26.3±5.4	25.2±5.8	25.0±6.4	29.3±7.7*
Total CBF AUC_{3min}	18.3±7.3	10.9±3.0	13.5±10.6	14.8±12.2

Values are mean±SD; Units are ml/min/100 ml tissue; *P≤0.05 vs. Baseline within-group; †P≤0.05 vs. CONTROL at same time-point; BF=blood flow; AUC=area under flow x time curve

Vasoactive Balance after Exercise Training

Plasma NOx and ET-1 in the CONTROL and TRAINED group after 12 weeks compared to baseline are displayed in Table 4-14 and Figure 4-24 and 4-25. There was no significant change in plasma NOx from baseline in the CONTROL (30.5 vs. 45.3

μmol/L, p=NS) or TRAINED group (42.7 vs. 56.1 μmol/L, p=NS). There was a was a trend but no significant change in ET-1 in the TRAINED group (-41.4%) after 12 weeks compared to baseline (3.8 vs. 2.2 pg/ml, p=0.09), and no significant change in ET-1 in the CONTROL group (-36.5%) after 12 weeks compared to baseline (4.4 vs. 2.8 pg/ml, p=0.15).

Table 4-14 Vasoactive balance at baseline and after exercise training or control

	CONTROL (n=7)		TRAINED (n=9)	
	Baseline	12 weeks	Baseline	12 Weeks
NOx (μmol/L)	30.5±41.6	45.3±14.2	42.73±23.7	56.1±41.2
ET-1 (pg/ml)	4.45±3.01	2.82±0.90	3.83±2.54	2.24±1.02

Values are mean±SD; *P≤0.05 vs. Baseline within-group; †P≤0.05 vs. CONTROL at same time-point; NOx=nitrate/nitrite; ET-1=endothelin-1.

Lipid Peroxidation, Antioxidant Enzyme Activity, and Endogenous Nitric Oxide Inhibition after Exercise Training

Plasma levels of 8-iso- $PGF_{2\alpha}$, SOD activity, and ADMA in the CONTROL and TRAINED group compared to baseline are displayed in TABLE 4-15 and Figure 4-26, 4-27, and 4-28. There was no significant change in 8-iso-$PGF_{2\alpha}$, SOD activity, or ADMA in the CONTROL or TRAINED group after 12 weeks compared to baseline.

Table 4-15 Lipid peroxidation, antioxidant enzyme activity, and endogenous nitric oxide inhibition at baseline and after exercise training or control

	CONTROL (n=7)		TRAINED (n=9)	
	Baseline	12 weeks	Baseline	12 Weeks
8-iso-$PGF_{2\alpha}$ (pg/ml)	1815.0±516.7	1711.3±904.1	1387.8±506	1535.0±514
SOD activity (U/ml)	1.67±0.36	1.75±0.48	1.86±0.35	1.80±0.67
ADMA (μmol/L)	0.73±0.26	0.72±0.25	0.60±0.17	0.68±0.23

Values are mean±SD; *P≤0.05 vs. Baseline within-group; †P≤0.05 vs. CONTROL at same time-point; $PGF_{2\alpha}$=prostaglandin $F_{2\alpha}$ isoprostane; SOD=superoxide dismutase; ADMA= asymmetric dimethylarginine

Inflammatory Markers after Exercise Training

Plasma CRP, IL-6, TNF-α, and sICAM-1 in the CONTROL and TRAINED group after 12 weeks compared to baseline are displayed in Table 4-16 and Figures 4-29, 4-30,

4-31, and 4-32. There was a non-significant 19% decrease in plasma CRP in the CONTROL group (5.62 vs. 4.54 mg/L, p=NS) and a non-significant 49% decrease in the TRAINED group (5.03 v. 2.55 mg/L, p=NS) after 12 weeks compared to baseline. Log transformation of CRP did not alter the results in either group. There was no change in IL-6 in the CONTROL group (6.65 vs. 6.16 pg/ml, p=NS) and a non-significant 35% decrease in IL-6 in the TRAINED group (5.02 vs. 3.25 pg/ml, p=NS) after 12 weeks compared to baseline. However, there was a significant increase in TNF-α (1.56 vs. 2.38 pg/ml, $p<0.05$) in the CONTROL group, but no significant change in TNF-α in the TRAINED group (1.83 vs. 1.79 pg/ml, p=NS) after 12 weeks. Lastly, there was no significant change in sICAM-1 in the CONTROL (269.2 vs. 295.6 ng/ml, p=NS) or TRAINED group (216.5 vs. 198.2 ng/ml, p=NS) after 12 weeks compared to baseline. Furthermore, TNF-α (1.79 vs. 2.38 pg/ml, $p<0.05$) and sICAM-1 (198.2 vs. 295.6 ng/ml, $p<0.05$) were significantly lower at 12 weeks in TRAINED compared to CONTROL at 12 weeks.

Table 4-16 Inflammatory markers at baseline and after exercise training or control

	CONTROL (n=7)		TRAINED (n=9)	
	Baseline	12 weeks	Baseline	12 Weeks
CRP (mg/L)	5.62±4.29	4.54±3.36	5.03±6.55	2.55±2.81
IL-6 (pg/ml)	6.65±5.03	6.16±2.72	5.02±3.15	3.25±2.55
TNF-α (pg/ml)	1.56±0.38	2.38±0.79*	1.83±0.59	1.79±0.50†
sICAM-1 (ng/ml)	269.2±104.8	295.6±86.1	216.5±64.0	198.2±39.1†

Values are mean±SD; *P≤0.05 vs. Baseline within-group; †P≤0.05 vs. CONTROL at same time-point; CRP=c-reactive protein; IL-6=interluekin-6; TNF-α=tumor necrosis factor-alpha; sICAM-1=soluble intercellular adhesion molecule-1

Peak Cardiopulmonary Exercise Testing Variables after Exercise Training

Peak cardiopulmonary variables during graded exercise testing with respiratory gas analysis in the CONTROL and TRAINED group are displayed in Table 4-17 and Figure 4-33 and Figure 4-34. There was no significant change in peak heart rate, peak systolic

blood pressure, peak diastolic blood pressure in the CONTROL or TRAINED group after 12 weeks compared to baseline. There was no significant change in peak oxygen uptake (VO_2) in the CONTROL group (16.2 vs. 16.8 ml/kg/min, p=NS), but there was a significant 26% increase in peak VO_2 in the TRAINED group (15.4 vs. 19.4 ml/kg/min, $p<0.01$) after 12 weeks compared to baseline. Moreover, there was no significant change in exercise duration in the CONTROL group (561.5 vs. 554.5 sec, p=NS), but there was a significant 44.5% increase in exercise duration in the TRAINED group (518.9 vs. 750.0 sec, $p<0.01$) after 12 weeks compared to baseline. Lastly, there was no significant difference in peak respiratory exchange ratio (RER) and rating perceived exertion (RPE) in the CONTROL or TRAINED group after 12 weeks compared to baseline.

Table 4-17 Peak cardiopulmonary graded exercise testing variables at baseline and after exercise training or control

	CONTROL (n=7)		TRAINED (n=9)	
	Baseline	12 weeks	Baseline	12 Weeks
Peak HR (b/min)	123.7±10.2	133.0±14.0	124.3±16.6	124.9±42.7
Peak systolic BP (mmHg)	173.7±21.0	178.3±18.1	147.1±23.7†	167.2±25.0
Peak diastolic BP (mmHg)	88.7±5.9	90.3±7.1	84.0±16.9	83.6±17.9
Peak VO_2 (ml/kgBW/min)	16.2±5.2	16.8±2.8	15.4±4.3	19.4±5.5*
Peak RER	1.05±0.07	1.01±0.08	1.05±0.08	1.08±0.06†
Peak RPE	16.2±1.6	16.3±1.2	15.4±1.6	15.3±1.5
Peak exercise duration (sec)	561.5±202.8	554.5±110.9	518.9±198.2	750.0±274.4*

Data are mean ± SD. *P≤0.05 vs. Baseline within-groups; †P≤0.05 vs. CONTROL at same time-point; HR=heart rate; BP=blood pressure; VO_2=rate of oxygen consumption; BW=body weight; RER=respiratory exchange ratio; RPE=rating of perceived exertion (Borg scale)

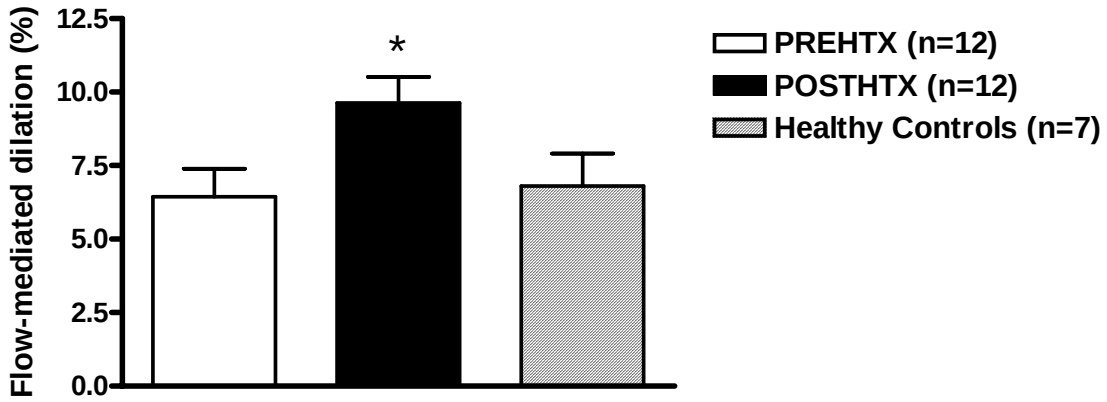

Figure 4-1. Brachial artery flow-mediated dilation before and after heart transplantation. *P≤0.05 vs. PREHTX.

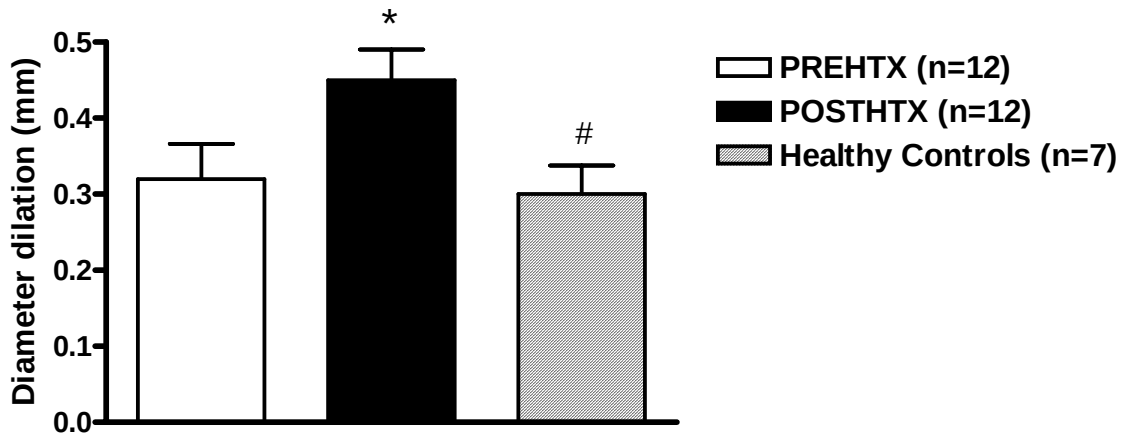

Figure 4-2. Brachial artery flow-mediated diameter dilation before and after heart transplantation. *P≤0.05 vs. PREHTX; #P≤0.05 vs. POSTHTX.

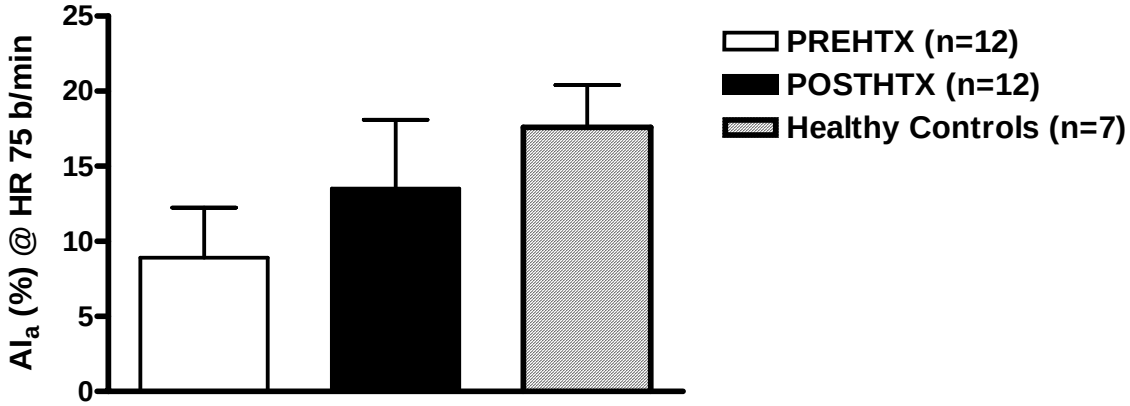

Figure 4-3. Aortic augmentation index (AI_a) corrected for heart rate=75 b/min before and after heart transplantation.

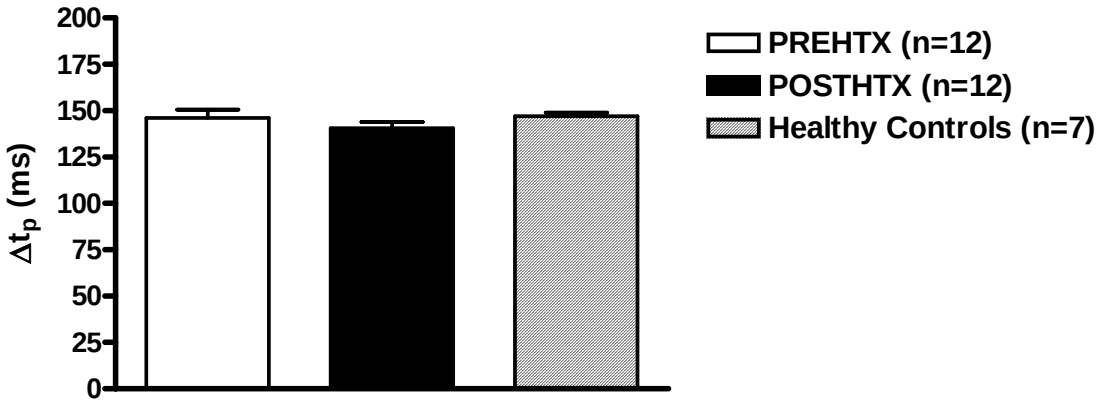

Figure 4-4. Roundtrip travel duration of reflected wave (Δt_p) before and after heart transplantation.

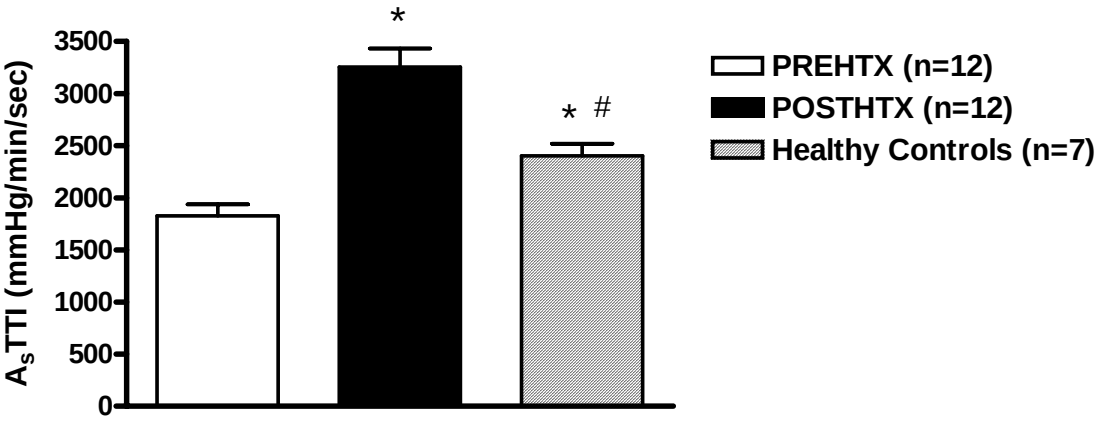

Figure 4-5. Aortic systolic tension-time index (A_sTTI) before and after heart transplantation. *P≤0.05 vs. PREHTX; #P≤0.05 vs. POSTHTX.

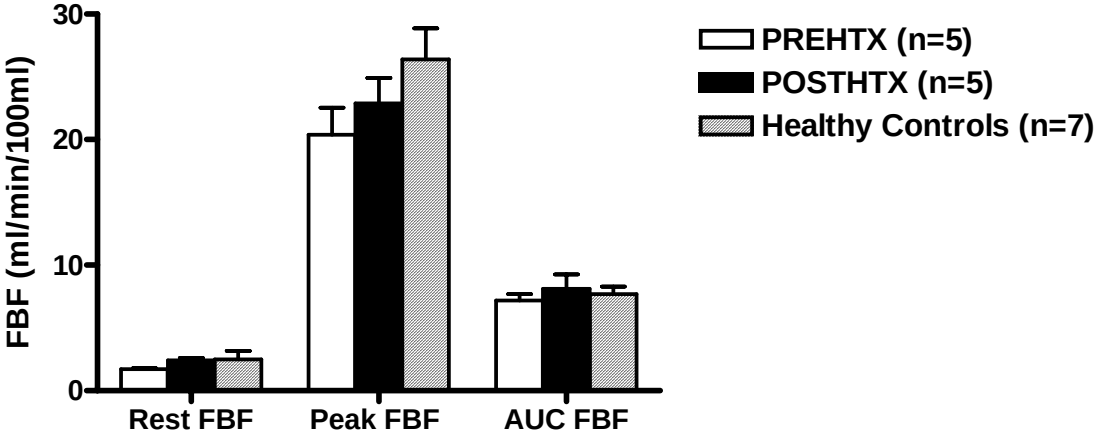

Figure 4-6. Forearm blood flow (FBF) before and after heart transplantation. *P≤0.05 vs. PREHTX; AUC=area under blood flow x time curve for 3 min

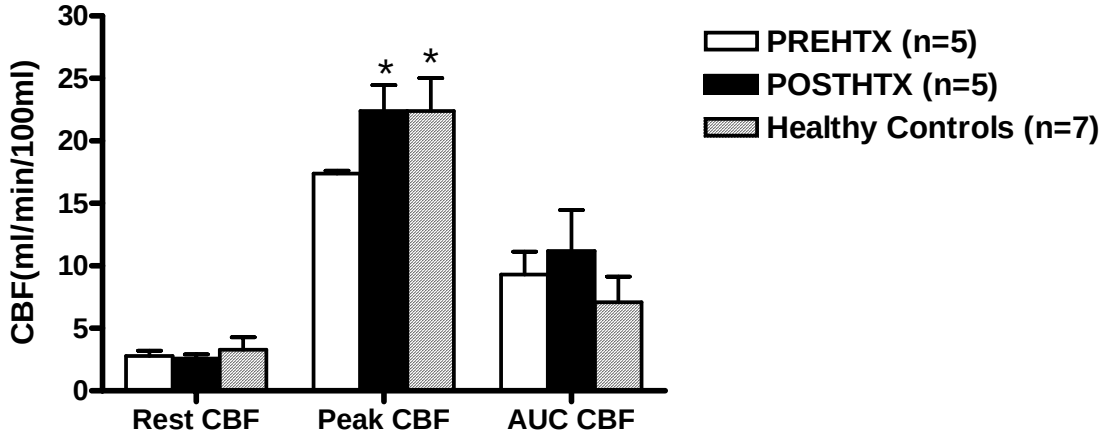

Figure 4-7. Calf blood flow (CBF) before and after heart transplantation. *P≤0.05 vs. PREHTX; AUC=area under blood flow x time curve for 3 min.

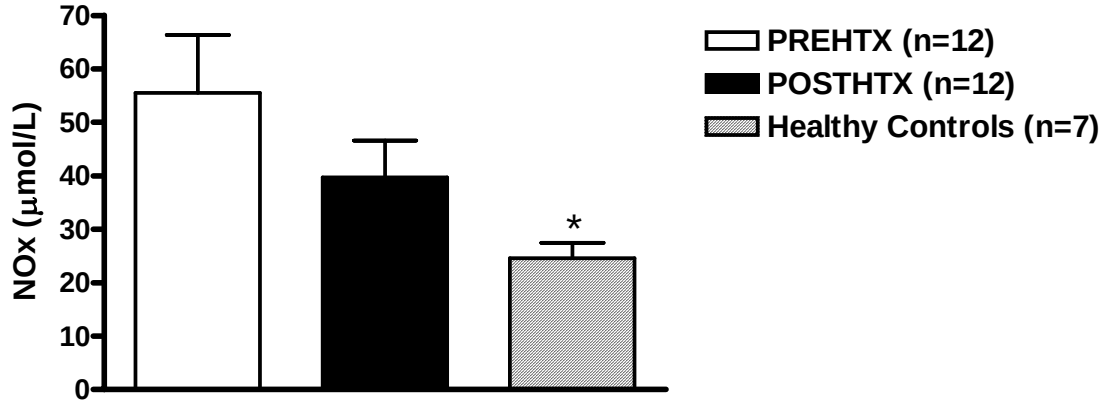

Figure 4-8. Nitrate/nitrite (NOx) before and after heart transplantation. *P≤0.05 vs. PREHTX.

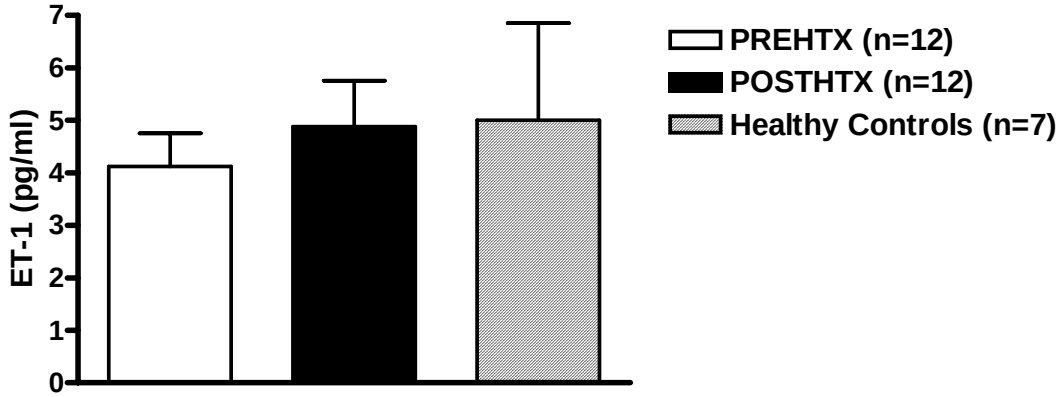

Figure 4-9. Endothelin-1 (ET-1) before and after heart transplantation.

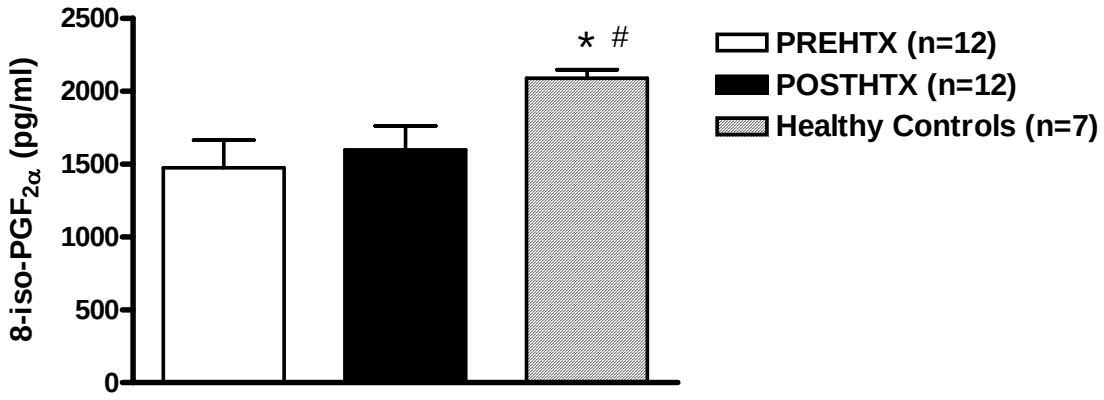

Figure 4-10. Eight (8)-iso-prostanglandin-F2α (PGF2α) before and after heart transplantation. *P≤0.05 vs. PREHTX; #P≤0.05 vs. POSTHTX.

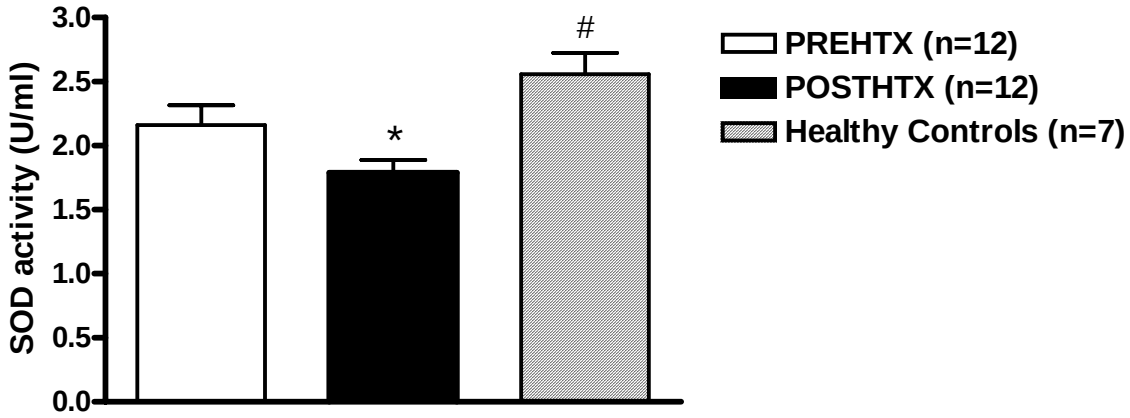

Figure 4-11. Superoxide dismutase (SOD) activity before and after heart transplantation. *P≤0.05 vs. PREHTX; #P≤0.05 vs. POSTHTX.

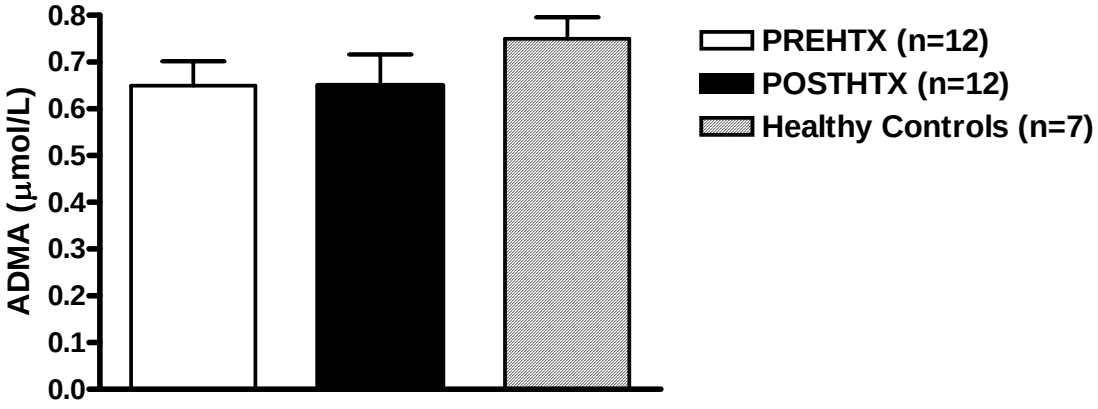

Figure 4-12. Asymmetric dimethylarginine (ADMA) before and after heart transplantation.

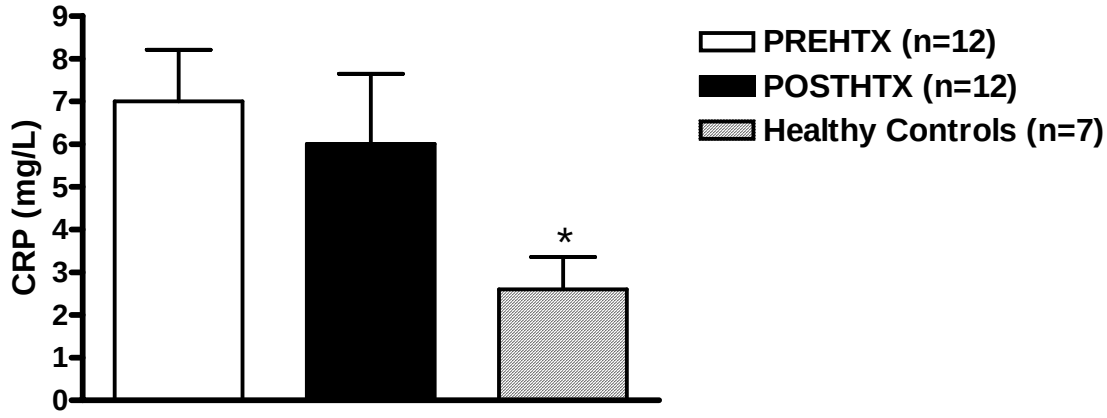

Figure 4-13. C-reactive protein (CRP) before and after heart transplantation. *P≤0.05 vs. PREHTX; #P≤0.05 vs. POSTHTX.

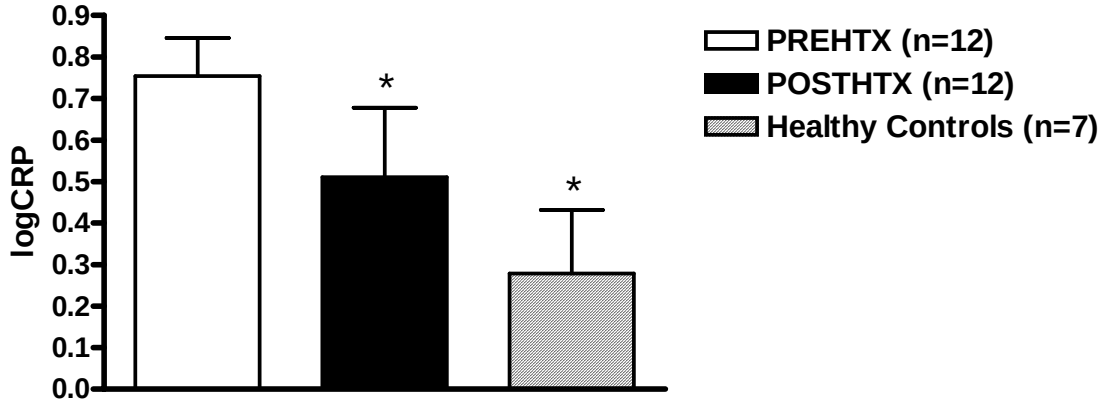

Figure 4-14. Log-transformed C-reactive protein (logCRP) before and after heart transplantation. *P≤0.05 vs. PREHTX.

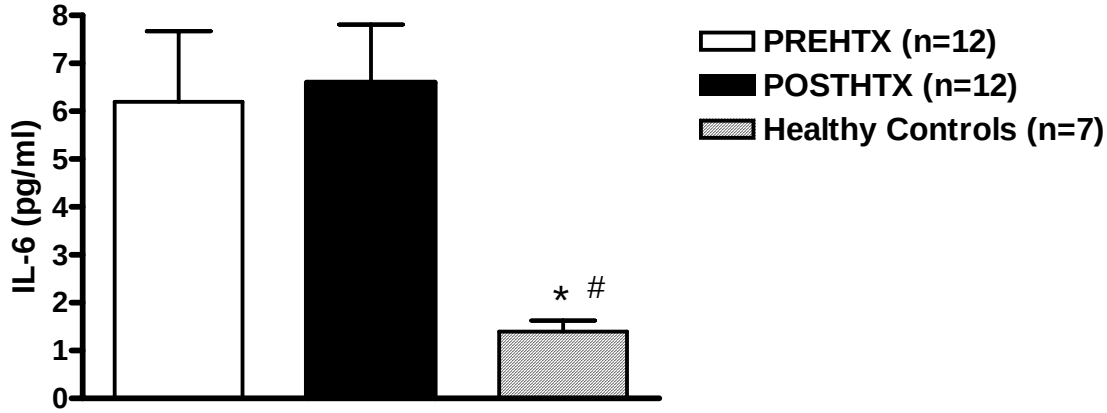

Figure 4-15. Interluekin-6 (IL-6) before and after heart transplantation. *P≤0.05 vs. PREHTX; #P≤0.05 vs. POSTHTX.

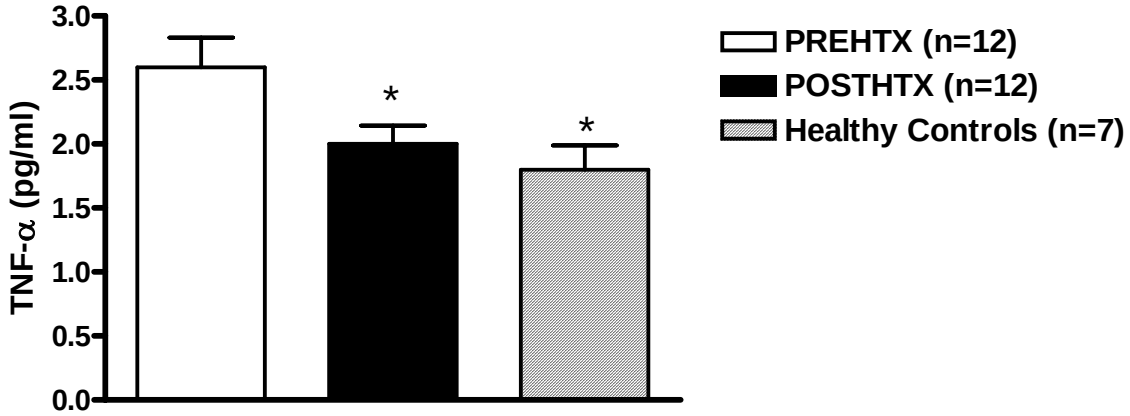

Figure 4-16. Tumor-necrosis factor-alpha (TNF-α) before and after heart transplantation. *P≤0.05 vs. PREHTX; #P≤0.05 vs. POSTHTX.

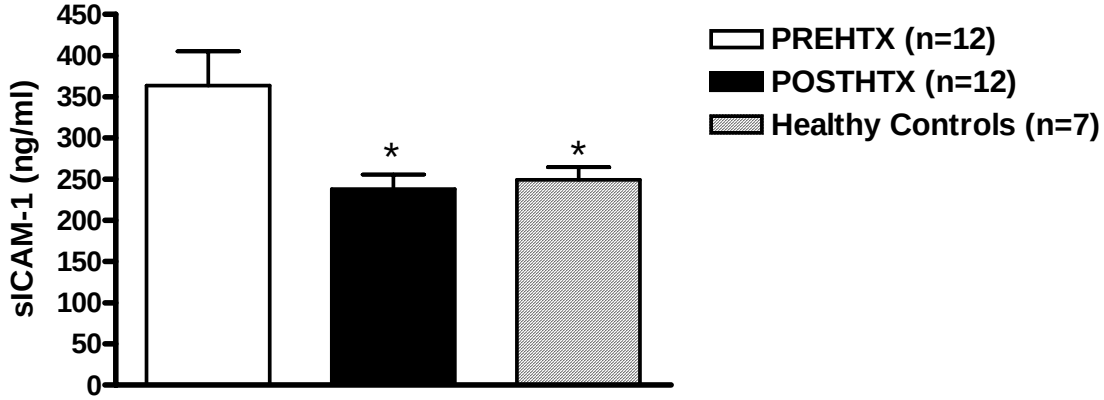

Figure 4-17. Soluble intercellular adhesion molecule-1 (sICAM-1) before and after heart transplantation. *P≤0.05 vs. PREHTX.

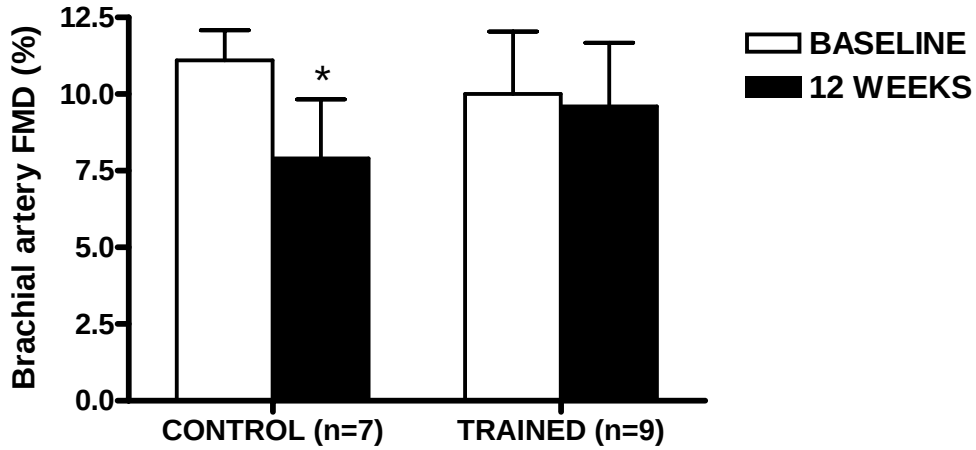

Figure 4-18. Brachial artery flow-mediated dilation (FMD) at baseline and after 12 weeks of exercise training or control. *P≤0.05 vs. BASELINE within-groups.

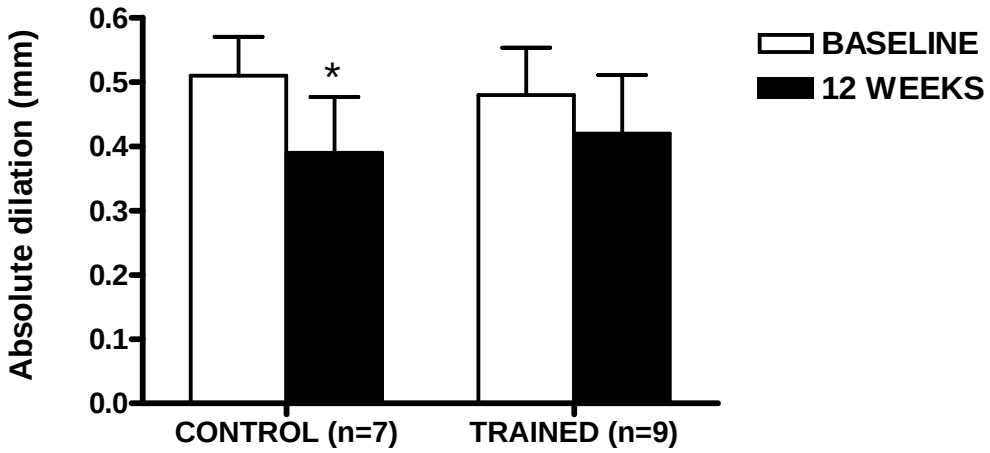

Figure 4-19. Brachial artery absolute diameter dilation at baseline and after 12 weeks of exercise training or control. *P≤0.05 vs. BASELINE within-groups.

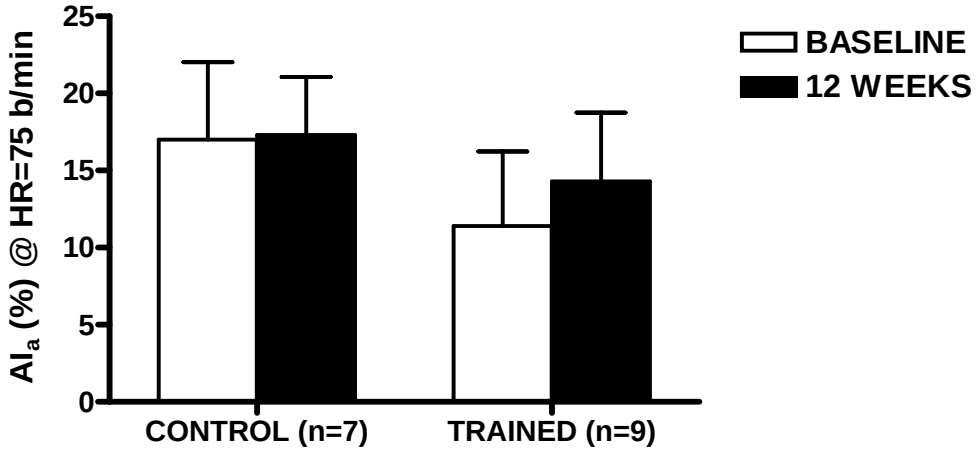

Figure 4-20. Aortic augmentation index (AI_a) normalized for heart rate at 75 b/min at baseline and after 12 weeks of exercise training or control. HR=heart rate.

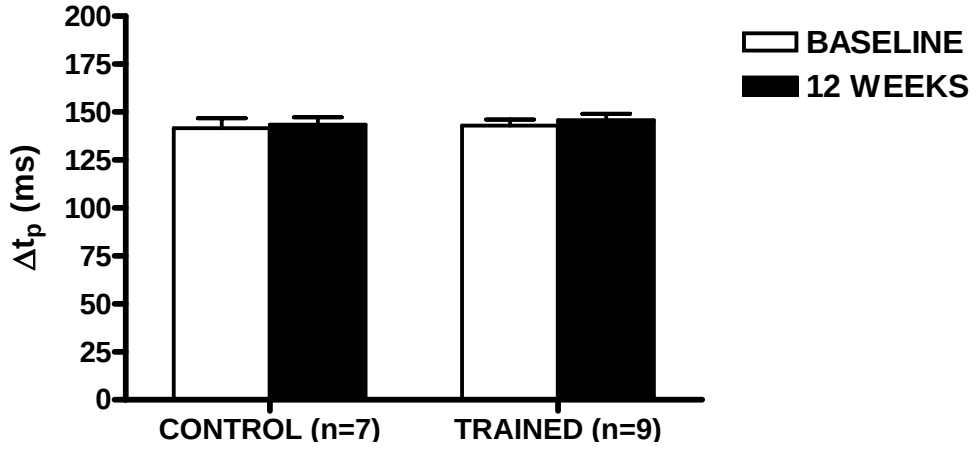

Figure 4-21. Roundtrip travel time of reflected wave (Δt_p) at baseline and after 12 weeks of exercise training or control.

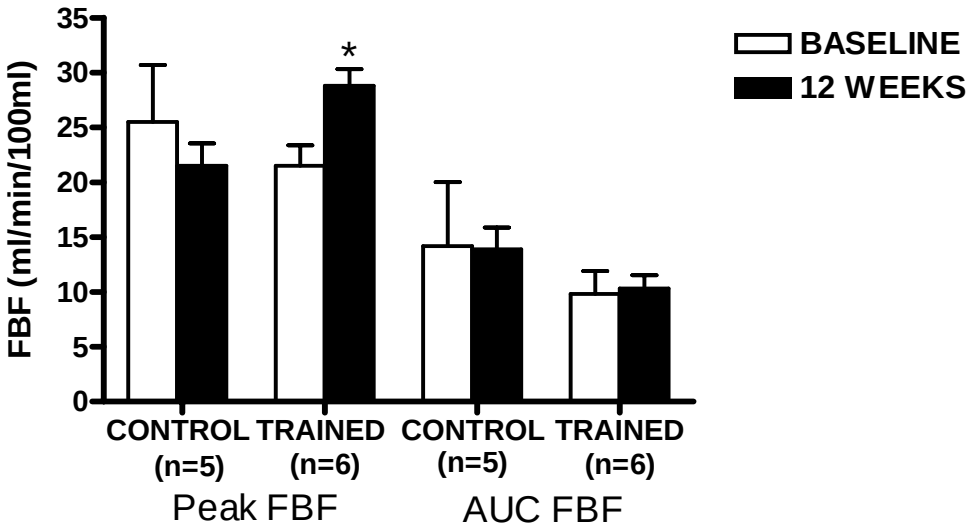

Figure 4-22. Peak and total area under curve (AUC) forearm blood flow (FBF) at baseline and after 12 weeks of exercise training or control. *P≤0.05 vs. BASELINE within-groups.

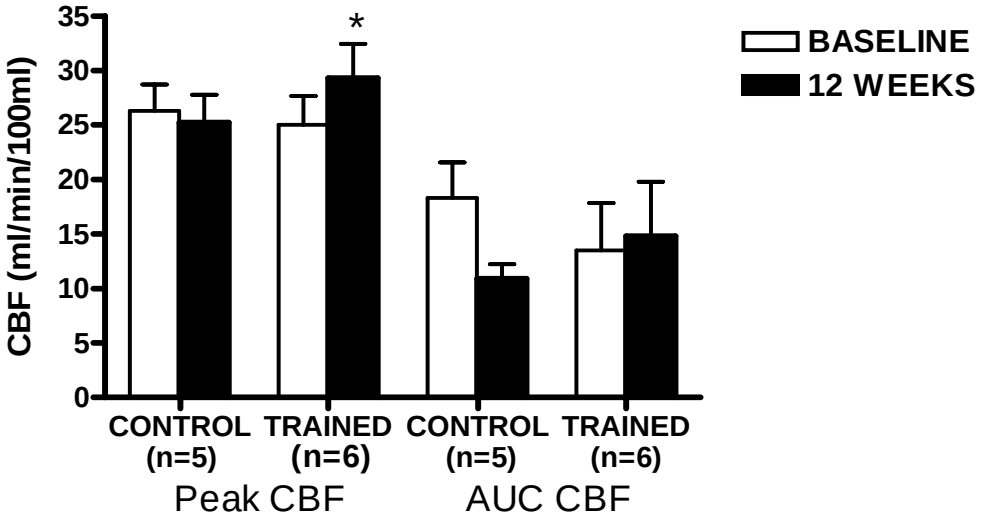

Figure 4-23. Peak and total area under curve (AUC) calf blood flow (CBF) at baseline and after 12 weeks of exercise training or control. *P≤0.05 vs. BASELINE within-groups.

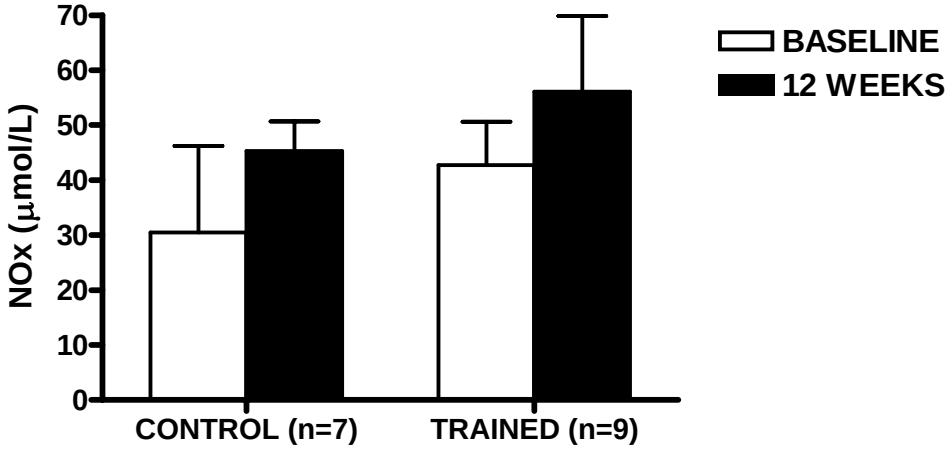

Figure 4-24. Nitrate/nitrite (NOx) at baseline and after 12 weeks of exercise training or control.

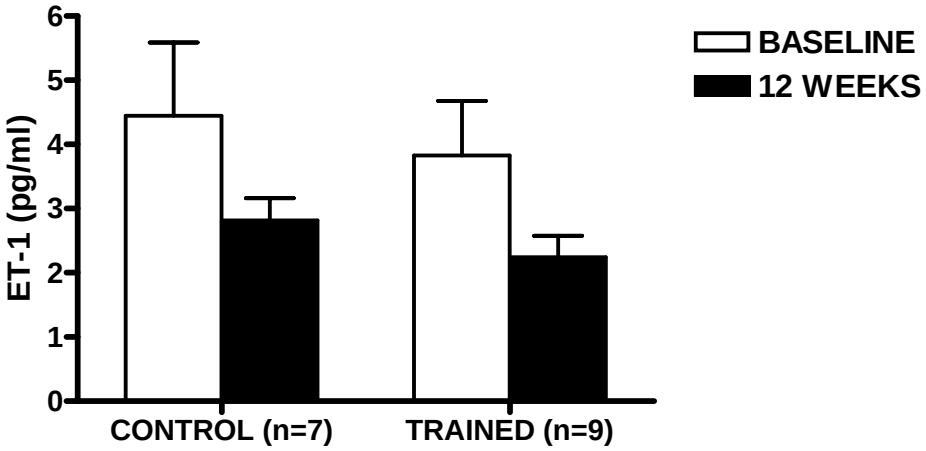

Figure 4-25. Endothelin-1 (ET-1) at baseline and after 12 weeks of exercise training or control. *P≤0.05 vs. BASELINE within-group.

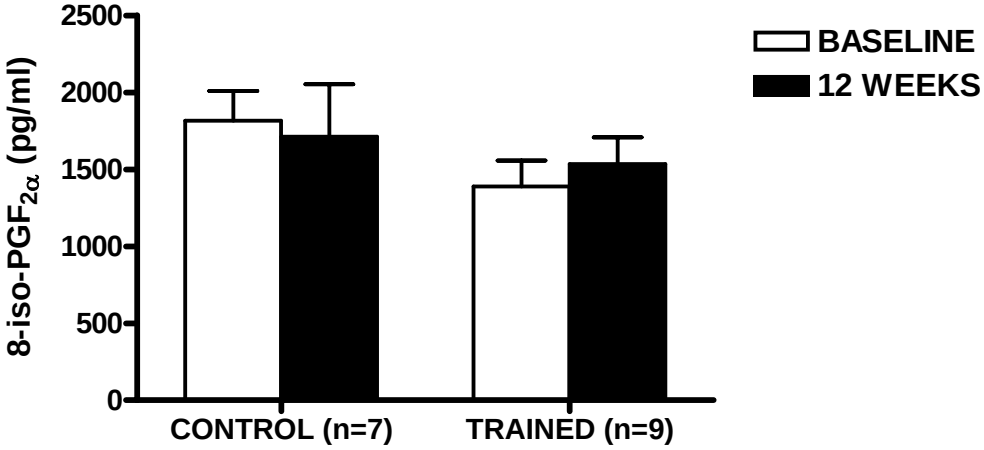

Figure 4-26. Eight (8)-iso-prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) at baseline and after 12 weeks of exercise training or control.

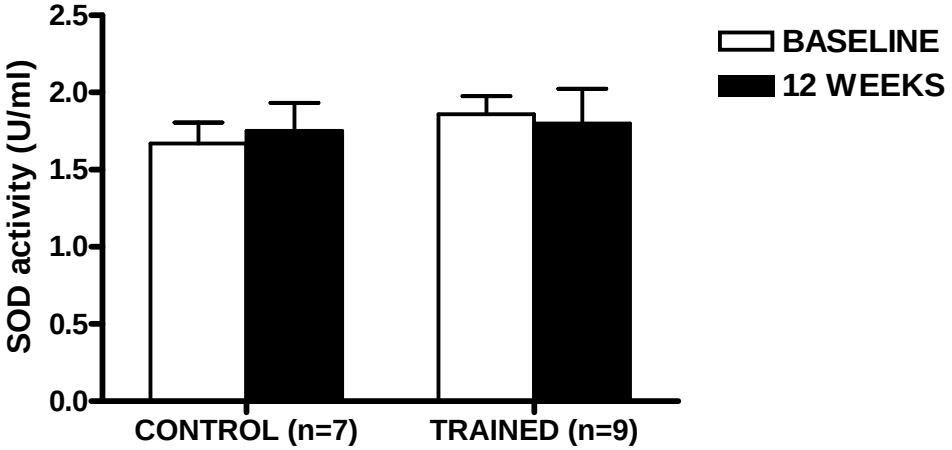

Figure 4-27. Superoxide dismutase (SOD) activity at baseline and after 12 weeks of exercise training or control.

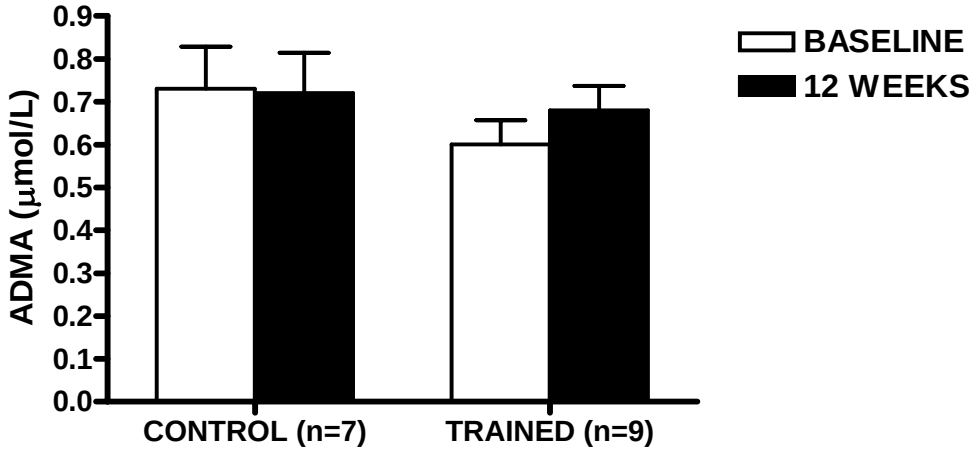

Figure 4-28. Asymmetric dimethylarginine (ADMA) at baseline and after 12 weeks of exercise training or control.

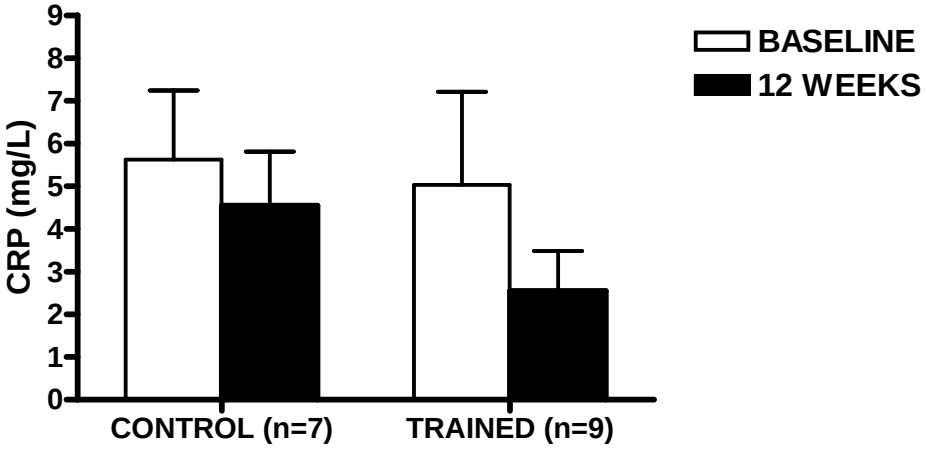

Figure 4-29. C-reactive protein (CRP) at baseline and after 12 weeks of exercise training or control.

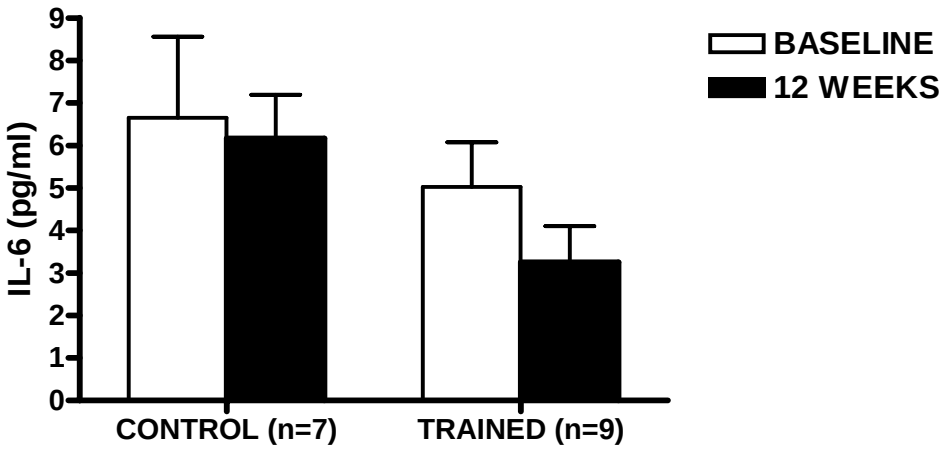

Figure 4-30. Interleukin-6 (IL-6) at baseline and after 12 weeks of exercise training or control.

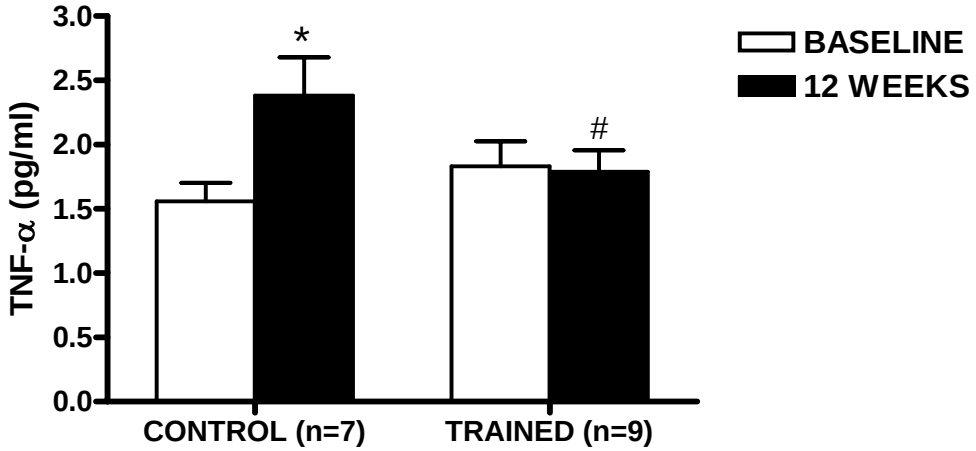

Figure 4-31. Tumor necrosis factor-alpha (TNF-α) at baseline and after 12 weeks of exercise training or control. *P≤0.05 vs. BASELINE within-groups; #P≤0.05 vs. CONTROL at same time-point.

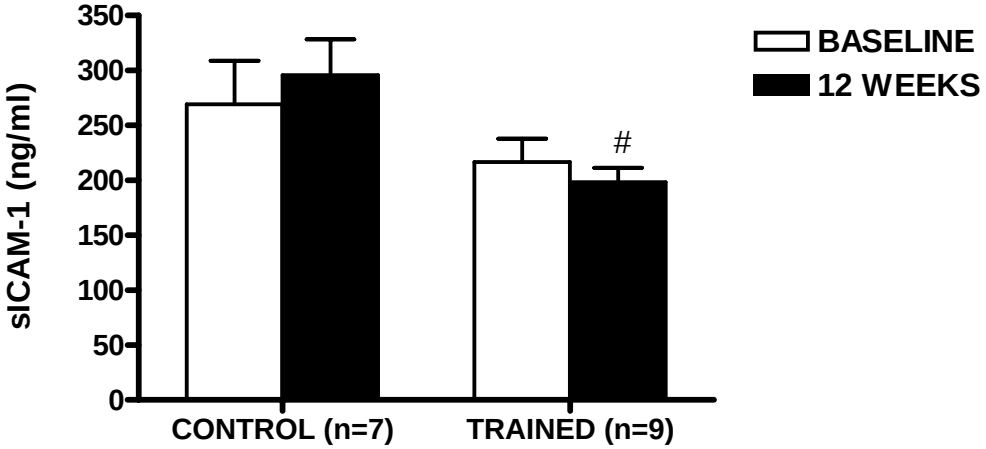

Figure 4-32. Soluble intercellular adhesion molecule-1 (sICAM-1) at baseline and after 12 weeks of exercise training or control. #P≤0.05 vs. CONTROL at same time-point.

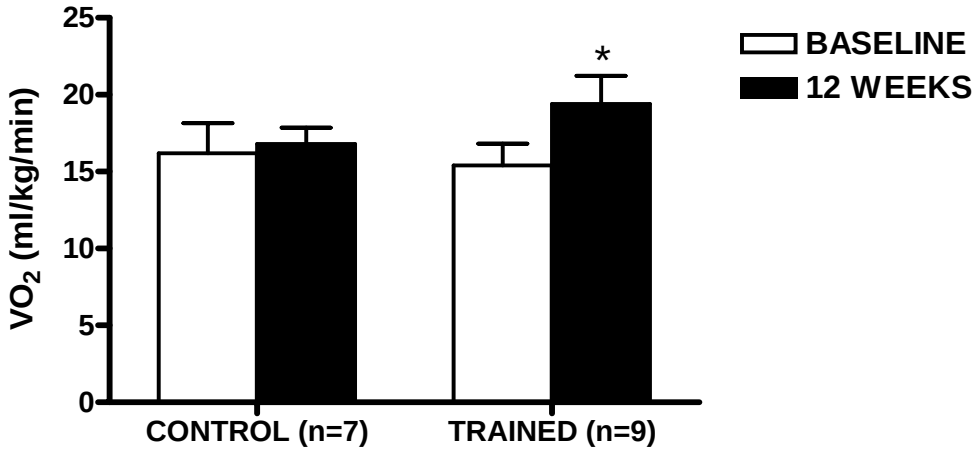

Figure 4-33. Peak exercise oxygen uptake (VO_2) on graded exercise test at baseline and after 12 weeks of exercise training or control. *P≤0.05 vs. BASELINE within-groups.

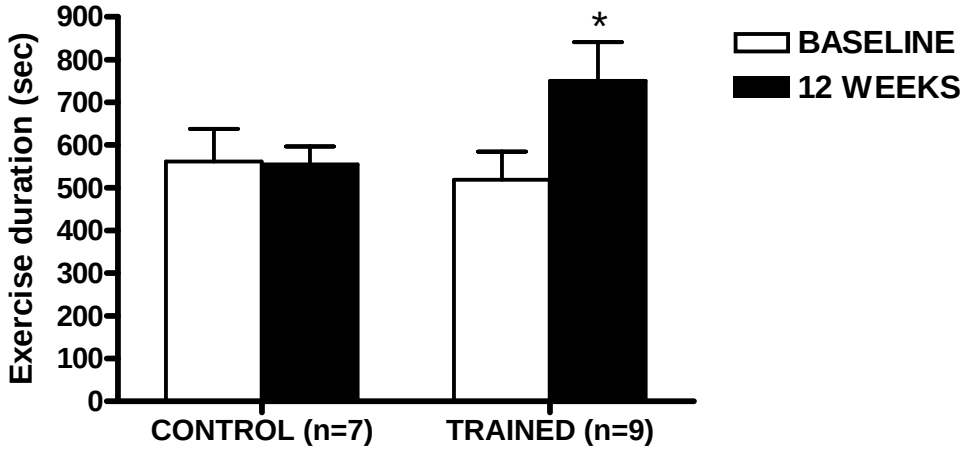

Figure 4-34. Peak exercise duration on graded exercise test at baseline and after 12 weeks of exercise training or control. *P≤0.05 vs. BASELINE within-groups.

CHAPTER 5
DISCUSSION

This is the first prospective study to evaluate the effects of HT on peripheral endothelial function, arterial stiffness, plasma vasoactive balance, oxidative stress, antioxidant enzyme activity, inflammation, and NO inhibition in end-stage HF patients. The first major finding is that upper limb conduit artery endothelial function (brachial FMD) is improved after HT. Second, peak vasodilatory capacity of resistance arteries of lower limb (peak calf BF) is increased after HT and comparable to values recorded in age-matched healthy controls, but peak vasodilatory capacity of upper limb (peak forearm BF) is not significantly changed. Third, peripheral and central systolic, diastolic, mean, and pulse blood pressures are increased after HT, but indices of arterial stiffness are not significantly altered. Fourth, plasma levels of inflammatory markers CRP, IL-6, TNF-α, and sICAM-1 are significantly elevated in end-stage HF subjects before HT compared to age-matched healthy control subjects. More importantly, CRP, TNF-α, and sICAM-1 are significantly decreased following HT. Fifth, there is a significant decrease in plasma SOD activity after HT to levels significantly lower than age-matched healthy controls, but there is no change in plasma 8-iso-$PGF_{2\alpha}$. Sixth, there is no significant change in plasma NOx or ET-1 after HT. Lastly, there is no significant change in plasma levels of the endogenous NO inhibitor, ADMA in HF patients after HT.

This is also the first prospective, controlled study to investigate the effects of supervised endurance exercise training on peripheral endothelial function, arterial stiffness, plasma vasoactive balance, oxidative stress, antioxidant enzyme activity,

inflammation, and endogenous NO inhibition in HTR. The major findings of this study are that 12 weeks of supervised endurance exercise training (walking) attenuates a progressive decline in upper limb conduit artery endothelial function (brachial FMD) in HTR. Second, 12 weeks of supervised endurance exercise training improves peak vasodilatory capacity of resistance arteries of upper (forearm) and lower (calf) peak blood flow in HTR, but peak limb vasodilatory capacity is unaltered in control HTR who do not participate in supervised exercise training. Third, 12 weeks of supervised exercise training results in a significant increase in peak exercise VO_2 and exercise duration, but remain unchanged in HTR who do not participate in supervised exercise training. Fourth, 12 weeks of supervised exercise training attenuates a progressive increase in the major inflammatory cytokine TNF-α in HTR, but does not alter the inflammatory proteins CRP, IL-6, or sICAM-1. Fourth, exercise training does not alter plasma levels of 8-iso-$PGF_{2\alpha}$, SOD activity, NOx, or ET-1 in HTR. Fifth, exercise training does not appear to alter peripheral or central systolic, diastolic, or pulse blood pressure, and does not alter arterial stiffness (AI_a) in HTR. Sixth, plasma levels of the endogenous competitive inhibitor of nitric oxide synthase, ADMA, is not altered following 12 weeks of supervised exercise training in HTR.

Peripheral Conduit Artery Endothelial Function and Heart Transplantation

There is conflicting data on whether peripheral endothelial function of upper limb conduit arteries improves after HT. One cross-sectional study reported that endothelial function in the brachial artery does not improve in HTR with antecedent ischemic HF etiology, but that brachial artery endothelial function does improve in HTR with non-ischemic HF etiology (Patel et al. 2001). In contrast, other studies report reduced

brachial artery FMD in HTR compared to healthy controls irrespective of etiology of HF (Saxonhouse et al. 2000; Lim et al. 2002; Schmidt et al. 2002; Cuppoletti et al. 2003). However, there have been no prospective, longitudinal studies of brachial artery endothelial function in the same cohort of HF subjects before and after HT compared to values in age-matched, healthy controls. The present study demonstrates that brachial artery FMD in end-stage HF subjects is not significantly different than age-matched healthy controls (6.44 vs. 6.81%, p=NS), but that brachial artery FMD improves significantly after HT (6.44 vs. 9.63%, $p<0.05$). In addition, there was a trend (p=0.06) in brachial artery FMD to be greater in HTR than age-matched healthy controls (9.63 vs. 6.81%). These data suggest that brachial artery FMD is improved early after HT to levels greater than age-matched healthy controls.

Several cross-sectional studies report that brachial artery FMD is impaired in HTR compared to healthy controls (Saxonhouse et al. 2000; Patel et al. 2001; Lim et al. 2002; Schmidt et al. 2002; Cuppoletti et al. 2003). Saxonhouse et al (2000) reported that brachial artery FMD in HTR, one to seven years post-transplant, was similar to stable class IV HF patients (4.4 vs. 3.3%, p=NS), but was decreased compared to age-matched healthy controls (4.4 vs. 9.8%, $p<0.01$). Patel et al. (2001) compared brachial FMD of ischemic vs. non-ischemic HF patients, to two groups of HTR with antecedent ischemic and non-ischemic HF etiology. HTR with ischemic HF etiology had significantly decreased brachial FMD compared to non-ischemic HTR (5.5% vs. 13.0%, p=0.002). FMD in non-ischemic HTR did not differ from healthy, age-matched controls (13.0% vs. 13.9%, p=NS). Lim et al. (2002) reported reduced brachial artery FMD in14 young HTR (mean age 18 years) with non-ischemic HF etiology, compared to age- and gender-

matched healthy controls (3.0% vs. 15.5%, $p<0.05$). Lastly, Schmidt et al. (2002) reported a reduced brachial FMD (1.4% vs. 8.4%, $p<0.05$) in sedentary HTR (age 60 ± 6 years) six years post-transplant, compared to age-matched sedentary, healthy controls.

The major reason for these discordant FMD results is likely due to the differences in study design and time after transplant that the measurements were obtained. The present study was a prospective, longitudinal design and brachial artery FMD measurements were obtained at approximately 8 weeks after HT in all subjects. In contrast, the above studies were cross-sectional designs and the time after HT that the measurements were obtained ranged from 1 to 6 years. Thus, selection bias and the heterogeneous length of time after HT likely affected the results. It is possible that early after HT brachial artery FMD is improved, but that brachial artery FMD progressively declines months to years after HT. The present study demonstrates that although FMD was improved after HT in all patients, the HTR who did not participate in exercise training, experienced significant declines in brachial artery FMD over a period of two to five months after HT (11.1 vs. 7.9%, $p<0.05$). Taken together, these data suggest that brachial artery FMD is improved early after HT, but progressively declines in the first year and persists indefinitely in sedentary HTR. The potential mechanisms for this phenomenon will be discussed in the sections that follow.

Peripheral Resistance Artery Endothelial Function and Heart Transplantation

Several studies have evaluated flow-mediated dilation of limb resistance arteries in HF subjects before and after HT using venous occlusion strain-gauge plethysmography. In a longitudinal study, Sinoway et al. (1988) reported that resting and peak hyperemic forearm blood flow did not increase immediately after transplant (21 vs.

25 ml/min/100ml, p=NS), but increased significantly at four months (21 vs. 43 ml/min/100ml,p <0.05). Kubo et al. (1993) measured forearm BF after methacholine and reactive hyperemia in the same six patients before and 4 months after HT. Resting forearm BF was similar before and after HT (3.3 vs. 3.7 ml/min/100ml), but peak FBF increased significantly at four months after HTX at each dose of methacholine and peak forearm BF during reactive hyperemia increased significantly after HTX from 19.0 to 44.8 ml/min/100 ml. These studies suggests that impaired forearm BF is not directly related to normalizing cardiac output and mean arterial pressure, but that it is increased after several months possibly as a result of resumption of daily physical activities. In addition, these data suggest that both agonist-mediated (methacholine) and flow-mediated (reactive hyperemia) forearm vasodilation of resistance vasculature is increased after HT.

In contrast, Cavero et al. (1994) reported that peak FBF during reactive hyperemia was increased significantly 24-36 hours after HT before cyclosporine therapy started (11.2 vs. 21.2 ml/min/100 ml, p<0.05), and despite initiation of cyclosporine therapy peak FBF was similar 6-8 days after HT (22.3 ml/min/100ml), and after 6 weeks post-HT (22.7 ml/min/100ml). One possible explanation of these discordant results is that the peak forearm BF of the pre-HT subjects in the study by Cavero and colleagues. (1994) were extremely low, therefore subjects may have had more peripheral edema, lower cardiac index, or have been more severely deconditioned than the subjects in the former studies. Furthermore, because there was no age-matched healthy controls in these studies, it was unknown whether the forearm vasodilatory capacity returns to “normal.” In the present study, we found that peak forearm BF in our small cohort of HTR was

significantly lower in end-stage HF subjects before HT, compared to healthy-age-matched control subjects, but did not increase significantly after HT.

Peak CBF was significantly lower in end-stage HF subjects compared to age-matched healthy controls. However, peak CBF significantly increased after HT. This is the first report of vasodilatory capacity of resistance arteries in the calf before and after HT. Thus, it appears that the calf resistance vasculature may be more responsive to the effects of normalized cardiac output and arterial pressure after HT, or affected by the resumption of daily physical activity. Although it must be emphasized that the cohort is small, it appears that forearm and calf resistance artery function returns to levels similar to age-matched-healthy controls.

Pulse Wave Analysis and Heart Transplantation

This was the first investigation that studied the effects of HT on systemic arterial stiffness in end-stage HF subjects. Our results demonstrate that arterial stiffness does not change significantly following HT. AI_a, a measure of stiffness in central elastic and peripheral muscular arteries, was not significant changed after HT (8.9 vs. 13.5%, p=NS). Additionally, roundtrip travel time of the reflected wave to the periphery and back to the aorta (Δt_p), an inverse of pulse wave velocity, was not significantly altered after HT (146 vs. 140 ms, p=NS). This positive outcome occurs in the setting of a significant increase in peripheral (brachial) and central (aorta) systolic, diastolic, and mean blood pressure after HT, but without a change in peripheral and central pulse pressure. Thus, restoration of cardiac output results in significant increase in mean arterial blood pressure without significantly altering arterial reflected pulse wave properties (AI_a and central pulse pressure) after HT. However, the increased aortic systolic blood pressure is accompanied

by increased aortic systolic tension time index (AsTTI), an indicator of LV myocardial oxygen demand during systole, after HT (1826.1 vs. 3254.3 mmHg/sec/min, $p<0.01$), without any change in diastolic tension time index (DTPI), an indicator of diastolic perfusion (2843.5 vs. 3079.7 mmHg/sec/min).

The study also demonstrates that augmentation pressure, pressure generated by reflected wave, is significantly higher in age-matched healthy controls than in end-stage HF subjects before HT (10.4 vs. 3.8 mmHg, $p<0.05$) and after HT (10.4 vs. 1.8 mmHg, $p<0.05$). However, there was no significant difference in AI_a normalized for heart rate, between age-matched healthy controls vs. before HT (17.6 vs. 8.9%, p=0.09), and vs. after HT (17.6 vs. 13.5%, p=NS). These data suggest that systemic arterial stiffness in age-matched healthy controls is not significantly different than end-stage HF and HTR subjects. It is possible that poor cardiac function and the combination of antihypertensive medications in HF subjects accounted for the lower mean blood pressure and lack of difference in arterial stiffness between HF and age-matched healthy controls. This is because muscular artery vasodilators such as ARB's, decrease pulse wave velocity and wave reflection amplitude resulting in reduced AI_a and central and systolic pulse pressure (Nichols 2005). Furthermore, antihypertensive medications which alter arterial pulse wave properties likely account for the lack of difference in AI_a between HTR and age-matched healthy controls.

Endothelial-Derived Vasoactive Balance and Heart Transplantation

In the present study, there was a 40% non-significant reduction in plasma NOx in end-stage HF subjects after HT (55.5 vs. 39.7 uM, p=NS). This finding did not support our hypothesis that plasma NOx would increase following HT reflecting improved endothelial function. Paradoxically, an improvement in conduit and resistance artery

flow-mediation vasodilation after HT occurred despite the 40% reduction in NOx. We suspect that these puzzling findings can be attributed to the multiple stimuli for NO. For example, the source of NO in end-stage HF subjects before HT is influenced by sources in addition to eNOS. It is well established that inflammatory cytokines, such as TNF-α and IL-6, both elevated in HF (Levine et al. 1990), stimulate expression of iNOS in vascular smooth muscle (Chester et al. 1998) and the failing myocardium (Drexler 1998) resulting in overproduction of NO. In this scenario, increased NO production has a negative inotropic effect on the myocardium (Habib et al. 1996; Drexler 1998) and impairs EDV (Kessler et al. 1997). Indeed, in the present study plasma NOx levels significantly higher in HF subjects than age-matched healthy controls.

Although TNF-α is decreased after HT levels, plasma NOx did not change significantly after HT. However, NOx levels may be confounded by several factors in HTR. Urinary NOx is elevated during acute endomyocardial rejection in animal models (Mugge et al. 1996), and also has been reported to increase before any clinical signs of rejection are evident (Winlaw et al. 1994). In contrast, the administration of the immunosuppressive agent cyclosporine inhibits induction of cytokine stimulated iNOS mRNA, and reduces the accumulation of NOx (Marumo et al. 1995). Thus, when HTR are not in acute clinical rejection, NOx levels likely reflect the competition between cytokine induced iNOS expression, “subclinical rejection,” and iNOS inhibition by cyclosporine. Moreover, we speculate that in healthy controls, where cytokine induction of iNOS is minimal, plasma levels of NOx more accurately reflect NO derived from the eNOS pathway in the vascular endothelium.

In the present study, plasma levels of ET-1 levels did not significantly change after HT, and ET-1 in end-stage HF and HTR were not significantly different than age-matched healthy controls. Cyclosporine has been demonstrated to increase ET-1 in vitro (Marsen et al. 2003), and acute oral dose of cyclosporine in transplant subjects results in an acute increase in plasma ET-1 levels by six hours. As such, Haas et al. (1993) reported that plasma ET-1 was elevated in HTR 2 years after HT (range 9 days to 3 years post-HT) compared to normal healthy controls (5.2 vs. 1.9 pg/ml). In contrast, Piquard et al. (2001) reported in a prospective study, that ET-1 increased significantly within the first 2 weeks after surgery, but normalized by 2 months post-HT to levels similar to healthy controls (2.8 vs. 1.4 pg/ml). These discordant results may be explained by the cross-sectional design and the heterogenous time after transplant which the samples were obtained by Haas and colleagues (1993). Although cyclosporine therapy may be a primary contributor to elevated ET-1 in some HTR, the data of Piquard et al. (2001) and the present study suggest that ET-1 levels may, in fact, be similar to healthy controls by two months after HT.

Lipid Peroxidation, Antioxidant Enzyme Activity, Endogenous Nitric Oxide Inhibition and Heart Transplantation

Administration of vitamin C, a known scavenger of superoxide anion radical, reverses impaired radial artery EDV in HF, suggesting that superoxide plays a significant role in endothelial dysfunction in HF patients (Hornig et al. 1999). In addition, lipid peroxidation products such as lipid peroxides (LPO), malonylaldehyde (MDA), and 8-iso-$PGF_{2\alpha}$ are elevated in chronic HF subjects (Keith et al. 1998; Polidori et al. 2004). In the present study, there was no significant difference in 8-iso-$PGF_{2\alpha}$ before and after HT. Surprisingly, 8-iso-$PGF_{2\alpha}$ was significantly greater in age-matched healthy controls than

in both end-stage HF subjects (2167.7 vs. 1474.8, $p<0.01$) and HTR (2167.7 vs. 1597.4 pg/ml, $p<0.05$). These data are in contrast to the study by Polidori et al. (2004) who reported significantly higher plasma F_2 isoprostanes in chronic HF than in healthy controls, and Keith et. al. (1998) who reported higher plasma MDA and LPO in chronic HF subjects. Although the precise mechanism for these discordant results are unclear from the present study, we propose that the HF and HTR subjects may have lower levels of lipid peroxidation due their pharmacological regimen which includes ACE inhibitors/ARB's and HMG-CoA reductase inhibitors (statins). Infusion of ANG II into pigs (Haas et al. 1999) and humans (Murphey et al. 2003) increases levels of lipid peroxidation in plasma, suggesting that ANG II is a significant contributor to plasma levels of oxidative stress. In the present study, all HF and HTR subjects were on ACE inhibitors or ARB's, both of which are known to inhibit ANG II stimulated production of superoxide by activation of NADPH oxidase via the AT-1 receptor in the vascular wall (Nickenbig and Harrison 2002). In addition to their lipid-lowering effects, statins also have a well documented antioxidant effect via upregulation of eNOS and decreased NADPH oxidase activity *in vitro* (Mason et al. 2004). In humans, Desideri et al. (2003) reported that statin therapy lowers urinary and plasma levels of 8-iso-$PGF_{2\alpha}$ and in the present study five subjects before HT and all subjects after HT were on statins. Therefore, these studies suggest that ACEI/ARB and statin pharmacological regimens may have influenced our measurement of lipid peroxidation in the HF and HTR in this study.

The present study also observed that plasma SOD activity is decreased after HT (2.16 vs. 1.79 U/ml, $p<0.05$), and is significantly lower than age-matched healthy

controls (1.79 vs. 2.56 U/ml, $p<0.01$). These data are consistent with animal studies that report that cyclosporine decreases plasma and tissue total antioxidant status, SOD activity, glutathione peroxidase (GPX) activity, and catalase activity in rats (Mun 2000). In humans, Perez et al. (2002) reported reduced plasma SOD activity in HTR compared to class III HF patients, but no difference in GPX or catalase activity (Perez et al. 2002). Thus, in the present study, the toxic effects of cyclosporine likely account, in part, for the decrease in plasma SOD activity after HT.

ADMA also did not change in end-stage HF subjects after HT. We hypothesized that ADMA would be elevated due to oxidative inactivation of DDAH, and therefore would result in accumulation of ADMA. Although our hypothesis was not supported, plasma lipid peroxidation did not increase after HT and therefore the lack of ADMA elevation was consistent with this finding.

Inflammatory Markers and Heart Transplantation

CRP is an acute phase protein released by liver cells in response to inflammatory cytokines IL-6 and TNF-α. CRP has been reported to be a strong independent predictor of coronary artery vasculopathy, cardiac allograft failure, and mortality in HTR (Eisenberg et al. 2000; Labarrare et al. 2002). In addition to being a marker of future cardiovascular risk, several *in vitro* studies suggest that CRP is directly involved in the development of endothelial dysfunction because endothelial cells incubated with CRP demonstrate decreased expression of eNOS mRNA, eNOS protein, and eNOS bioactivity (Venupogal et al. 2002), as well as increased expression of vascular adhesion molecules VCAM-1 and ICAM-1 (Pasceri et al. 2000). Fichtlscherer et al. (2000) reported that elevated CRP correlated inversely with impaired EDV in a cohort of CAD patients, and a

reduction in CRP after 3 months was associated with improved EDV. In the present, study CRP and IL-6 were significantly higher in end-stage HF subjects than in age-matched healthy controls. Following HT, IL-6 remained higher than age-matched controls, but CRP (log transformed) decreased significantly after HT to levels comparable to healthy controls. To our knowledge, this is the first report of plasma IL-6 and CRP in same cohort of subjects before and after HT which clearly illustrates the hyperinflammatory state of end-stage HF subjects compared to normal healthy controls.

TNF-α is an inflammatory cytokine produced from activated macrophages, T-lymphocytes (Blake and Ridker 2003), and adipose tissue (Kern et al. 2001). In end-stage HF subjects, TNF-α is expressed in the failing myocardium (Torre-Amione et al. 1996b) which can be shed into the circulation and increase plasma levels of TNF-α as severity of HF increases (Torre-Amione et al. 1996a). TNF-α has a negative inotropic effect on myocardium and increases expression of iNOS in myocardium (Habib et al. 1996; Wildhirt et al. 2001a) and vascular smooth muscle (Chester et al. 1998). Importantly, *in vitro* studies demonstrate that TNF-α has a direct effect on endothelial function by causing post-transcriptional modification of eNOS mRNA leading to early mRNA degradation (Yoshizumi et al. 1993). In chronic HF subjects, Katz et al. (1994) found TNF-α levels to be highly correlated with impaired forearm EDV in response to acetylcholine. Moreover, Weis et al. (2001) reported that treatment with simvastatin lowered IL-6 and TNF-α and was associated with improved acetylcholine-induced coronary EDV. In the present study, TNF-α was significantly elevated in end-stage HF subjects compared to age-matched healthy controls, and was significantly lower after HT. Thus, it is possible that the reduction in TNF-α after HT is one mechanism that

contributed to the improvement in conduit and resistance artery endothelial function after HT.

Expression of sICAM-1 and VCAM-1 on the endothelial wall in response to activation by ROS, CRP, TNF-α, and decreased NO, is a critical step in the development and progression of atherosclerosis (Hope and Meredith 2003). sICAM-1 is the circulating form of the endothelial adhesion molecule ICAM-1 which has been shed from the activated endothelium. When the endothelium is activated, ICAM-1 and VCAM-1 expression on the endothelial surface are involved in the adherence and transendothelial migration of circulating leukocytes into the subintimal space at sites of inflammation, including developing atheroslerotic plaques (Hope and Meredith 2003). The present study demonstrates that plasma sICAM-1 is elevated in end-stage HF subjects compared to age-matched healthy controls, and is significantly reduced after HT.

The mechanism for the decreases in CRP, TNF-α, and sICAM-1 and not IL-6 may be due to several factors. First, a major source of TNF-α, during end-stage HF is the failing myocardium (Torre-Amione et al. 1996b). Therefore, transplantation removes this source and therefore is likely a major contributor to the reduction in TNF-α after HT. Second, the calcineurin inhibitor cyclosporine inhibits IL-2 gene expression in T-lymphocytes, which prevents allograft rejection by inhibiting T-lymphocyte proliferation and stimulation of specific cytokines during allograft rejection. Cyclosporine suppresses the post-surgical increase in CRP and IL-6 usually observed after cardiac surgery (Van Lente et al. 1985) and decreases cytokine induced ICAM-1 and VCAM-1 expression on endothelial cells *in vitro* (Markovic et al. 2002). Therefore, chronic cyclosporine therapy in HTR may account for the decrease in CRP, TNF-α, and sICAM-1 after HT.

Peripheral Conduit Artery Endothelial Function and Exercise Training

The present study demonstrates that 12 weeks of supervised endurance exercise training in HTR preserves but does not improve brachial artery endothelial function (FMD: 10.0 vs. 9.6%, p=NS). However, HTR who do not participate in supervised exercise training show a progressive decline in endothelial function of the brachial artery (FMD: 11.1% vs. 7.9%, $p<0.05$). Two studies have reported that that brachial artery FMD is improved in CAD patients following supervised exercise training (Walsh et al. 2003; Edwards et al. 2004). Walsh et al. (2003) recently reported that 8 weeks of cross-training (aerobic/resistance training) in CAD patients resulted in improved brachial artery FMD (3.0% to 5.7%, $p<0.05$). Edwards et al. (2004a) found that 12 weeks of treadmill walking as part of a cardiac rehabilitation program resulted in an improvement in brachial artery FMD (7.9 vs. 11.2%, $p<0.05$). In contrast, Gokce et al. (2002) reported that 12 weeks of lower body endurance exercise training resulted in a significant increase in FMD of the posterior tibialis artery of the lower leg (7.9 vs. 11.2%, $p<0.05$), but only a non-significant trend in improvement in brachial artery FMD (6.4% vs. 8.3%, $p>0.05$) in CAD patients. Our results are consistent with Gokce et al. and suggest that 12 weeks of endurance training may not be sufficient to improve upper limb endothelial function. However, the finding of a progressive decrease in brachial artery FMD after 12 weeks in HTR who did not participate in supervised exercise training is a novel finding. One cross-sectional study evaluated brachial artery FMD in trained and untrained HTR six years after HT (Schmidt et al. 2002). Brachial artery FMD was significantly higher in HTR who participated in 6 months of cycling for 40 minutes 2 to 3 times per week compared to sedentary HTR (7.1% vs. 1.4%, $p<0.05$). Our prospective study support the results of Schmidt and colleagues (2002) and suggest that exercise training may have, in

fact, attenuated a progressive decline in peripheral endothelial function of upper limb conduit arteries that occurs early after HT. Taken together, these data demonstrate that supervised exercise training has a beneficial effect on the peripheral vasculature in HTR early after HT by attenuating a progressive decline in upper limb endothelial dysfunction demonstrated by a decrease in brachial artery FMD.

Peripheral Resistance Artery Endothelial Function and Exercise Training

Our study demonstrates that welve weeks of endurance exercise training improved the maximal flow-mediated vasodilatory capacity of forearm and the calf resistance arteries in a subgroup of HTR. Peak FBF during reactive hyperemia increased 34% (21.5 vs. 28.8 ml/min/100 ml, $p<0.05$) in HTR who performed supervised exercise training, but there was a non-significant 14% increase in peak FBF (25.5 vs. 29.2 ml/min/100ml, p=NS) in the control group who did not perform supervised training. Peak CBF increased 17% in HTR following 12 weeks of exercise training (25.0 vs. 29.3 ml/min100 ml, p=0.05), but there was no change in peak CBF in control HTR (26.3 vs. 25.2 ml/min/100ml, p=NS) who did not participate in supervised training. These data support the hypothesis that maximal vasodilatory capacity of the forearm and calf resistance arteries are improved following 12 weeks of lower body endurance exercise training in HTR.

Two prospective studies in subjects with essential hypertension reported improvements in maximal vasodilatory capacity of forearm resistance arteries during reactive hyperemia. In 20 subjects with essential hypertension, Higashi et al. (1999) reported a 23% increase in peak FBF during reactive hyperemia using plethysmography after a 12-week exercise training intervention. In subjects who showed an improvement in peak FBF, the increased FBF was abolished by the NO inhibitor, L-NMMA,

suggesting that the increase in peak FBF during reactive hyperemia of forearm resistance arteries was NO mediated. Tanaka et al. (1998) reported a 17% increase in peak VO_2, a 17.5% increase in peak FBF, and 18.9% increase in peak CBF during reactive hyperemia in older, hypertensive subjects after exercise training (walking) for 6 months 3-4 days per week.

Two studies have reported conflicting results on forearm and calf resistance artery vasodilatory capacity in subjects with chronic HF. Testa et al. (2000) reported a 50% increase in peak CBF during reactive hyperemia along with a 23% increase in peak VO_2 following 12 weeks of supervised exercise training in chronic HF subjects. In contrast, Dziekan and colleagues (1998) reported a decrease in peak forearm BF and an increase in peak calf BF in both trained and control HF subjects. The investigators suggest that that exercise training had no additional benefit in forearm or calf despite a 25% increase in peak VO_2 in the trained HF subjects. In aggregate, our blood flow data suggest that lower body dynamic endurance exercise training results in a systemic improvement in maximal flow-mediated vasodilatory capacity of limb resistance arteries likely due to in part to improvements in endothelial function in HTR. The possible mechanisms for this improvement in both forearm and calf resistance artery endothelial function will be discussed in the sections to follow.

Pulse Wave Analysis, Blood Pressure, and Exercise Training

Twelve weeks of supervised exercise training resulted in no significant change in arterial stiffness in HTR in the present study. Moreover, exercise training resulted in no change in AI_a or the round trip travel time of the reflected pressure wave (Δt_p). These findings were surprising due to the recent reports of exercise-induced improvements in

arterial stiffness in healthy sedentary, young men (Cameron et al. 1994), healthy, sedentary middle- aged and older men (Tanaka et al. 2000), and men with coronary artery disease (Edwards et al. 2004). The study by Edwards et al. (2004) reported a significant decrease AI_a and an increase in Δt_p with no change in systolic or mean blood pressure. Their results suggest that endurance exercise training improved systemic arterial stiffness independent of blood pressure in men with CAD. Consistent with our results, however, Parnell et al. (2002) reported that 8 weeks of endurance exercise training in chronic HF patients resulted in no change in aortic pulse pressure, aortic AI_a, and aortic pulse wave velocity after the exercise intervention.

It is possible antihypertensive medications confounded the results of the present study (Table 4-9). Angiotensin receptor blockers and calcium channel blockers decrease the amplitude and timing of the reflected wave returning to the aorta, and thus decrease AI_a, pulse wave velocity, aortic systolic and pulse blood pressure (Nichols 2005). Although the number of the antihypertensive medication was not different between TRAINED and CONTROL group at baseline and after 12 weeks, individual variability in response to medication may have confounded the results.

Twelve weeks of supervised endurance exercise training also resulted in no change in peripheral and central blood pressure components in HTR early after HT. Specifically, 12 weeks of exercise training resulted in no change in peripheral or central systolic, diastolic, pulse, or mean blood pressure in HTR. These results are consistent with the randomized, controlled study Kobashigawa et al. (1999) who randomized twenty-seven HTR two weeks after HT to an exercise or control group. Subjects performed supervised endurance exercise training as part of a cardiac rehabilitation

program for six-months. Resting brachial systolic blood pressure did not significantly change in the exercise trained group (126 vs. 121 mmHg, p=NS) or the control group (130 vs. 114 mmHg, p=NS) after 6 months of endurance exercise training beginning early after HT. As in the pulse wave analysis, these findings may be due to the confounding effects of antihypertensive medications being taken by all HTR in the study. An additional confounding factor could be the effects of cyclosporine on blood pressure (Ventura et al. 1997; Scherrer et al. 1990). Although the dose and serum trough levels of cyclosporine were not significantly different at baseline and at 12 weeks, the individual effects of cyclosporine on blood pressure at the time of each measurement is unknown. Alternatively, it is possible that the exercise training stimulus (frequency, intensity, or duration) was not sufficient to elicit changes in peripheral vascular resistance and thus alter mean or systolic blood pressure.

Endothelial-Derived Vasoactive Balance and Exercise Training

It has been well documented in animals (Sessa et al. 1994; Fukai et al. 2000; Spier et al. 2004) and humans (Hambrecht et al. 2003) that chronic exercise training increases eNOS mRNA and eNOS protein leading to increased synthesis of NO. Previous studies have reported that endurance exercise training results in an increase in plasma NOx in both healthy (Jungersten et al. 1997) and CAD subjects (Edwards et al. 2004), reflecting the increase in vascular NO production from the endothelial wall. In the present study, twelve weeks of exercise training resulted in a non-significant 31% increase in resting plasma nitrate/nitrite (NOx) levels in HTR. In addition, plasma NOx levels increased 48.5% over 12 weeks in the HTR who did not perform exercise training, but this change was not statistically different. Schaefer et al. (2001) found an acute increase in plasma NOx following a 30 minute bout of cycle exercise in healthy humans, but no change in

plasma NOx following exercise in HTR. The authors suggested that this was due to endothelial dysfunction and the inability of HTR to increase shear-stress mediated endothelial-derived NO synthesis. In support of this hypothesis, they noted that exercise capacity was significantly correlated to NOx in the healthy subjects ($r=0.95$, $p<0.01$), but not in the HTR. The lack of change in exercise-induced plasma NOx in the study by Schaefer (2001) and the large non-significant increase in NOx in both groups in the present study, suggests that an additional source of NO, in addition to endothelial-derived NO, contributes to plasma NOx levels in HTR. In light of the fact that resistance artery endothelial function increased after 12 weeks of exercise training, but the NO-mediated brachial artery FMD did not, it is possible that a non-NO mediated mechanism may have accounted for this increase in forearm and calf resistance artery endothelial function. Although several studies confirm that limb resistance artery vasodilation during reactive hyperemia is NO-dependent (Higashi et al. 1999; Meredith et al. 1996), indeed several studies suggest that peak vasodilation of limb resistance vasculature during reactive hyperemia is prostacyclin-dependent (Engelke et al. 1996; Tagawa et al. 1994).

As stated in a previous section, urinary NOx has been reported to be elevated during acute allograft rejection in HTR (Mugge et al. 1996). In the present study no HTR were in active rejection on endomyocardial biopsy at the time the blood sample was obtained. However, Winlaw et al. (1994) reported that an increase in urinary NOx precedes clinical evidence of allograft rejection in rats. Also, Wildhirt et al. (2001a) reported that 26% of HTR at 1 month post-transplantation had increased endomyocaridal iNOS expression which was associated with impaired coronary endothelial function and increased transcardiac NOx release. In a separate study, Wildhirt et al. (2001b) reported

that transcardiac TNF-α levels correlated to NOx levels at one month (r=0.081, p=0.001) and 12 months (r=0.62, p=0.04) post-HT suggesting that an inflammatory process is involved in coronary microvasculature dysfunction. Thus, increased NOx may be an early indicator of coronary artery vasculopathy and contribute to plasma NOx levels in HTR. Therefore, we conclude that in the present study it is possible that NO production from smooth muscle of coronary microvasculature iNOS expression and non-clinical allograft rejection contributed to the variable NOx levels and temporal trend for NOx to increase over 12 weeks in both trained and control HTR.

In the present study, plasma ET-1 levels did not significantly change after 12 weeks of exercise training or control period. However, there was a trend for a decrease (-41%) in ET-1 (p=0.09) and a non-significant decrease (-36%) in the control group. Endurance exercise training has been reported to reduced plasma ET-1 levels in young, healthy subjects (Maeda et al. 2001), and older women (Maeda et al. 2003). However, Callaerts-Vegh et al. (1998) reported that 12 weeks of exercise training did not alter ET-1 in chronic HF patients. It has been suggested that NO produced from the endothelium during chronic exercise training decreases ET-1 production, and that plasma NOx correlates inversely with plasma ET-1 in healthy young subjects (Maeda et al. 2001). Therefore, we hypothesized that exercise training would decrease ET-1 levels in HTR due to an increase in NO synthesis or bioavailablilty. Thus, our study demonstrates that endurance exercise training does not significantly alter plasma levels of ET-1 in HTR.

Lipid Peroxidation, Antioxidant Enzyme Activity, Endogenous Nitric Oxide Inhibition and Exercise Training

F_2-isoprostanes are a family of prostaglandin products of free-radical oxidation of polyunsaturated fatty acids which circulate esterified to lipid membranes in plasma and in

free plasma form. Using the F_2-isoprostane isomer, 8-iso-$PGF_{2\alpha}$ as a marker of in vivo lipid peroxidation, we hypothesized that 12 weeks of supervised endurance exercise training would reduce plasma lipid peroxidation primarily by an increase in activity of the extracellular antioxidant enzyme SOD. SOD is the primary SOD isoform found in the vascular wall and is the principal regulator of superoxide radical production and the primary mechanism for preservation of NO from superoxide-induced degradation (Fukai et al. 2002). As mentioned, chronic exercise training has been reported in animals and humans to enhance NO production by increased eNOS protein production. In addition, Fukai et al. (2000) reported that three weeks of exercise training in eNOS wildtype (+/+) mice resulted in a 3-fold increase in aortic eNOS protein which was paralleled by a 3-fold increase in aortic ecSOD. However, this increased ecSOD protein expression was not observed in aortas from eNOS knockout (-/-) mice suggesting that NO production modulates ecSOD expression in the vascular wall via a feed-forward mechanism, therefore increasing its own biological effects. Therefore, we hypothesized that exercise training would enhanced ecSOD activity and subsequently lower levels of free-radical induced lipid peroxidation and therefore preserve NO. However, twelve weeks of supervised endurance exercise training did not alter basal levels of plasma 8-iso-$PGF_{2\alpha}$ or ecSOD activity in HTR. The reasons for the lack of change in NOx data were explained in the previous section, however, the lack of change in SOD activity are surprising in light a recent report that exercise training enhanced SOD activity and lowered lipid peroxidation in humans (Edwards et al. 2004). The lack of improvement in brachial artery FMD in HTR who participated in supervised exercise training is consistent with the lack of change in SOD activity and 8-iso-$PGF_{2\alpha}$. However, we would have expected

that a decrease in SOD activity and an increase in 8-iso-$PGF_{2\alpha}$ would have paralleled the decrease in brachial artery FMD in the control HTR after 12 weeks. However, we speculate that additional factors may have contributed to the decrease in brachial artery FMD in the control HTR. For example, sympathetic activation increases in HTR due to chronic cyclosporine therapy. Thus, one hypothesis is that exercise training attenuated a progressive increase in sympathetic activation in HTR because endurance training decreases muscle sympathetic nerve activity (MSNA) in chronic heart failure subjects (Roveda et al. 2003). Although we did not measure MSNA in this study, studies show that elevated sympathetic activation in end-stage HF subjects normalizes immediately after HT (Rundqvist et al. 1996), but chronic cyclosporine therapy contributes to enhanced sympathetic activation months to years after HT (Scherrer et al. 1990). Indeed, Hijmering et al. (2002) reported that acute enhanced sympathetic activation results in a decreased brachial artery FMD, but has no effect in peak forearm vasodilatory capacity or resistance arteries during reactive hyperemia (Hijmering et al. 2002). Hence, we speculate that this could explain the decline in brachial artery FMD in control HTR, but not in the peak forearm vasodilatory BF and calf BF.

Lastly, the endogenous NO inhibitor, ADMA, was not altered by 12 weeks of exercise training in HTR. This is not surprising because we hypothesized that oxidative stress following exercise training would decrease ADMA by decreasing oxidant inactivation of DDAH, the enzyme that degrades ADMA and the primary pathway of ADMA regulation (Sydow and Munzel 2003). Thus, the lack of change in plasma 8-iso-$PGF_{2\alpha}$ and SOD activity suggest that oxidative stress was not altered following HTR and support the lack of change in ADMA.

Inflammatory Markers and Exercise Training

As mentioned, it is now known that CRP has a direct pro-inflammatory effect on the endothelium in addition to its clinical value as a predictor of future cardiovascular disease. Therefore pharmacological or non-pharmacological interventions which modify levels of CRP may have important clinical implications. There have only been two prospective studies on effects of exercise training on CRP in patients with CAD (Milani et al. 2004; Edwards et al. 2004). Milani et al. (2004) reported a 41% decrease in CRP in 277 CAD patients after 12 weeks of exercise training and Edwards et al. (2003) reported that 12 weeks of endurance exercise training lowered CRP by 45% in CAD patients. However, there have been no prospective studies on the effects of exercise training on CRP in HTR. In the present study, 12 weeks of exercise training resulted in a non-significant 49% decrease in plasma CRP, and a non-significant 19% decrease in CRP in the control HTR. The relative change in CRP is similar to that reported by Milani et al. (2004) and Edwards et al. (2003), but the lack of a statistical significant change is likely due to the large inter-individual variation in CRP within each group and the small number of subjects. However, the decrease in CRP in the trained HTR in the present study may be clinically significant because Eisenberg et al. (2000) reported that for every 2 fold increase in CRP increased the risk of allograft failure by 32%. Furthermore, Pethig et al. (2000) reported that HTR with significant coronary artery vasulopathy had significantly higher CRP (4.1 vs. 1.8 mg/L) than HTR with no coronary vasculopathy. Thus, exercise training did not significantly alter CRP in this small cohort of HTR, but more research is needed with larger cohort to confirm these findings.

There have been two prospective studies on the effects of exercise training on inflammatory cytokines in chronic HF patients (Adamopoulos et al. 2002; Adamopoulos

et al. 2001). Adamopoulos and colleagues conducted a 12-week, randomized, controlled, cross-over design study in 24 chronic HF patients and 20 healthy controls. They reported a significant 29% decrease in plasma levels of IL-6 and 39% decrease in TNF-α (Adamopolous et al. 2002). Second, Larsen et al. (2001) reported a 12.5% decrease in TNF-α but no change in IL-6 in 28 patients with chronic HF. In CAD patients, Edwards et al. (2003) reported that 12 weeks of endurance exercise training lowered IL-6 by 32%. In the present study, the effect of 12 weeks exercise training in HTR resulted in a non-significant 35% decrease in IL-6 (5.02 vs. 3.25 pg/ml) and no significant change in TNF-α. However, in the control HTR, there was a significant 53% increase in plasma TNF-α (1.56 vs. 2.38 pg/ml, $p<0.05$) after 12 weeks. These data suggest that endurance exercise training attenuates a progressive increase in TNF-α over 12 weeks early after HT. The mechanism for this increase in the control HTR is unknown, but a possible explanation is that the greater increase in body weight in the control HTR than in trained HTR contributed to this finding, since TNF-α is correlated modestly to body weight and body fat (Kern et al. 2001). As mentioned in a previous section, *in vitro* studies demonstrate that TNF-α has a direct effect on endothelial function by causing post-transcriptional modification of eNOS mRNA leading to early mRNA degradation (Yoshizumi et al. 1993), and in humans, TNF-α levels are highly correlated with impaired forearm EDV in response to acetylcholine in HF patients (Katz et al. 1994). Therefore, in the present study we propose that this increase in circulating TNF-α over 12 weeks in the control HTR, may have contributed to the progressive decrease in brachial artery endothelial function during the control period.

In HTR, the development of coronary artery vasculopathy is preceded by expression of ICAM-1 on the endothelium (Labarrere et al. 2000). Also, serum levels of sICAM-1 are a significant predictor of coronary vasculopathy, post-transplant ischemic events, and graft failure in HTR (Labarrere et al. 2000; Labarrere et al. 2002). For example, HTR with sICAM-2 of 308 ng/ml or greater had a 2.6 fold increase risk of CAV, and 3.6 fold increased risk of graft failure (Labarrere et al. 2000). Thus, a pharmacological or non-pharmacological intervention which lowers sICAM-1 may be indicator of improved endothelial function or decreased CV risk in HTR. Two studies on the effects of exercise on plasma sICAM-1 levels have been reported in chronic HF patients (Adamapolous et al. 2001; Niebauer et al. 2005). Adamapolous et al. (2001) reported a 14.4% decrease in sICAM-1 (367 vs. 314 ng/ml, $p<0.01$) after 12 weeks of endurance exercise training in chronic HF patients and Niebauer et al. (2005) reported no change in basal plasma levels of sICAM-1 after 8 weeks of a home-based endurance cycling program in chronic HF. Prior to the present study, the effects of exercise training on plasma sICAM-1 in HTR were unknown. The present study demonstrates that twelve weeks of endurance exercise training in HTR does not significantly alter plasma levels of sICAM-1 (216.5 vs. 198.2 ng/ml, p=NS). Additionally, there was no significant change in the control HTR (269.2 vs. 295.6 ng/ml, p=NS) after 12 weeks. These results are consistent with the study by Niebauer (2005) but not with Adamopoulos et al. (2001). It is possible that since TNF-α and the measure of brachial artery endothelial function did not change with training, that endothelial activation was not altered significantly and therefore did not change circulating sICAM-1 levels. However, sICAM did not increase in the control group where TNF-α increased, however, ICAM-1 expression is regulated

by several factors in addition to TNF-α which may have influenced plasma sICAM-1 levels.

Exercise Capacity and Exercise Training

There has been only one randomized, controlled study of the effect of endurance exercise training on exercise capacity in HTR. Kobashigawa et al. (1999) randomized twenty-seven HTR two weeks after HT to an exercise or control group. They reported that 24 weeks of supervised exercise training as part of a cardiac rehabilitation program increased peak VO_2 by 49% and exercise duration 59%, compared to 18% and 18% in the non-supervised control HTR, respectively. In the present study, HTR who performed 12 weeks of endurance exercise training increased peak VO_2 by 26%, whereas there was no significant change in peak VO_2 in the HTR who did not perform supervised exercise training. Furthermore, exercise duration on the graded treadmill test increased 45% in the trained HTR, but there was no change in exercise duration in the control HTR. Although Kobashigawa and colleagues (1999) reported greater changes in peak VO_2 and exercise duration, these differences are likely due to the longer duration of their exercise training program (24 weeks) than the program in our study (12 weeks).

Conclusions

HT results in an improvement in brachial artery FMD and forearm and calf vasodilatory capacity. The mechanisms responsible for these post-HT are not clear from the present study, however attenuation of the hyper-inflammatory state after HT present in end-stage HF subjects may be a significant contributor. Although, it is possible that NO mediated the improvement in endothelial function, the confounding influences of HT pharmacology and low-grade non-clinical allograft rejection on plasma NOx levels prevent such conclusions. Additionally, although lipid peroxidation was unchanged after

HT, but is suppressed in HTR compared to normal healthy controls possibly due to pharmacological therapy which attenuates oxidative stress. In contrast, SOD activity is significantly depressed after HT to levels below healthy controls likely due to chronic cyclosporine therapy. Although peripheral and central blood pressure components increase after HT due to normalization of cardiac function, reflected wave properties and arterial stiffness does not appear to change significantly.

This study was the first study to report that 12 weeks of exercise training attenuates a progressive decline in brachial artery endothelial function in HTR. Although the mechanism for this finding is unclear, it may be due a progressive increase in the inflammatory mediator TNF-α in HTR who do not perform endurance exercise training. This study also demonstrates for the first time the 12 weeks of exercise training improved upper and lower limb resistance artery vasodilation. The mechanism for this finding is unclear because we could not confirm whether increases in NOx were due to endothelial-derived NO or non-endothelial sources of NO. It is possible that the increase is a non-NO-mediated mechanism, because several studies suggest that limb resistance vasculature during reactive hyperemia is prostacyclin-dependent. However, we cannot confirm this in the present study. Lastly, HTR who participated in 12 weeks of supervised exercise training experienced a 26% increase in peak VO_2 and 44% increase in exercise duration, but there was no significant changes in peak VO_2 or exercise duration in the control group. This may be the most important clinical finding because it suggests that the HTR who are instructed to initiate self-monitored, rather than supervised, exercise training programs, likely adopt a sedentary lifestyle despite their recent life-saving surgery.

Limitations and Future Research

The present study was designed to investigate the effects of HT on endothelial function and arterial stiffness in end-stage HF patients. In addition, we sought to investigate the effects of endurance exercise training on endothelial function and arterial stiffness in HTR. The study is limited by the small sample size. Also, it is possible that our findings are a result of selection bias or confounding variable such as dietary influences, or antihypertensive medications. However, we believe that a larger sample size would have supported the present results, and perhaps teased out some of the additional mechanisms that may be involved in changes in endothelial function following exercise training.

Future research should include more invasive studies including infusion of pharmacological agonists and inhibitors of endothelial-derived substances via brachial artery catheterization to better pharmacodissect the mechanisms of endothelial function after exercise training in HTR. Furthermore, novel techniques such as human endothelial cell biopsies would allow superior isolation of changes of protein expression in endothelial cells than measurement by traditional blood plasma samples.

LIST OF REFERENCES

ACSM Guidelines for Exercise Testing and Prescription, 6th edition. Lippincott Williams and Wilkins, 2000.

Adamopoulos S, Parissis J, Karatzas D, Kroupis C, Georgiadis M, Karavolias G, Paraskevaidis J, Koniavitou K, Coats AJ, Kremastinos DT. Physical training modulates proinflammatory cytokines and the soluble Fas/soluble Fas ligand system in patients with chronic heart failure. J Am Coll Cardiol 2002;39:653-663.

Adamopoulos S, Parissis J, Kroupis C, Georgiadis M, Karatzas D, Karavolias G, Koniavitou K, Coats AJ, Kremastinos DT. Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. Eur Heart J 2001;22:791-797.

Anderson TJ, Uehata A, Gerhard MD, Meredeth IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC, Selwyn AP. Close relationship of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995;26:1235-1241.

Arnold JM, Marchiori GE, Imrie JR, Burton GL, Pflugfelder PW, Kostuk WJ. Large artery function in patients with chronic heart failure: studies of brachial artery diameter and hemodynamics. Circulation 1991;84:2418-2425.

Berger R, Stanek B, Hulsmann M, Frey B, Heher S, Pacher R, Neunteufl T. Effects of endothelin A receptor blockade on endothelial function in patients with chronic heart failure. Circulation 2001;103:981-986.

Blake GJ, Ridker PM. C-reactive protein and other inflammatory markers in acute coronary syndromes. J Am Coll Cardiol 2003;41:37S-42S.

Bonapace S, Rossi A, Cicoira M, Franceschini L, Golia G, Zanolla L, Marino P, Zardini P. Aortic distensibility independently affects exercise tolerance in patients with dilated cardiomyopathy. Circulation 2003;107:1603-1608.

Braith RW, Magyari PM, Pierce GL, Hill JA, Edwards DG, Hill JA, Aranda JM. Resistance exercise reverses glucocorticoid-induced skeletal muscle myopathy in heart transplant recipients. Am J Cardiol 2005;95:1192-1198.

Braith RW, Mills RM Jr, Wilcox CS, Davis GL, Wood CE. Breakdown of blood pressure and body fluid homeostasis in heart transplant recipients. J Am Coll Cardiol 1996;27:375-383.

Braith RW, Plunkett MB, Mills RM Jr. Cardiac output responses during exercise in volume-expanded heart transplant recipients. Am J Cardiol 1998a;81:1152-1156.

Braith RW, Welsch MA, Feigenbaum MS, Kluess HA, Pepine CJ. Neuroendocrine activation in heart failure is modified by endurance exercise training. J Am Coll Cardiol 1999;34:1170-1175.

Braith RW, Welsch MA, Mills RM, Keller JW, Pollock ML. Resistance exercise prevents glucocorticoid-induced myopathy in heart transplant recipients. Med Sci Sports Exerc 1998b;30:483-489.

Bunchman TE, Brookshire CA. Cyclosporine-induced synthesis of endothelin by cultured human endothelial cells. J Clin Invest 1991;88:310-314.

Butler R, Morris AD, Belch JJF, Hill A, Struthers AD. Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. Hypertension 2000;35:746-751.

Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circulation 2000;87:840-844.

Callaerts-Vegh Z, Wenk M, Goebbels U, Dziekan G, Myers J, Dubach P, Haefeli WE. Influence of intensive physical training on urinary nitrate elimination and plasma endothelin-1 levels in patients with congestive heart failure. J Cardiopulm Rehab 1998;18:450-457.

Cameron JD, Dart AM. Exercise training increases total systemic arterial compliance in humans. Am J Physiol 1994;266:H693-701.

Cameron JD, McGrath B, Dart AM. Use of radial artery tonometry and a generalized transfer function to determine aortic pressure augmentation in subjects with treated hypertension. J Am Coll Cardiol 1998;32:1214-1220.

Cavero PG, Suhir K, Galli F, DeMarco T, Keith F, Chatterjee K. Effect of orthotopic cardiac transplantation on peripheral vascular function in congestive heart failure: influence of cyclosporine therapy. Am Heart J 1994;127:1581-1587.

Chen CH, Nevo E, fetics B, Pak PH, Yin FCP, Maughn WL, Kass DA. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Circulation 1997;95:1827-1836.

Chester AH, Borland JA, Buttery LK, Mitchell JA, Cunningham DA, Hafizi S, Hoare GS, Springall DR, Polak JM, Yacoub MH. Induction of nitric oxide synthase in human vascular smooth muscle: interactions between proinflammatory cytokines. Cardiovasc Res 1998;38:814-821.

Church TS, Barlow CE, Earnest CP, Kampert JB, Priest EL, Blair SN. Associations between cardiorespiratory fitness and C-reactive protein in men. Arterioscler Thromb Vasc Biol 2002;22:1869-1876.

Cooke JP, Rossitch E, Andon NA, Loscalzo J, Dzau VJ. Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. J Clin Invest 1991;88:1663-1671.

Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. *J Amer Coll Cardiol* 2002;39:257-265.

Cracowski JL, Durand T, Bessard G. Isoprostanes as a biomarker of lipid peroxidation in humans: physiology, pharmacology and clinical implications. Trends Pharmacol Sci 2002;23:360-366.

Cuppoletti A, Sitges M, Perez Villa F, Orus J, Magrina J, Roig E. Impairment in forearm endothelial-dependent vasodilation after heart transplantation. Transpl Proc 2003;35:2011-2013.

Davis SF, Yeung AC, Meredith IT, Charbonneau F, Ganz P, Selwyn AP, Anderson TJ. Early endothelial dysfunction predicts the development of transplant coronary artery disease at 1 year posttransplant. Circulation 1996;93:457-462.

Desideri G, Croce G, Tucci M, Passacquale G, Broccoletti S, Valeri L, Santucci A, Ferri C. Effects of bezafibrate and simvastatin on endothelial activation and lipid peroxidation in hypercholesterolemia: evidence of different vascular protection by different lipid-lowering treatments. J Clin Endocrinol Metab 2003;88:5341-5347.

Dietrich D, Skopec J, Diederich A, Dai FX. Cyclosporine produces endothelial dysfunction by increased production of superoxide. Hypertension 1994;23(2): 957-961.

Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase by Akt-dependent phosphorylation. Nature 1999;399:601-605.

Drexler H. Endothelial dysfunction: clinical implications. Prog Cardiovasc Dis 1997;34:287-324.

Drexler H, Kästner S, Strobel A, Studer R, Brodde OE, Hasenfuß G. Expression, activity and functional significance of inducible nitric oxide synthase in the failing human heart. J Am Coll Cardiol 1998;32:955-963.

Dziekan G, Myers J, Goebbels U, Muller P, Reinhart W, Ratti R, Hafeli W, Dubach P. Effects of exercise training on limb blood flow in patients with reduced ventricular function. Am Heart J 1998;136:22-30.

Edwards DG, Davis M, Brubaker PH, Phillips T, Leeuwenburgh C, Braith RW. Markers of inflammation are modified by exercise training in coronary artery disease (abstract). Med Sci Sports Exerc 2002;34(suppl 5): S180.

Edwards DG, Schofield RS, Lennon SL, Pierce GL, Nichols WW, Braith RW. Effect of exercise training on endothelial function in men with coronary artery disease. Am J Cardiol 2004a;93:617-620.

Edwards DG, Schofield RS, Magyari PM, Nichols WW, Braith RW. Effect of exercise training on central aortic pressure wave refelction in coronary artery disease. Am J Hypertens 2004b;17:540-543.

Eisenberg MS, Chen HJ, Warshofsky MK, Sciacca RR, Wasserman HS, Schwartz A, Rabbani LE. Elevated levels of plasma c-reactive protein are associated with decreased graft survival in cardiac transplant recipients. Circulation 2000;102:2100-2104.

Engelke KA, Halliwell JR, Proctor DN, Dietz NM, Joyner MJ. Contribution of nitric oxide and prostaglandins to reactive hyperemia. J Appl Physiol 1996;81:1807-1814.

Faraci FM, Didion SP. Vascular protection: superoxide dismutase isoforms in the vessel wall. Arertioscler Thromb Vasc Biol 2004;24:1367-1373.

Farquharson CAJ, Butler R, Hill A, Belch JJF, Struthers AD. Allopurinol improves endothelial dysfunction in chronic heart failure. Circulation 2002;106:221-226.

Fearon WF, Wang B, Nakamura M, Potena L, Valantine HA, Yeung AC, Cooke JP. Coronary endothelial dysfunction in cardiac transplant recipients is related to elevated levels of ADMA (abstract). Circulation 2004;110:supplement.

Feron O, Saldana F, Michel JB, Michel T. The endothelial nitric-oxide synthase-caveolin regulatory cycle. J Biol Chem 1998;273:3125-3128.

Fichtlscherer S, Rosenberger G, Walter DH, Breuer S, Dimmeler S, Zeiher AM. Elevated c-reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease. Circulation 2000;102:1000-1006.

Fish RD, Nabel, Selwyn AP, Ludmer PL, Mudge GH, Kirshenbaum JM, Schoen FJ, Alexander RW, Ganz P. Responses of coronary arteries of cardiac transplant patients to acetylcholine. J Clin Invest 1988;81:21-31.

Fukai T, Folz RJ, Landmesser U, Harrison. Extracellular superoxide dismutase and cardiovascular diseases. Circ Res 2002;55:239-249

Fukai T, Siegfried MR, Ushio-Fukai M, Cheng Y, Kojda G, Harrison DG. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. J Clin Invest 2000;105:1631-1639.

Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373-376.

Givertz M, Hartley LH, Colucci WS. Long-term sequential changes in exercise capacity and chronotropic responsiveness after cardiac transplantation. Circulation 1997;96:232-237.

Goetz KL, Wang BC, Madwed JB, Zhu JL, Leadley RJ. Cardiovascular, renal, and endocrine responses to intravenous endothelin in conscious dogs. Am J Physiol 1988;255:R1064-R1068.

Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, Vita JA. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. J Am Coll Cardiol 2003;41:1769-1775.

Gokce N, Vita JA, Bader DS, Sherman DL, Hunter LM, Holbrook M, O'Malley C, Keaney JF, Balady GJ. Effect of exercise on upper and lower extremity endothelial function in patients with coronary artery disease. Am J Cardiol 2002;90:124-127.

Greenfield ADM, Whitney RJ, Mowbray JF. Methods for the investigation of peripheral blood flow. Br Med Bull 1963;19:101-109.

Grieff M, Loertscher R, Al Shohaib S, Stewert DJ. Cyclosporine-induced elevation in circulating endothelin-1 in patients with solid-organ transplants. Transplantation 1993;56:880-884.

Gudmundsson GS, Sinkey CA, Chenard CA, Stumbo PJ, Haynes WG. Resistance vessel endothelial function in healthy humans during transient postprandial hypertriglyceridemia. Am J Cardiol 2000;85:381-5

Guthikonda S, Sinkey C, Barenz T, Haynes WG. Xanthine oxidase inhibition reverses endothelial dysfunction in heavy smokers. Circulation 2003;107:416-421.

Haas GJ, Wooding-Scott M, Binkley PF, Myerowitz PD, Kelly R, Cody RJ. Effects of successful cardiac transplantation on endothelin-1 in heart failure patients. Am J Cardiol 1993;71:237-240.

Haas JA, Krier JD, Bolterman RJ, Juncos LA, Romero JC. Low-dose angiotensin II increases free isoprostane levels in plasma. Hypertension. 1999;34:983-986.

Habib FM, Springall DR, Davies GJ, Oakley CM, Yacoub MH, Polak JM. Tumour necrosis factor and inducible nitric oxide synthase in dilated cardiomyopathy. Lancet 1996;347:1151–1155.

Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KR, Quyyumi AA. Prognostic value of coronary vascular endothelial dysfunction. Circulation 2002;106:653-658.

Hambrecht R, Adams V, Erbs S, Linke A, Krankel N, Shu Y, Baither Y, Gielen S, Thiele H, Gummert JF, Mohr FW, Schuler G. Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. Circulation 2003;107:3152-3158.

Hambrecht R, Fiehn E, Weigl C, Gielen S, Hamann C, Kaiser R, Yu J, Adams V, Niebauer J, Schuler G. Regular exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. Circulation 1998;98:2709-2715.

Hambrecht R, Gielen S, Linke A, Fiehn E, Yu J, Walther C, Schoene N, Schuler G. Effects of exercise training on left ventricular function and peripheral resistance in patients with chronic heart failure: A randomized trial. JAMA 2000a;283:3095-3101.

Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S, Schoene N, Schuler G. Effects of exercise on coronary endothelial function in patients with coronary artery disease. N Engl J Med 2000b;342:454-460.

Harrison DG. Cellular and molecular mechanisms of endothelial cell dysfunction. J Clin Invest 1997;100:2153-2157.

Haynes WG, Ferro CJ, O'Kane KP, Somerville D, Lomax CC, Webb DJ. Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in humans. Circulation 1996;93:1860-1870.

Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673-2678.

Hertz MI, Taylor DO, Trulock EP, Boucek MM, Mohacsi PJ, Edwards LB, Keck BM. The registry of the international society for heart and lung transplantation: nineteenth official report- 2002. J Heart Lung Transplant 2002;21:950-970.

Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Kajiyama G, Oshima T. A noninvasive measurement of reactive hyperemia that can be used to assess resistance artery endothelial function in humans. Am J Cardiol 2001;87:121-125.

Higashi Y, Sasaki S, Sasaki N, Nakagawa K, Matsuura H, Ueda T, Yoshimizu A, Kuisu S, Matsuura H, Kajiyama G, Oshima T. Daily aerobic exercise improves reactive hyperemia in patients with essential hypertesnion. Hypertension 1999;33:591-597.

Hijmering ML, Stroes ESG, Olijhoek J, Hutten BA, Blankestijn PJ, Rabelink TJ. Sympathetic activation markedly reduces endothelium-dependent, flow-mediated dilation. J Amer Coll Cardiol 2002;39:683-688.

Hokanson DE, Sumner DS, Strandness DE. An electrically calibrated plethysmograph for direct measurement of limb blood flow. IEEE Trans Biomed Eng 1975;22: 25-29.

Hollenberg SM, Klein LW, Parillo JE, Scherer M, Burns D, Tamburro P, Oberoi M, Johnson MR, Costanzo MR. Coronary endothelial dysfunction after heart transplant predicts allograft vasculopathy and cardiac death. Circulation 2001;104: 3091-3096.

Holm T, Andreassen AK, Ueland T, Kjekshus J, Froland SS, Kjekshus E, Simonen S, Aukrust P, Gullestad L. Effect of pravastatin on plasma markers of inflammation and peripheral endothelial function in male heart transplant recipients. Am J Cardiol 2001;87:815-818.

Holm T, Aukrust P, Andreassen AK, Ueland T, Brosstad F, Froland SS, Simonen S, Gullestad L. Peripheral endothelial dysfunction in heart transplant recipients: possible role of proinflammatory cytokines. Clin Trans 2000 14:218-225.

Hope SA, Meredith IT. Cellular adhesion molecules and cardiovascular disease. Part I. Their expression and role in atherogenesis. Intern med J 2003;33:380-386.

Hornig B, Arakawa N, Kohler C, Drexler H. Vitamin C improves endothelial function of conduit arteries in patients with chronic heart failure. Circulation 1998;97:363-368.

Hornig B, Volker M, Drexler H. Physical training improves endothelial function in patients with chronic heart failure. Circulation 1996;93:210-214.

Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelial-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 1987;84:9265-9269.

Inoue N, Ramasamy S, Fukai T, Nerem RM, Harrison DG. Shear stress modulates expression of Cu/Zn superoxide dismutase in human aortic endothelial cells. Circ Res 1996;79:32-37.

Iuchi T, Akaike M, Mitsui T, Ohshima Y, Shintani Y, Azuma H, Matsumoto T. Glucocorticoid excess induces superoxide production in vascular endothelial cells and elicits vascular endothelial dysfunction. Circ Res 2003;92:81-87.

Joyner MJ. Effect of exercise on arterial compliance. Circulation 2000;102: 1214-1215.

Joyner MJ, Dietz NM, Shepard JT. From Belfast to Mayo and beyond: the use and future of plethysmography to study blood flow in human limbs. J Appl Physiol 2002; 91:2431-2441.

Julien J, Farge D, Kreft-Jais C, Guyene T, Plouin PF, Houssin D, Carpentier A, Corvol P. Cyclosporine-induced stimulation of the renin-angiotensin system after liver and heart transplantation. Transplantation 1993;56:885-891.

Jungersten L, Ambring A, Wall B, Wennmalm A. Both physical fitness and acute exercise regulate nitric oxide formation in healthy humans. J Appl Physiol 1997;82:760-764.

Jungersten L, Edlund A, Petersen AS, Wennmalm A. Plasma nitrate as an index of nitric oxide formation in man: analysis of kinetics and confounding factors. Clin Physiol 1996;16:369-379.

Kao AC, Van Trigt P, Shaeffer-McCall GS, Shaw JP, Kuzil BB, Page RD, Higginbotham MB. Central and peripheral limitations to upright exercise in untrained cardiac transplant recipients. Circulation 1994;89:2605-2615.

Katz SD, Biasucci L, Sabba C, Strom JA, Jondeau G, Galvao M, Solomon S, Nikolic SD, Forman R, LeJemtel TH. Impaired endothelium-mediated vasodilation in the peripheral vasculature of patients with congestive heart failure. J Am Coll Cardiol 1992;19:918-925.

Katz SD, Rao R, Berman JW, Schwarz M, Demopoulos L, Bijou R, LeJemtel TH. Pathophysiological correlates of increased serum tumor necrosis factor in patients with congestive heart failure. Relation to nitric oxide-dependent vasodilation in the forearm circulation. Circulation 1994;90:12-16.

Katz SD, Yuen J, Bijou R, LeJemtel TH. Training improves endothelium-dependent vasodilation in resistance vessels of patients with heart failure. J Appl Physiol 1997;82:1488-1492.

Keith M, Geranmayegan A, Sole MJ, Kurian R, Robinson A, Omran AS, Jeejeebhoy KN. Increased oxidative stress in patients with congestive heart failure. J Am Coll Cardiol 1998;31:1352-1356.

Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab 2001;280:E745-751.

Kessler P, Bauersachs J, Busse R, Schini-Kerth VB. Inhibition of inducible nitric oxide synthase restores endothelium-dependent relaxations in proinflammatory mediator-induced blood vessels. Arterioscler Thromb Vasc Biol 1997;17:1746-1755.

Kingwell BA, Cameron JD, Gillies K, Jennings G, Dart A. Arterial compliance may influence baroreflex function in athletes. Am J Physiol 1995;268:H411-418.

Kobashigawa JA, Leaf DA, Lee N, Gleeson MP, Liu H, Hamilton MA, Moriguchi JD, Kawata N, Eihhorn K, Herlihy E, Laks H. A controlled trial of exercise rehabilitation after heart transplantation. N Eng J Med 1999;340:272-277.

Kubo SH, Rector TS, Bank AJ, Tschumperlin LK, Raij L, Leopoldo R, Brunsvold N, Kraemer MD. Effects of cardiac transplantation on endothelium-dependent dilation of the peripheral vasculature in congestive heart failure. Am J Cardiol 1993;71:88-93.

Kubo SH, Rector TS, Bank AJ, Williams RE, Heifetz SM. Endothelium-dependent vasodilation is attenuated in patients with heart failure. Circulation 1991;84:1589-1596.

Labarrere CA, Lee JB, Nelson DR, Al-Hassani M, Miller SJ, Pitts DE. C-reactive protein, arterial endothelial activation, and development of transplant coronary artery disease: a prospective study. Lancet 2002;360; 1462-1467.

Labarrere CA, Nelson DR, Miller SJ, Nieto JM, Conner JA, Pitts DE, Kirlin PC, Halbrook HG. Value of serum soluble intercellular adhesion molecule-1 for the noninvasive risk assessment of transplant coronary artery disease, posttransplant ischemic events, and cardiac graft failure. Circulation 2000;102; 1549-1555.

Lage SG, Kopel L, Monachini MC, Medeiros CJ, Pileggi F, Polak JF, Creager MA. Carotid arterial compliance in patients with congestive heart failure secondary to idiopathic dilated cardiomyopathy. Am J Cardiol 1994;74:691-695.

LaMonte MJ, Durstine JL, Yanowitz FG, Lim T, DuBose KD, Davis P, Ainsworth BE. Cardiorespiratory fitness and C-reactive protein among a tri-ethnic sample of women. Circulation 2002;106:403-406.

Landmesser U, Drexler H. Allopurinol and endothelial function in heart failure- future or fantasy. Circulation 2002;106:173-175.

Larsen AI, Aukrust P, Aarsland T, Dickstein K. Effect of aerobic exercise training on plasma levels of tumor necrosis factor in patients with heart failure. Am J Cardiol 2001;88:805-808.

Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an indepenedent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension 2001;37:1236-1241.

Lerman A, Kubo SH, Tschumperlin LK, Burnett JC. Plasma endothelin concentrations in humans with end-stage heart failure and after heart transplantation. J Am Coll Cardiol 1992;20: 849-853.

Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med 1990;323:236-241.

Lieberman EH, Gerhard MD, Uehata A, Selwyn AP, Ganz P, Yeung AC, Creager MA. Flow-induced vasodilation of the human brachial artery is impaired in patients <40 years of age with coronary artery disease. Am J Cardiol 1996;78:1210-1214.

Lim DS, Gomez CA, Goldberg CS, Crowley DC, Rocchini AP, Charpie JR. Systemic arterial pressure and brachial arterial flow-mediated dilation in young heart transplant recipients. Am J Cardiol 2002;90:1035-1037.

Linke A, Schoene N, Gielen S, Hofer J, Erbs S, Schuler G, Hambrecht R. Endothelial dysfunction in patients with heart failure: systemic effects of lower-limb exercise training. J Am Coll Cardiol 2001;37:392-397.

London GM, Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME. Arterial wave reflections and survival in end-stage renal failure. Hypertension 2001;38: 434-438.

Ludmer PL, Selwyn A, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;315:1046-1051.

Maeda S, Miyauchi T, Kakiyama T, Sugawara J, Iemitsu M, Irukayama-Tomobe Y, Murakami H, Kumagai Y, Kno S, Matsuda M. Effects of exercise training of 8 weeks and detraining on plasma levels of endothelium-derived factors, endothelin-1 and nitric oxide, in young healthy humans. Life Sci 2001;69:1005-1016.

Maeda S, Tanabe T, Miyauchi T, Otsuki T, Sugawara J, Iemitsu M, Kuno S, Ajisaka R, Yamaguchi I, Matsuda M. Aerobic exercise training reduces plasma endothelin-1 concentration in older women. J Appl Physiol 2003;95:336-341.

Markovic S, Raab M, Daxecker H, Griesmacher A, Karimi A, Muller MM. In vitro effects of cyclosporin A on the expression of adhesion molecules on human umbilical vein endothelial cells. Clinica Chimica Acta 2002;316:25-31.

Marumo T, Nakaki T, Hishikawa K, Suzuki H, Kato R, Saruta T. Cyclosporin A inhibits nitric oxide synthase induction in vascular smooth muscle. Hypertens 1995;25:764-768.

Mason RP, Walter MF, Jacob RF.Effects of HMG-CoA reductase inhibitors on endothelial function: role of microdomains and oxidative stress. Circulation. 2004;109:II34-II41

Matthews JNS, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. Br Med J 1990;300:230-235.

Mayer B, Hemmens B. Biosynthesis and action of nitric oxide in mammalian cells. TIBS 1997;22:477-481.

Meredith IT, Currie KE, Anderson TJ, Roddy MA, Ganz P, Creager MA. Postischemic vasodilation in human forearm is dependent on endothelium-derived nitric oxide. Am J Physiol 1996;270:H1435-1440.

Milani RV, Lavie CJ, Mehra MR. Reduction in c-reactive protein through cardiac rehabilitation and exercise training. J Am Coll Cardiol 2004;43:1056-1061.

Mills RW, Billett JM, Nichols WW. Endothelial dysfunction early after heart transplantation. Circulation 1992;86:1171-1174.

Mitchell GF, Tardif JC, Arnold JMO, Marchiori G, O'Brien TX, Dunlap M, Pfeffer MA. Pulsatile hemodynamics in congestive heart failure. Hypertension 2001;38:1433-1439.

Mombouli JV, Vanhoutte PM. Endothelial dysfunction: from physiology to therapy. J Mol Cell Cardiol 1999;31:61-74.

Moncada S, Higgs EA. The L-arginine-nitric oxide pathway. N Engl J Med 1993;329:2002-2012.

Mugge A, Kurucay S, Boger RH, Bode-Boger SM, Schafers HJ, Wahlers T, Frolich JC, Lichtlen PR. Urinary nitrate excretion is increased in cardiac transplanted patients with acute graft rejection. Clin Transpl 1996;10:298-305.

Mun KC. Total antioxidant status and antioxidant enzymes in cyclosporine- treated rats. Transpl Proc 2000;32:2007-2008.

Munzel T, Harrison DG. Increased superoxide in heart failure: a baroreflex gone awry. Circulation 1999;100:216-218.

Murgo JP, Westerhof N, Giolma JP, Altobelli SA. Aortic imput impedance in normal man: relationship to pressure wave forms. Circulation 1980:62:105-116.

Murphey LJ, Morrow JD, Sawathiparnich P, Williams GH, Vaughan DE, Brown NJ. Acute angiotensin II increases plasma F2-isoprostanes in salt-replete human hypertensives. Free Radic Biol Med 2003;35:711-718.

Nakamura M, Sugawara S, Arakawa N, Nagano M, Shizuka T, Shimoda Y, Sakai T, Hiramori K. Reduced vascular compliance is associated with impaired endothelium-dependent dilatation in the brachial artery of patients with congestive heart failure. J Card Fail 2004;10:36-42.

Navarro-Antolin J, Lopez-Munoz MJ, Klatt P, Soria J, Michel T, Lamas S. Formation of peroxynitrite in vascular endothelial cells exposed to cyclosporine A. FASEB J 2001;13: 1291-1293.

Navarro-Antolin J, Rey-Campos J, Lamas S. Transcriptional induction of endothelial nitric oxide gene by cyclosporine A. J Biol Chem 2000;275:3075-3080.

Niebauer J, Clark AL, Webb-Peploe KM, Coats AJS. Exercise training in chronic heart failure: effects on pro-inflammatory markers. Eur J Heart Fail 2005;7:189-193.

Nichols WW. Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms. Am J Hypertens 2005;18:3S-10S.

Nichols WW, O'Rourke MF. McDonald's Blood Flow in Arteries: Clinical, Theoretical and Experimental Principles, 4th edition. Arnold, London, 1998.

Nichols WW, Pepine CJ. Ventricular/vascular interaction in health and heart failure. Comprehens Ther 1992;18:12-19.

Nichols WW, Singh BM. Augmentation index as a measure of peripheral vascular disease state. Curr Opin Cardiol 2002;17:543-551.

Nickenbig G, Harrison DG. The AT_1-type angiotensin receptor in oxidative stress. Part I: Oxidative stress and artherogenesis. Circulation 2002;105:393-396.

O'Rourke MF, Pauca AL. Augmentation of the aortic and central arterial pressure. Blood Press Monit 2004;9:179-185.

O'Rourke MF, Pauca A, Jiang XJ. Pulse wave analysis. Br J Clin Pharmacol 2001;51:507-522.

Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelial-derived relaxing factor. Nature 1987;327:524-526.

Pannala AS, Mani AR, Spencer JPE, Skinner V, Bruckdorfer KR, Moore KP, Rice-Evans CA. The effect of dietary nitrate on salivary, plasma, and urinary nitrate metabolism in humans. Free Radic Biol Med 2003;34:576-584.

Parnell MM, Holst DP, Kaye DM. Exercise training increases arterial compliance in patients with congestive heart failure. Clin Sci 2002;102:1-7.

Pasceri V, Willerson JT, Yeh ETH. Direct proinflammatory effect of c-reactive protein on human endothelial cells. Circulation 2000;102: 2165-2168.

Patel AR, Kuvin JT, DeNofrio D, Kinan D, Sliney KA, Eranki KP, Pandian NG, Udelson JE, Konstam MA, Karas RH. Peripheral vascular endothelial function correlates with exercise capacity in cardiac transplant recipients. Am J Cardiol 2003;91:897-899.

Patel AR, Kuvin JT, Pandian NG, Smith JJ, Udelson JE, Mendelsohn ME, Konstam MA, Karas RH. Heart failure etiology affects peripheral vascular endothelial function after heart transplantation. J Am Coll Cardiol 2001;37:195-200.

Patrono C, Fitzgerald GA. Isoprostanes: potential markers of oxidant stress in atherothrombotic disease. Arteriosclero Thromb Vasc Biol 1997;17:2309-2315.

Pepine CJ, Nichols WW, Conti CR. Aortic input impedance in heart failure. Circulation 1978;58:460-465.

Perez O, Castro P, Diaz-Araya G, Nettle D, Moraga F, Chiong M, Jalil J, Zalaquett R, Moran S, Becker P, Corbalan R, Lavandero S. Persistence of oxidative stress after heart transplantation: a comparative study of patients with heart transplant versus chronic stable heart failure. Rev Esp Cardiol 2002;55:831-837.

Perez-Villa F, Roig E, Ferrer E, Cuppoletti A, Llancaqueo M, Jimenez W, Sanz G. Neurohormonal activation in congestive heart failure: does it normalize after heart transplantation. Rev Esp Cardiol 2004;57:725-731.

Pethig K, Heublein B, Kutschka I, Haverich A. Systemic inflammatory response in cardiac allograft vasculopathy: high-sensitive c-reactive protein is associated with progressive luminal narrowing. Circulation 2000;102 (suppl III):233-236.

Pierce GL, Mering MC, Casey D, Braith RW. Reproducibility of forearm and calf blood flow during reactive hyperemia in young, healthy subjects. (abstract) Med Sci Spor Exer 2004;36:supplement.

Pierce GL, Schofield RS, Nichols WW, Aranda JM, Pauly DF, Hill JA, Braith RW. Arterial stiffness is not increased after cardiac transplantation in patients with severe heart failure (abstract). Circulation 2004;110:supplement.

Piquard F, Richard R, Doutreleau S, Epailly E, Thiranos JCI, Lonsdorfer E, Eisenmann B, Mettauer B, Geny B. Generally increased circulating endothelin can normalize after heart transplantation. Transpl Proc 2001;33:3358-3560.

Plotnick GD, Corretti MC, Vogel RA. Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal. JAMA. 1997;278:1682-6

Polidori CM, Practico D, Savino K, Rokach J, Stahl W, Mecocci P. Increased F_2 isoprostane levels in patients with congestive heart failure are correlated with antioxidant status and disease severity. J Card Fail 2004;10:334-338.

Pratico D. F_2-isoprostanes: sensitive and specific non-invasive indices of lipid peroxidation in vivo. Atherosclerosis 1999;147:1-10.

Ramsey MW, Goodfellow J, Jones CJ, Luddington LA, Lewis MJ, Henderson AH. Endothelial control of arterial distensibility is impaired in chronic heart failure. Circulation 1995;92:3212-3219.

Roberts LJ, Morrow JD. Measurement of F_2-isoprostanes as an index of oxidative stress in vivo. Free Radic Biol Med 2000;28:505-513.

Roveda F, Middlekauff HR, Rondon MU, Reis SF, Souza M, Nastari L, Barretto AC, Krieger EM, Negrao CE. The effects of exercise training on sympathetic neural activation in advanced heart failure: a randomized controlled trial. J Am Coll Cardiol 2003;42:854-60.

Rundqvist B, Elam M, Eisenhofer G, Friberg P. Normalization of total body and regional sympathetic hyperactivity in heart failure after heart transplantation. J Heart Lung Transpl 1996;15:516-526.

Safar ME, Levy BI, Struijker-Boudier H. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. Circulation 2003;107:2864-2869.

Savidge ST. Prostaglandin H synthase and vascular function. Circ Res 2001;89:650-660.

Saxonhouse SJ, Edwards D, Schofield RS, Nichols WW. Effects of cardiac transplantation on flow-mediated arterial vasodilation of the peripheral vasculature (abstract). J Am Coll Cardiol 2000;35(suppl 1):167A.

Schaefer A, Piquard F, Doutreleau S, Mettauer B, Epailly E, Eisenmann B, Londsdorfer J, Geny B. Reduced exercise capacity is associated with reduced nitric oxide production after heart transplantation. J Thorac Cardiovasc Surgery 2001;122:821-822.

Scherrer U, Vissing SF, Morgan BJ, Rollins JA, Tindall RSA, Ring S, Hanson P, Mohanty PK, Victor RG. Cyclosporine-induced sympathetic activation and hypertension after heart transplantation. N Engl J Med 1990;323:693-699.

Schmidt A, Pleiner J, Bayerle-Eder M, Wiesinger GF, Rodler S, Quittan M, Mayer G, Wolzt M. Regular physical exercise improves endothelial function in heart transplant recipients. Clin Transplant 2002;16:137-143.

Schofield RS, Schuler BT, Edwards DG, Aranda JM, Hill JA, Nichols WW. Amplitude and timing of central aortic pressure wave reflections in heart transplant recipients. Am J Hyperten 2002;15:809-815.

Schofield RS, Edwards DG, Schuler BT, Estrada J, Aranda JM, Pauly DF, Hill JA, Aggarwal R, Nichols WW. Vascular effects of sildenafil in hypertensive cardiac transplant recipients. Am J Hypertens 2003;16:874-877.

Schwedhelm E, Bartling A, Lenzen H, Tsikas D, Maas R, Brummer J, Gutzki FM, Chem I, Berger J, Frolich JC, Boger RH. Urinary 8-iso-prostaglandin $F_{2\alpha}$ as a risk marker in patients with coronary heart disease. A matched case-control study. Circulation 2004;109:843-848.

Sinoway LI, Minotti JR, Davis D, Pennock JL, Burg JE, Musch TI, Zelis R. Delayed reversal of impaired vasodilation in congestive heart failure after heart transplantation. Am J Cardiol 1988;61: 1076-1079.

Smith CJ, Sun D, Hoegler C, Roth BS, Zhang X, Zhao G, Xu XB, Kobari Y, Pritchard K, Sessa WC, Hintze TH. Reduced gene expression of vascular endothelial NO synthase and cyclooxygenase-1 in heart failure. Circ Res. 1996;78:58-64.

Spier SA, Delp MD, Meninger CJ, Donato AJ, Ramsey MW, Muller-Delp JM. Effects of ageing and exercise training on endothelium-dependent vasodilation and structure of rat skeletal muscle arterioles. J Physiol 2004;556:947-958.

Sudhir K, MacGregor JS, DeMarco T, De Groot CJM, Taylor RN, Chou TM, Yock PG, Chatterjee K. Cyclosporine impairs release of endothelium-derived relaxing factors in epicardial and resistance coronary arteries. Circulation 1994;90:3018-3023.

Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000;101:948-954.

Sydow K, Munzel T. ADMA and oxidative stress. Atheroslcerosis Supplements 2003;4:41-51.

Taddei S, Virdis A, Ghiadoni L, Sudano I, Magagna A, Salvetti A. Role of endothelin in the control of peripheral vascular tone in human hypertension. Heart Fail Rev 2001;6:277-285.

Tagawa T, Imaizumi T, Endo T, Shiramoto M, Harasawa Y, Takeshita A. Role of nitric oxide in reactive hyperemia in human forearm vessels. Circulation 1994;90:2285-2290.

Takase B, Uehata A, Nagai T, Nishioka T, Hamabe A, Satomura K, Ohsuzu F, Kurita A. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. Am J Cardiol 1998;82:1535-1539.

Tanaka H, Dinneno FA, Monahan KD, Clevenger CM, DeSouza CA, Seals DR. Aging, habitual exercise, and dynamic arterial compliance. Circulation 2000;102:1270-1275.

Tanaka H, Reiling MJ, Seals DR. Regular walking increases peak limb vasodilatory capacity of older hypertensive humans: implications for arterial structure. J Hypertens 1998;16:423-428.

Testa M, Ennezat PV, Vikstrom KL, Demopoulos L, Gentilucci M, Loperfido F, Fanelli R, Kitsis RN, Leinwand LA, LeJemtel TH. Modulation of vascular endothelial gene expression by physical training in patients with chronic heart failure. Ital Heart J 2000;1:426-430.

Torre-Amione G, Kapadia S, Benedict C, Oral H, Young JB, Mann DL. Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD). J Am Coll Cardiol 1996a;27:1201-1206.

Torre-Amione G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB, Mann DL. Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. Circulation. 1996b;93:704-711.

Tran CTL, Leiper JM, Vallance P. The DDAH/ADMA/NOS pathway. Atheroslcerosis Supplements 2003;4:33-40.

Triggle CR, Ding H, Anderson TJ, Pannirselvam M. The endothelium in health and disease: A discussion of the contribution of non-nitric oxide endothelium-derived vasoactive mediators to vascular homeostasis in normal vessels and in type II diabetes. Mol Cell Biochem 2004;263:21-27.

Uematsu M, Ohara Y, Navas JP, Nishida K, Murphy TJ, Alexander RW, Nerem RM, Harrison DG. Regulation of endothelial nitric oxide synthase mRNA expression by shear stress. Am J Physiol 1995;269:C1371-1378.

Usui M, Matsuoka H, Miyazaki H, Ueda S, Okuda S, Imaizumi T. Increased endogenous nitric oxide synthase inhibitor in patients with congestive heart failure. Life Sci 1998;62:2425-2430.

Vaitkevicius PV, Fleg JL, Engel JH, O'Conner FC, Wright JG, Lakatta LE, Yin FCP, Lakatta EG. Effects of aging and aerobic capacity on arterial stiffness in healthy adults. Circulation 1993;88:1456-1462.

Vallance P, Chan N. Endothelial function and nitric oxide: clinical relevance. Heart 2001:342-350.

Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arterial tone in man. Lancet 1989;330:997-1000.

Vallance P, Hingorani A. Endothelial nitric oxide in humans in health and disease. Int J Exp Path 1999;80:291-303.

Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 1992;339:572-575.

Vanhees L, Fagard R, Lijnen P, Moerman E, De Geest H, Amery A. Influence of physical training on blood pressure, plasma renin, angiotensin and catecholamines in patients with ischemic heart disease. Eur J Appl Occup Physiol 1984;53:219-224.

Van Lente F, Castellani W, Abbott LB. Changes in concentrations of C-reactive protein in serum after kidney or heart transplantation Clin Chem 1986;32:633-636.

Vassalle C, Botto N, Andreassi MG, Berti S, Biagini A. Evidence for enhanced 8-isoprostane plasma levels of oxidative stress *in vivo*, inpatients with coronary artery disease. Coron Artery Dis 2003;14:213-218.

Ventura HO, Malik FS, Mehra MR, Stapleton DD, Smart FW. Mechanisms of hypertension in cardiac transplantation and the role of cyclosporine. Curr Opin Cardiol 1997;12:375-381.

Venupogal SK, Devaraj S, Yuhanna I, Shaul P, Jialal I. Demonstration that c-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. Circulation 2002;106:1439-1441.

Walsh JH, Bilsborough W, Maiorana A, Best M, O'Driscoll GJ, Taylor RR, Green DJ. Exercise training improves conduit vessel function in patients with coronary artery disease. J Appl Physiol 2003;95:20-25.

Weber T, Auer J, O'Rourke MF, Kvas E, Lassnig E, Berent R, Eber B. Arterial stiffness, wave reflections, and the risk of coronary artery disease. Circulation 2004;109:184-189.

Weis M, Kledal TN, Lin KY, Panchal SN, Gao SZ, Valantine HA, Mocarski ES, Cooke JP. Cytomegalovirus infection impairs the nitric oxide synthase pathway- role of asymmetric dimethlyarginine in transplant arterioscleosis. Circulation 2004;109:500-505.

Weis M, Pehlivanli S, Meiser BM, von Scheidt W. Simvastatin treatment is associated with improvement in coronary endothelial function and decreased cytokine activation in patients after heart transplantation. J Am Coll Cardiol 2001;38:814-818.

Wildhirt SM, Weis M, Schulze C, Conrad N, Pehlivanli S, Reider G, Enders G, von Scheidt W, Reichart B. Expression of endomyocardial nitric oxide synthase coronary endothelial function in human cardiac allografts. Circulation 2001a;104[suppl I]:I336-I343.

Wildhirt SM, Weis M, Schulze C, Conrad N, Pehlivanli S, Reider G, Enders G, von Scheidt W, Reichart B. Coronary flow reserve and nitric oxide synthases after cardiac transplantation in humans. Eur J Cardiothorac Surg 2001;19:840-847.

Wilkinson IB, Franklin SS, Cockcroft JR. Nitric oxide and the regulation of large artery stiffness: from physiology to pharmacology. Hypertension 2004;44:112-116.

Wilkinson IB, Fuchs SA, Jansen IM, Spratt JC, Murray GD, Cockcroft JR, Webb DJ. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. J Hypertens 1998;16:2079-2084.

Wilkinson IB, MacCallum H, Flint L, Cockroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. J Physiol 2000;525:263-270.

Wilkinson IB, Webb DJ. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. Br J Clin Pharmacol 2001;52:631-646.

Winlaw DS, Schyvens CG, Smythe GA, Du Z, Rainer SP, Keogh AM, Mundy JA, Lord RS, Spratt PM, MacDonald PS. Urinary nitrate excretion is a non-invasive indicator of acute cardiac allograft rejection and nitric oxide production in the rat. Transplantation 1994;58:1031-1036.

Woodman CR, Muller JM, Laughlin MH, Price EM. Induction of nitric oxide synthase mRNA in coronary resistance arteries isolated from exercise-trained pigs. Am J Physiol 1997;273:H2575-2579.

Woodman CR, Muller JM, Rush JW, Laughlin MH, Price EM. Flow regulation of ecNOS and Cu/Zn SOD mRNA expression in porcine coronary arterioles. Am J Physiol 1999;276:H1058-1063.

Yoshizumi M, Perrella MA, Burnett JC, Lee ME. Tumor necrosis factor downregulates an endothelial nitric oxide synthase mRNA by shortening its half-life. Circ Res 1993;73:205–209.

Yousufuddin M, Yamani MH. The renin-angiotensin hypothesis for the pathogenesis of cardiac allograft vasculopathy. Int J Cardiol 2004;95:123-127.

BIOGRAPHICAL SKETCH

Gary Leon Pierce was born October 9, 1971 in Fitchburg, MA and graduated from Fitchburg High School in 1989. He attended Worcester State College in Worcester, MA and graduated in 1994 with a B.S. in biology. He then received an M.S. in clinical exercise physiology in 1997 from Northeastern University, and worked from 1997 to 2001 as the supervising clinical exercise physiologist in the Exercise Testing Laboratory in the Division of Cardiovascular Medicine, Brigham and Women's Hospital in Boston. He was also an adjunct faculty in the Department of Exercise Physiology at Lasell College from 1999-2001, and in the Sargent College of Health Sciences at Boston University in 2000.

In 2001, he enrolled in the doctoral program in Applied Physiology and Kinesiology in the College of Health and Human Performance (HHP) at the University of Florida (UF) in Gainesville, FL and received the Jane Edmonds Predoctoral Fellowship. He was a graduate teaching assistant and adjunct instructor in the Department of Applied Physiology and Kinesiology at UF from 2001-04, and adjunct faculty in the Department of Health Sciences at Santa Fe Community College in Gainesville, FL in 2000. He received a Predoctoral Research Fellowship from the American Heart Association Florida/Puerto Rico Affiliate from 2003-05, and earned a Ph.D. in exercise physiology from UF in 2005. In summer 2005, he began work as a post-doctoral research associate in the Department of Integrative Physiology at the University of Colorado in Boulder,

CO. He is married to Catherine (King) Pierce of Rochester, MN, and they have a daughter, Carolyn Louise Pierce, born Sept. 27, 2004.