2-29-2016

Effects of Resistance Exercise Training on Doxorubicin-Induced Cardiotoxicity

Keith B. Pfannenstiel

UNIVERSITY OF NORTHERN COLORADO

Greeley, Colorado

The Graduate School

EFFECTS OF RESISTANCE EXERCISE TRAINING ON DOXORUBICIN-INDUCED CARDIOTOXICITY

A Dissertation Submitted in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

Keith B. Pfannenstiel

College of Natural and Health Sciences
School of Sport and Exercise Science
Exercise Physiology

December 2015

This Dissertation by: Keith B. Pfannenstiel

Entitled: *Effects of Resistance Exercise Training on Doxorubicin-Induced Cardiotoxicity*

has been approved as meeting the requirement for the Degree of Doctor of Philosophy in College of Natural and Health Sciences in School of Sport and Exercise Science, Program of Exercise Science

Accepted by the Doctoral Committee

_____
Reid Hayward Ph.D., Research Advisor

_____
David S. Hydock Ph.D., Committee Member

_____
Gary Heise, Ph.D., Committee Member

_____
Mark Thomas, Ph.D., Faculty Representative

Date of Dissertation Defense July 9, 2015

Accepted by the Graduate School

_____
Linda L. Black, Ed.D.
Associate Provost and Dean
Graduate School and International Admissions

ABSTRACT

Pfannenstiel, Keith B. *Effects of Resistance Exercise Training on Doxorubicin-Induced Cardiotoxicity*. Published Doctor of Philosophy Dissertation, University of Northern Colorado, 2015.

In recent decades cancer survivorship has steadily increased; however, the adverse side effects associated with chemotherapy treatment can diminish a patient's overall quality of life. One of the most effective and widely used chemotherapeutic agents is doxorubicin (DOX). Though highly effective, its use is limited by a dose-dependent cardiotoxicity. While it is known that exercise preconditioning with endurance training models provide a cardioprotective effect to DOX treatment, little focus has been placed on the effects of a resistance training (RT) model on DOX-induced cardiac dysfunction.

The purpose of the study was to determine the effects of a 12-week RT model on DOX-induced cardiac dysfunction to determine if any cardioprotective effects are a result of a reduction in lipid peroxidation and to determine if any cardioprotective effects are a result of a preservation of the cardiac myosin heavy chain (MHC) isoform distribution.

Ten-week-old male Sprague-Dawley rats were randomly selected to undergo 12 weeks of RT or remain sedentary (SED). Twenty-four hours following the completion of the exercise training or sedentary period, animals

received a 12.5 mg/kg bolus intraperitoneal injection of DOX or a bolus intraperitoneal injection of 0.9% saline. Five days following injection, animals were sacrificed. Cardiac function was assessed both *in vivo* and *ex vivo* and the left ventricle tissue was used to assess lipid peroxidation, as measured by malondialdehyde (MDA) + 4-hydroxyalkenal (HAE) and percentage of β-MHC.

The DOX treatment induced cardiac dysfunction when measured both *in vivo* and *ex vivo*. The RT provided a cardioprotective effect, evident by significant increases in end systolic pressure, left ventricular developed pressure, and the maximal rate of developed pressure. No significant difference existed between RT+DOX and SED+DOX in lipid peroxidation; however, RT did attenuate the α- to β-MHC shift that occurs with DOX treatment.

These data suggest that 12 weeks of the RT model used provided cardioprotection against DOX-induced cardiac dysfunction and may be a result of preservation of the cardiac MHC isoform distribution.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Cancer is one of the leading causes of morbidity and mortality throughout the world. In 2015, it is estimated that there will be more than 1.6 million new cases of cancer diagnosed in the United States (Siegel, Miller, & Jemal, 2015). In addition, it is estimated that more than 580,000 people are expected to succumb to the disease in 2015, thus statistically making cancer the second most common cause of death in the United States. The risk of dying from cancer has steadily declined over the past two decades, including a 22% decrease between 1991 and 2011 (Siegel et al., 2015). Increased survival rates for cancer patients are due, in part, to progressive methods of prevention, detection, and treatment options available to patients. Increased survival rates have led to a greater emphasis on improving overall quality of life in cancer survivors, with a particular focus on reducing or attenuating treatment-related side effects.

A Food and Drug Administration approved chemotherapeutic agent successful in the treatment of a variety of cancers is doxorubicin (DOX), clinically known as Adriamycin®. Clinicians began prescribing DOX to cancer patients as a chemotherapy treatment in 1974. Since then, it has been used to treat a variety of malignancies, both solid and hematological (Weiss, 1992).

While substantially effective at treating cancer, the implementation of DOX into routine cancer treatment regimens has been limited due to its known detrimental cardiotoxicity. A well-characterized side effect of DOX is its dose-dependent cardiotoxicity, which can be manifested as cardiac dysrhythmias, contractile dysfunction, or even congestive heart failure (Steinherz, Steinherz, Tan, Heller, & Murphy, 1991). Mechanistic actions of DOX that have been reported to induce cardiotoxicity include interference with deoxyribonucleic acid (DNA) strand separation during replication (Bachur et al., 1992), induction of apoptosis (Wonders, Hydock, Greufe, Schneider, & Hayward, 2009), and excess generation of reactive oxygen species (ROS) (Minotti, Menna, Salvatorelli, Cairo, & Gianni, 2004)

Aside from examining ways to prevent, diagnose, and treat cancer, researchers have diligently investigated interventions aimed at the reduction or attenuation of burdensome side effects commonly accompanying cancer treatment. Purported to counteract many of the transient and chronic effects associated with cancer treatments, the role of regular exercise in the prevention and treatment of cancer has been the focus of much clinical research over the past 15 years.

Studies have suggested that exercise preconditioning may reduce DOX-induced cardiotoxicity following treatment (Hydock, Lien, Jensen, Schneider, & Hayward, 2011; Hydock, Lien, Schneider, & Hayward, 2008; Jensen, Lien, Hydock, Schneider, & Hayward, 2013). However, not fully understood are the mechanisms involved with the induction of exercise-mediated cardioprotection. Exercise-induced cardioprotection against DOX

toxicity may be explained by upregulation of heat shock proteins (HSP) (Chicco, Schneider, & Hayward, 2005), downregulation of pro-apoptotic pathways (Wonders et al., 2009), increased antioxidant enzymes (Ascensão et al., 2005; Ashrafi, Roshan, & Mahjoub, 2012; Chicco, Hydock, Schneider, & Hayward, 2006; Kanter, Hamlin, Unverferth, Davis, & Merola, 1985), decreased lipid peroxidation (Marques-Alexio et al., 2014; Wonders, Hydock, Schneider, & Hayward, 2008), decreased cardiac DOX accumulation (Jensen et al., 2013), and/or by increasing the expression of multi-drug resistance protein (Krause et al., 2007).

Although a substantial amount of research has focused on the effects of endurance training, little attention has been placed on the effects of resistance training (RT) on DOX-induced cardiotoxicity. Cancer rehabilitation programs have begun to incorporate RT due to the fact that this type of training can result in significant improvements in muscular strength, aerobic capacity, and quality of life (Schwartz & Winters-Stone, 2009; Segal et al., 2003; Winters-Stone et al., 2012). Additionally, RT has been utilized as a rehabilitative therapy for heart failure, which may accompany DOX treatment, to counteract damaging peripheral side effects and improve quality of life (Alves, Nunes, Stefani, & Dal Lago, 2014). To our knowledge, no studies have been conducted which examine the effects of RT on DOX-induced cardiotoxicity.

Statement of Purpose

The purpose of this study was threefold: (a) to examine the effects of chronic RT prior to the administration of DOX on *in vivo* and *ex vivo* cardiac function in male Sprague Dawley rats, (b) to determine if any cardioprotective

effects are a result of a reduction in lipid peroxidation, and (c) to determine if any cardioprotective effects are a result of a preservation of the cardiac myosin heavy chain (MHC) isoform distribution.

Research Hypotheses

H1 Resistance training will provide protection against the cardiac dysfunction associated with doxorubicin treatment.

H2 Resistance training will mitigate the increase in cardiac lipid peroxidation associated with doxorubicin treatment.

H3 Resistance training will attenuate the cardiac α- to β-myosin heavy chain shift that occurs with doxorubicin treatment.

Abbreviations

Table 1.1 shows the abbreviations used in this study.

Table 1.1

Abbreviations Used in This Study

Abbreviation	Explanation	Abbreviation	Explanation
4-HAE	4-hydroxyalkenal	MDA	malondialdehyde
4-HNE	4-hydroxynonenal	MHC	myosin heavy chain
ATP	adenosine triphosphate	MnSOD	manganese superoxide dismutase
CA^{2+}	calcium	MPTP	mitochondrial permeability transitional pore
CAT	catalase	NADH	nicotinamide adenine dinucleotide
CHF	chronic heart failure	NO	nitric oxide
CuSOD	copper zinc superoxide dismulase	O_2	oxygen
DNA	deoxyribonucleic acid	$O_2•^-$	superoxide anion
DOX	doxorubicin	OH	hydroxyl radical
+dP/dt	maximum rate of developed pressure	ONOO-	peroxynitrite
-dP/dt	minimum rate of developed pressure	PWd	posterior wall thickness during diastole
EDP	end diastolic pressure	PWs	posterior wall thickness during systole
EF	ejection fraction	RNS	reactive nitrogen species
ESP	end systolic pressure	ROS	reactive oxygen species
FS	fractional shortening	RYR	ryanodine receptor
GPX	glutathione peroxidase	SERCA2	sarcoendoplasmic reticulum ATPase 2
HSP	heat shock protein	SED	sedentary
H_2O_2	hydrogen peroxide	SOD	superoxide dismutase
i.p.	intraperitoneal	SWd	septal wall thickness during diastole
LV	left ventricular	SWs	septal wall thickness during systole
LVDd	LV end diastolic diameter		
LVDP	LV developed pressure		
LVDPd	LVDP during diastole		
LVDs	LV end systolic diameter		

Definition of Terms

Apoptosis. Programmed cell death.

Cardiomyopathy. Disease of the heart when the myocardium becomes enlarged, thick, or rigid and can lead to heart failure.

Cardioprotection. Adaptations in the heart that provide increased tolerance to ischem or chemical-induced injury.

Cardiotoxocity. Damage to the heart from cytotoxic drugs.

Cytochrome c. A small essential protein located at outside of inner mitochondria membrane attaching to cardiolipin, transports electrons (between complex III and IV) during aerobic energy production, and initiates intrinsic apoptosis pathway.

Dilated cardiomyopathy. A pathological condition of the heart where the chamber enlarges and the walls thin, interfering with ejection of blood from the heart.

Doxorubicin. Chemotherapy agent approved by the Food and Drug Administration for the treatment of a variety of cancers including non-Hodgkin's lymphoma, acute leukemia, and multiple myeloma and cancers of the breast, adrenal cortex, endometrium, lung, and ovary.

Echocardiogram. An ultrasonic technique used to image real-time cardiac tissue dimensions and blood flow velocities.

M-mode. An imagining mode used to determine cardiac geometry during echocardiography.

CHAPTER II

REVIEW OF LITERATURE

Doxorubicin

In 1958, *Streptomycin peucetius* was isolated from soil by the Farmitalia Research Laboratory in southern Italy. From *Streptomycin peucetius*, researchers were ultimately able to derive doxorubicin (DOX) (Di Marco et al., 1964). The DOX, trade name Adriamycin®, is a 14-hydroxy analog of daunorubicin. The DOX is a member of the family of anthracyclines that has been established as a highly effective antineoplastic agent administered as a treatment for multiple cancers. These antineoplastic agents are clinically prescribed to treat leukemia, non-Hodgkin lymphomas, breast cancer, Hodgkin's disease, and sarcomas (Young, Ozols, & Myers, 1981). Although effective, DOX is oftentimes accompanied by a number of deleterious side effects on cardiac tissue, which may include arrhythmias, contractile abnormalities, dilated cardiomyopathy, and chronic heart failure (Narula et al., 1996; Singal, Li, Kumar, Danelisen, & Iliskovic, 2000; Zhang, Shi, Li, & Wei, 2009). The mechanisms underlying the toxic effects of DOX still remain unclear; however, proposed actions include its interference with topoisomerase-II (Tewey, Chen, Nelson, & Liu, 1984), induction of apoptosis, and an increased generation of ROS (Minotti et al., 2004).

The DOX can intercalate DNA leading to the inhibition of topoisomerase-II by stabilizing the DNA-topoisomerase-II complex. Under normal conditions, topoisomerase-II is able to cleave the DNA double helix, thereby allowing DNA fragments to pass through the break before rejoining the two strands. The formation of the ternary topoisomerase-II-DOX-DNA cleavage complex inhibits the function of topoisomerase-IIβ (Lyu et al., 2007). Topoisomerase-IIβ inhibition leads to DNA strand breakage and ultimately cellular apoptosis (Hilmer, Cogger, Muller, & Le Couteur, 2004; Zhang et al., 2012). The DOX can also induce cell death by activation of two apoptotic pathways, extrinsic and intrinsic.

The extrinsic and intrinsic pathways are the two main pathways that stimulate the caspases. Activated when extracellular signals bind with transmembrane death receptors, such as the tumor necrosis factor, the extrinsic pathway can lead to apoptosis beginning with the activation of caspase-8 (Tait & Green, 2010). The intrinsic pathway begins through the release of mitochondrial cytochrome c. After release of cytochrome c into the cytosol, it forms an apoptosome complex by combining with apoptosis activating factor and procaspase-9. This formation, in turn, leads to the activation of caspase-9. Caspase-9 cleaves downstream caspases, thereby initiating apoptosis (Acehan et al., 2002; Salvesen & Dixit, 1997).

Another proposed mechanism of DOX's antineoplastic effects is oxidative stress. Normal cells generate a modicum of ROS, which may play a positive role in the regulation of cell growth and intercellular signaling (Mantovani et al., 2003). In order to combat the overproduction of ROS, cells

produce antioxidant enzymes that can control the concentration of free radicals to mitigate ROS-mediated damage. These enzymes include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). In a physiological milieu, the administration of DOX has been reported to generate a significant increase in the production of ROS (Balanehru & Nagarajan, 1992). The condition of an imbalance between the antioxidant and pro-oxidant forces within and outside the cell is referred to as oxidative stress. This imbalance may lead to the damage of proteins, DNA, and lipids (Nordberg & Arnér, 2001).

A common form of a ROS-induced damage is lipid peroxidation (Hrelia et al., 2002; Tam et al., 2006). The ROS attack and degrade polyunsaturated fatty acids leading to damage of the membrane structure which, in turn, compromises membrane integrity. This cascade of events is referred to as lipid peroxidation. Compromised membrane integrity can alter membrane receptor function, calcium homeostasis, and eventually induce apoptosis.

Doxorubicin-Induced Cardiotoxicity

The DOX-induced cardiotoxicity is multifactorial and complex, involving both ultrastructural and functional changes. Evidence indicates that the risk of cardiotoxicity rises with an increase in the cumulative dose of DOX (Shapiro et al., 1998; Von Hoff et al., 1979). The DOX-induced cardiotoxicity can be broken down into three distinct categories: acute myocardial injury, chronic cardiotoxicity, and late-onset cardiac dysfunction. Occurring immediately following a single dose of DOX, acute myocardial injury has been observed in the forms of hypotension, tachycardia, and arrhythmias (Herman, Mhatre, Lee,

Vick, & Waravdeker, 1971; Schimmel, Richel, van den Brink, & Guchelaar, 2004). The second type of DOX-induced cardiotoxicity, chronic cardiotoxicity, has been reported to be an established, clinically prevalent form on which great importance has been placed. Chronic cardiotoxicity is characterized by tachycardia, left ventricular (LV) cavity dilation, exercise intolerance, and progressive cardiac injury (Ferrans, 1978). Increased awareness has been made regarding the third category of DOX-induced cardiotoxicity, late-onset cardiac dysfunction. Late-onset cardiac dysfunction becomes evident in the years to decades after treatment with DOX. Key features of late-onset cardiac dysfunction include arrhythmias, LV systolic dysfunction, cardiomyocyte damage, and chronic heart failure (Schwartz et al., 1987; Steinherz et al., 1991). The dose-related significance of chronic and late-onset cardiotoxicity relating directly to increased mortality rates indicates that if a patient is given a cumulative dose in excess of 550 mg/m^2 of body surface, morbidity rates dramatically increase (Singal & Iliskovic, 1998).

The heart is a high-energy organ reliant upon a constant supply of adenosine triphosphate (ATP) via mitochondrial oxidative phosphorylation. The targeted organelle by DOX-induced cardiotoxicity is the cardiomyocyte mitochondria. Studies have demonstrated that DOX can accumulate in the mitochondria, and this may be due to the high affinity DOX has for the mitochondrial phospholipid cardiolipin (Goormaghtigh, Chatelain, Caspers, & Ruysschaert, 1980). Cardiolipin is a phospholipid specific to the inner mitochondrial membrane and plays an integral part in electron transport chain assembly. The attachment of DOX to cardiolipin creates a complex displacing

cardiolipin from key mitochondrial proteins. This DOX-cardiolipin complex inhibits cytochrome c oxidase and mitochondrial enzymatic complex I and III, reducing ATP production and triggering apoptotic pathways (Goormaghtigh et al., 1980; Goormaghtigh, Huart, Praet, Brasseur, & Ruysschaert, 1990).

A central mechanism associated with DOX-induced cardiotoxicity is the overproduction of ROS and reactive nitrogen species (RNS). As stated previously, cardiomyocytes are highly aerobic and require a constant supply of ATP by oxidation phosphorylation. The DOX administration may lead to an overproduction of ROS and RNS, which leads to cardiotoxicity by the disruption of the cardiac sarcoplasmic reticulum and the mitochondrial respiratory chain. The increased generation of ROS results mainly in the mitochondria where it undergoes redox cycling at complex I of the electron transport chain (Marcillat, Zhang, & Davies, 1989).

The quinone form of DOX can be converted to a semiquinone free radical through univalent reduction. The semiquinone radical can then be re-oxidized by a one-electron transfer to molecular oxygen, regenerating the parent quinone with superoxide anion ($O_2^{\bullet-}$) as a byproduct (Nohl, Gille, & Staniek, 1998). Large amounts of $O_2^{\bullet-}$ will be generated through redox cycling between the quinone and semiquinone forms of DOX. The DOX-induced redox imbalance may lead to the excess generation of superoxide.

The superoxide radical can be converted to hydrogen peroxide (H_2O_2) by SOD. From here, the anti-oxidative enzymes, CAT and GPX, reduce H_2O_2 to water (H_2O) (Harrison et al., 2005). The overproduction of $O_2^{\bullet-}$ can lead to increased production of H_2O_2. The two radicals can react with one another,

forming a hydroxyl radical (OH), via the Harber-Weiss reaction. This reaction can lead to lipid peroxidation (Radi, Beckman, Bush, & Freeman, 1991). The H_2O_2 is able to traverse the mitochondrial membrane and generate an OH in the presence of iron, copper, cobalt, and chromium via the Fenton reaction (Henkler, Brinkmann, & Luch, 2010).

Ferritin is the main iron storage protein. Release of iron from ferritin can occur in the presence of $O_2^{\bullet-}$. It achieves this dissociation by disassembling the 4Fe-4S cluster. In doing so, this phenomenon has been reported to increase the activity of iron regulating protein-1, leading to increased iron uptake. The increases in free iron at the cellular level can react with H_2O_2, which is known as the Fenton reaction, forming an OH (Corna, Galy, Hentze, & Cairo, 2006).

The DOX can also induce the generation of RNS, such as nitric oxide (NO) and peroxynitrite (ONOO−). The ONOO−, which is formed from NO and $O_2^{\bullet-}$, is a highly reactive radical, capable of damaging tissues and cells (Mccord, 1985). The ONOO− also damages DNA by several mechanisms, such as single-strand breaks, leading to additional stress on the cardiovascular system (Szabó & Ohshima, 1997).

Oxidative stress can also disrupt calcium homeostasis by impairing the ryanodine receptor (RYR) and sarcoendoplasmic reticulum ATPase 2 (SERCA2). The ROS promotes an open state of the RYR and a diminished effect of SERCA2. This will cause a rise in intracellular calcium (Ca^{2+}) and, in turn, may induce mitochondrial Ca^{2+} overload (Kim et al., 2006). Mitochondrial Ca^{2+} overload triggers a mitochondrial permeability transition, which leads to

additional rise in calcium levels by altering the accessibility of mitochondria permeability transition pores (MPTP). The Ca^{2+} overload may induce swelling of the mitochondria, rupturing of the outer mitochondrial membrane, resulting in the release of cytochrome c into the cytosol (Childs, Phaneuf, Dirks, Phillips, & Leeuwenburgh, 2002). Release of cytochrome c will lead to the formation of an apoptosome complex which eventually leads to apoptosis (Sokolove & Shinaberry, 1988). Oxidative stress can also alter the permeability of MPTP through the downregulation of anti-apoptotic proteins, such as B-cell lymphoma 2 and activation of pro-apoptotic proteins, Bcl-2-like protein 4, and Bcl-2 homologous antagonist/killer (Crompton, 1999).

Doxorubicin-Induced Cardiac Dysfunction

Diminished LV function may be observed within the first six months after DOX treatment (Ali, Ewer, Gibbs, Swafford, & Graff, 1994). Cardiac function may be assessed *in vivo* and *ex vivo*. *In vivo* cardiac function can be analyzed by echocardiography, which assesses left ventricular end diastolic (LVDd) and end systolic diameter (LVDs), posterior wall thickness during diastole (PWd) and systole (PWs), septal wall thickness during diastole (SWd) and systole (SWs), and fractional shortening (FS). *Ex vivo* function can be analyzed using an isolated working heart. Measurements obtained from an isolated working-heart model include left ventricular developed pressure (LVDP), maximum rate of developed pressure (+dP/dt), minimum rate of developed pressure (-dP/dt), end systolic pressure (ESP), and end diastolic pressure (EDP).

Studies suggest that acute DOX treatment causes cardiac dysfunction (Chicco et al., 2006; Wonders et al., 2008). In a study by Wonders et al. (2008) it was found that DOX caused significant reductions in LVDP, ESP, and +dP/dt five days after a single bolus injection of 15 mg/kg. The DOX treatment also led to significant increases in EDP and +dP/dt. Cardiac dysfunction has also been noticeable with smaller acute doses of DOX (Hydock et al., 2008). Hydock et al. (2008) reported significant *in vivo and ex vivo* cardiac dysfunction 5 and 10 days following single bolus injections of DOX at 10 mg/kg. *In vivo* assessment indicated a significant depression of FS and significant decreases in SWs and SWd at both time points. *Ex vivo* assessment showed that following treatment, LVDP and +dP/dt were significantly reduced at both time points. Along with this, significant reductions in –dP/dt were also observed 10 days post injection.

Along with acute DOX treatment, studies have also examined altering the time course of treatment and cardiac function (Hayward & Hydock, 2007; Hydock et al., 2011). A cornerstone study was carried out by Hayward and Hydock (2007) in which they examined *in vivo* cardiac function for acute and chronic dosing schedules. Animals received 10 mg/kg of DOX by a bolus injection (group 1), 10 daily injections of 1 mg/kg/day (group 2), or five weekly injections of 2 mg/kg/wk (group 3). Cardiac dysfunction was evident within the acute and chronic dosing groups. Evidence of dysfunction appeared for all groups in the form of decreased FS. Morphological changes resembling dilated cardiomyopathy were reported for all three groups. Decreases in septal and posterior ventricular wall thicknesses were observed in the three

groups. However, for groups 2 and 3 there was a delayed response in changes of cardiac geometry.

Exercise Protection

It is widely acknowledged that chronic exercise training provides wide-ranging benefits to a multitude of organs including the heart. Studies have reported that different forms of exercise, such as treadmill running, wheel-running, and swimming can attenuate DOX-induced cardiotoxicity. Mechanisms underlying the cardioprotection afforded by exercise may be dependent upon a number of factors including the dose of DOX, the modality of exercise, the temporal relationship between exercise and DOX treatment, subject comorbidities, gender, or even accompanying cancer therapies. Proposed mechanisms of cardioprotection have included reduced DOX accumulation within the cardiomyocyte (Jensen et al., 2013), attenuating unfavorable shifts towards β-myosin heavy chain (β-MHC) (Hydock et al., 2008), decreased oxidative stress (Ascensão et al., 2005), and increased HSP expression (Ascensão, Ferreira, Oliveira, & Magalhães, 2006; Kavazis, Smuder, Min, Tümer, & Powers, 2010). From available evidence, it appears that multiple mechanisms may be simultaneously involved with the protective effects of chronic aerobic exercise.

Oxidative Stress

One of the main mechanisms for cardiotoxicity is the overproduction of ROS, which can lead to DNA damage and/or lipid peroxidation. Various studies have presented results suggestive of exercise as a positive modulator of oxidative stress, which can be examined by the expression of key

antioxidant enzymes (Lennon et al., 2004; Somani, Frank, & Rybak, 1995) and one of the principle byproducts of lipid peroxidation, malondialdehyde (MDA) (Marques-Aleixo et al., 2014).

An early study by Quintanilha (1984) was one of the first to report an increase in antioxidant enzyme activity due to exercise training, and since then literature on the subject has expanded. A study by Powers et al. (1993) was the first to examine the influence of intensity and duration of exercise training on the activity antioxidant enzymes. Animals treadmill trained at one of the combinations of duration (30, 60, or 90 minutes) and intensity (low, moderate, or high) for 10 weeks. Intensity was characterized as low (55% of maximal O_2 uptake), moderate (65% of maximal O_2 uptake), and high (75% of maximal O_2 uptake). Results indicated that high intensity exercise for all durations resulted in an increase SOD activity within the LV. An increase in SOD activity was also observed in both low and moderate intensity when training was 60 minutes or greater.

Along with these studies, an extensive amount of research has been conducted examining exercise-induced adaptations in antioxidant activity, which is believed to play a role in the attenuation or reduction of DOX-induced cardiotoxicity (Ascensão et al., 2006; Ascensão et al., 2005; Powers et al., 1993). Wonders et al. (2008) found that an acute bout of treadmill exercise significantly reduced lipid peroxidation in animals receiving a 15 mg/kg bolus injection of DOX. Likewise, a more recent study by Ascensão et al. (2011), examined the effects of an acute bout of exercise on DOX-treated rats. Animals in the exercise group performed a 60-minute treadmill exercise bout

24 hours prior to receiving a DOX injection (20 mg/kg). Results indicated that exercise provided a cardioprotective response from DOX treatment by increasing cardiac mitochondrial SOD activity and reducing caspase activity.

An early study by Kanter et al. (1985) was one of the first to report the effects of chronic aerobic exercise on DOX-induced oxidative stress. The study examined the effects of a 21-week swim training program on antioxidant enzymes and DOX-associated cardiotoxicity. The investigators reported that chronic exercise training led to physiological adaptations eliciting cardioprotective effects counteracting DOX, which may have been attributed to the upregulation of antioxidant enzymes. This has been substantiated by studies examining different modes of exercise and the time course of treatment. Kavazis et al. (2010) found that a short-term exercise training (10 sessions) prior to a single bolus injection of DOX (20 mg/kg) increased manganese superoxide dismutase (MnSOD), copper zinc superoxide dismutase (CuSOD), CAT, and GPX while attenuating DOX-induced 4-hydroxynonenal formation. A study by Chicco et al. (2006) examined the effects of a long-term training program (12 weeks) prior to DOX treatment and reported a significant decrease in lipid peroxidation. The role of oxidative stress on intracellular damage has been well-established. The data suggest that exercise is an effective countermeasure to intracellular damage caused by oxidative stress. This, in part, could be due to increase myocardial antioxidants.

Heat Shock Proteins

One mechanism by which exercise may protect the myocardium is through the induction of HSPs. The HSPs serve as molecular chaperones during both protein assembly and membrane translocation. An elevation of HSPs may play a protective role by preventing the denaturing of regulatory proteins, stabilizing newly denatured proteins, and protecting against apoptosis (Powers & Demirel, 2001).

Demirel et al. (1998) conducted a 10-week treadmill training program with rats and measured HSP72 expression. Exercised animals ran 4 days per week and up to 90 minutes per day at 75% of maximal oxygen consumption (VO_{2max}). The investigators reported that exercise training was associated with a large (450%) increase in HSP72 in the LV. A study conducted by Harris and Starnes (2001) had animals performing a three-, six-, or nine-week chronic exercise program. After a week habituation of running on the treadmill at 6% incline for 10 minutes, the duration of the exercise was gradually increased to 60 minutes per day for five days a week by the end of week three. The duration and intensity was maintained throughout the remaining weeks. Animals in the exercise trained groups produced an 8.5-fold, 10.7-fold, and 12.3-fold increase in HSP70 in the LV, for three-, six-, and nine-week training groups, respectively. Similarly, studies have examined cardiac HSP expression posttraining as a method to attenuate DOX-induced cardiac dysfunction. A study by Chicco et al. (2006) reported an increase expression of HSP72 in an exercise group, which attenuated DOX-induced cardiac dysfunction. Ample evidence has indicated that exercise can elicit altered and

increased expression of HSPs; however, evidence to support variations of intensity-related physiological adaptations has yet to be determined (Locke et al., 1995; Noble et al., 1999). Despite evidence that increased HSP expression may provide a cardioprotective effect, studies have reported that this exercise-induced cardioprotection may be mechanistically independent to the observed increase in HSPs (Kavazis et al., 2010; Taylor, Harris, & Starnes, 1999).

Myosin Heavy Chain Expression

The DOX treatment has been shown to induce an α-β shift in MHC isoforms, ultimately resulting in impaired cardiac function. Aerobic exercise prior to and during DOX treatment has been shown to preserve α-MHC expression and attenuate DOX-induced cardiac dysfunction (Hydock et al., 2012). Hydock, Wonders, Schneider, and Hayward (2005) examined the effects of voluntary wheel running on MHC distribution during six weeks of DOX treatment. It was concluded that exercise during DOX treatments leads to a significant increase in the expression of α-MHC when compared to the SED DOX group. Furthermore, Hydock et al. (2008) examined if exercise preconditioning would provide a cardioprotective effect against a single bolus injection of DOX (10 mg/kg) and whether this was associated with altered MHC expression. Animals performed a progressive treadmill training program for 10 weeks followed by DOX treatment and what was observed was a preservation of MHC isoform distribution that could be linked to cardioprotection. DOX-induced cardiac dysfunction may be influenced by a noticeable shift in MHC expression from α to β isoforms. Evidence suggests

that preservation of α-MHC expression may play a mechanistic role in exercise-induced cardioprotection. Although the percentage of cardiac α-MHC isoform differs greatly between humans (< 3%) and rats (> 90%), its expression plays an important role in human cardiac function (Miyata, Minobe, Bristow, & Leinwand, 2000). A study by Lowes et al. (1997) reported a downregulation of α-MHC with a corresponding upregulation of β-MHC in failing ventricular myocardium. The α to β shift is linked to significant decreases to cardiac contractility (Tardiff et al., 2000).

Doxorubicin Accumulation

The DOX treatment can lead to its accumulation in tissues including skeletal and cardiac muscle which may explain functional decrements (Hayward et al., 2013). Recent studies from our laboratory have reported that exercise, both voluntary wheel running and treadmill training, resulted in decreased DOX accumulation (Gibson, Quinn, Pfannenstiel, Bashore, et al., 2013; Jensen et al., 2013). From these studies, it appears as though preservation of cardiac function may be influenced by a decrease in DOX accumulation (Jensen et al., 2013).

The accumulation of DOX within cardiomyoctyes may be a result of multidrug resistance protein (MRP) regulation. MRPs are a part of the superfamily of ATP binding cassette (ABC) transporters that function as drug efflux pumps. Multiple types of MRPs have been reported to be expressed in cardiac tissue, including MRP-1, MRP-2, and MRP-7 (Gibson, Quinn, Pfannenstiel, Hydock, & Hayward, 2013). Evidence has indicated that exercise can increase expression of myocardial MRP-1, MRP-2, and MRP-7,

which can lead to the preservation of cardiac function as a result of decreases in DOX accumulation (Gibson, Quinn, Pfannenstiel, Bashore, et al., 2013; Krause et al., 2007).

Resistance Training

The benefits of acute and chronic aerobic exercise on cardiac dysfunction have received a great deal of attention. Since myocardial tissue relies heavily on oxidative metabolism, much of the research has focused on aspects of oxidative metabolism; there is a limited amount of research investigating the cardioprotective effects of resistance exercise training. Though limited, some studies have indicated beneficial effects to both physiological and pathological processes.

The beneficial effects of RT training may be influenced by the adaptations that occur to the cardiovascular system. Similar to aerobic training, physiological cardiac hypertrophy can occur with RT. Characteristics of the cardiac hypertrophy, however, differ between the two due to the type of circulatory overload that occurs with RT (Pluim, Zwinderman, van der Laarse, & van der Wall, 2000). Possible adaptations that may occur include increased interventricular septum and wall thickness, which has led to this type of hypertrophy being termed concentric cardiac hypertrophy. Concentric cardiac hypertrophy has been observed in pathological conditions such as hypertension, which can be linked to systolic and diastolic dysfunction (Barauna, Rosa, Irigoyen, & de Oliveira, 2007). However, it has been indicated that the cardiac hypertrophy observed from RT will not lead to

abnormalities in function but may enhance systolic function (Colan, Sanders, & Borow, 1987; Levinger, Bronks, Cody, Linton, & Davie, 2005).

Not only can RT influence hypertrophy, it can also provide cardioprotection against intracellular damage. In order to induce adaptions that will, in turn, attenuate oxidative stress, an acute bout of exercise must actually elicit oxidative stress. Studies have reported that an acute bout of a resistance exercise can cause an increase in ROS as well as can lead to an attenuation of oxidative damage (Goldfarb, Bloomer, & McKenzie, 2005; McBride, Kraemer, Triplett-McBride, & Sebastianelli, 1998; Vincent, Vincent, Braith, Lennon, & Lowenthal, 2002). The above studies do indicate that an acute bout of resistance exercise can induce oxidative stress as well as serve as a stimuli, which will attenuate it; however, studies that look specifically at myocardial oxidative stress are extremely limited. Chicco et al. (2006) examined the effects of a six-week RT program on cardiac oxidative stress caused by alcohol consumption. Rats were assigned to one of four groups: SED, SED plus alcohol treatment, RT, or RT plus alcohol treatment. The exercise groups trained by standing on their hind limbs while wearing a weighted vest. Training activity was repeated 30 times per session three days a week. The load lifted was incrementally increased by 20% of the animal's body weight each week. Results of the study indicated that this type of RT attenuated ethanol-induced cardiac lipid peroxidation. The studies above indicate that resistance exercise can induce cellular damage, which in turn can lead to an adaptation whereby the cell is protected against oxidative stress.

The RT-induced cardioprotection, in the form of improved cardiac function, has also been observed with other models. A study by Soufi, Saber, Ghiassie, and Alipour (2011) showed the cardioprotective effects of a 12-week RT program against ischemia-reperfusion-induced injury in rats. Training was performed on a squat-training apparatus in which rats would stand on their hind legs. Through an electrical stimulation of the rat's tail, hind limbs would contract, raising a weighted piston that was located above the animals head. Animals performed four sets of 12 repetitions per day, with a 90-second rest period between each set, five times per week for 12 weeks. The investigators noted that the observed significant ($p < .05$) increases in LVDP indicated that this type of RT model can provide cardioprotection against myocardial injuries. This RT-induced improvement in cardiac function has also been observed in patients with mild and chronic congestive heart failure (Levinger et al., 2005; Palevo, Keteyian, Kang, & Caputo, 2009). These studies suggest that resistance exercise training may provide protection against treatments and conditions that cause myocardial injury.

Summary

Although DOX is limited by a dose-dependent cardiotoxicty, it still remains one of the most effective antieoplastic agents used to treat a variety of cancers. Acute and chronic DOX treatments may elicit varying degrees of cardiac dysfunction including arrhythmias, LV cavity dilation, and chronic heart failure. Though we continue to learn more about the mechanisms behind the development of cardiotoxicity, it has been proposed that uncontrolled generation of ROS plays a key role. Exercise has been shown to mitigate

DOX-induced cardiotoxicity and preserve cardiac function. This exercise-induced attenuation is thought to be linked to a variety of positive physiological adaptations including increased HSP capacity, decreased caspase activity, preservation of the cardiac MHC isoform distribution, and increased antioxidant activity. Multiple studies have implemented treadmill or voluntary wheel running as the modality of exercise intervention, while few researchers have examined the effects of RT on DOX-associated toxicity. Therefore, the purpose of this study was to determine the effects of chronic resistance exercise preconditioning on DOX-induced cardiac dysfunction and DOX-induced oxidative injury.

CHAPTER III

METHODS

Experimental Design

The exercise modality implemented in this study mimicked resistance exercise training and consisted of a raised-caged model, which forces animals to assume an erect bipedal stance while feeding and drinking. Following 12 weeks of training, a bolus 12.5 mg/kg doxorubicin (DOX) injection was administered 24 hours after trained animals had been placed into non-elevated cages. Control animals received a bolus intraperitoneal injection of 0.9% SAL. Rats were sacrificed five days after DOX treatment, and cardiac function was analyzed both *in vivo* and *ex vivo*. Following *ex vivo* cardiac function assessment, the LV was isolated and flash frozen in liquid nitrogen for biochemical analysis.

Subjects

Ten-week-old male Sprague Dawley rats (N = 48) were randomly assigned to sedentary (SED) (n = 24) or RT (n = 24) groups in a temperature-controlled facility with a 12:12-hour light-dark cycle. Rats were provided standard rat chow and water *ad libitum*. All protocols used for the study were approved by the University of Northern Colorado Institutional Animal Care and

Use Committee and was in compliance with the Animal Welfare Act guidelines (see Appendix).

Exercise Training

Animals that were randomly assigned to the RT group were placed in cages that allow for a progressive elevation of food and water, encouraging the animals to rise to a bipedal stance each time they eat and drink. Cage height was raised to specified heights using specially designed plastic spacers that sat between the standard cage and the standard cage lid. Placement of the spacers between the cage and the lid raises the food and water to the desired height. This RT model has been shown to increase hind limb muscle mass and increased tibial cortical bone (Yao, Jee, Chen, Li, & Frost, 2001). On day one of the training protocol, cage height was raised from the standard 20.32 cm to a height of 28 cm. Cage height remained at 28 cm for one week. Following the first week, the height was raised by 2.5 cm. From there, every third day cages were raised 2.5 cm until they reach the final height of 35.5 cm. This height was maintained for an additional 10 weeks for a total of 12 weeks of training. Food and body weight were recorded daily, and adequate water consumption was confirmed for the first three weeks of training to ensure animals were able to reach both food and water. Following the first three weeks of training, animals had their food measured and water monitored three days per week, and body weight was measured weekly.

At the completion of the 12-week training period, all plastic spacers were removed so that that all food and water returned to the standard cage height. This was done to ensure that there were no effects of acute exercise

on the observed dependent variables. All animals remained in these standard cages for 24 hours prior to DOX treatment. Following the 24-hour period, each animal was randomly assigned to one of four groups: SED+SAL (*n* = 9), SED+DOX (*n* = 15), RT+SAL (*n* = 9), and RT+DOX (*n* = 15). The SAL groups received a bolus intraperitoneal 0.9% SAL injection, and the DOX group received a bolus intraperitoneal 12.5 mg/kg DOX injection.

Various exercise modalities have been used to mimic RT. These include tower climbing (Notomi et al., 2001), an erected bipedal stance exercise with a weighted vest (Westerlind et al., 1998), and an erected bipedal stance whole-body exercise (Yao et al., 2000). Consistent findings have observed that an exercise modality that mimics RT can cause adaptation to bone, skeletal muscle, and cardiac muscle (Barauna et al., 2007; Duncan, Williams, & Lynch, 1998; Notomi et al., 2001). There is no standard or set protocol for laboratory animal weight-bearing exercise modalities. The range of total repetitions and total resistance varies greatly between studies.

The RT model has been used by others (Mo et al., 2002; Rosa, Firth, Blair, Vickers, & Morel, 2011; Yao et al., 2000) and pilot studies in our laboratory. Recently in our laboratory, the RT model has been associated with positive functional adaptations in skeletal muscle, which have also been observed following resistance protocols discussed previously. These data, along with the pilot study, suggest that the exercise modality may be an effective model for exercise-induce cardiac adaptations.

Assessment of Cardiac Function

In Vivo Cardiac Function

In vivo cardiac function was analyzed five days after DOX treatment via echocardiography. Animals were sedated with ketamine (40 mg/kg, intraperitoneal), and the anterior and left lateral thoracic regions were shaved. Transthoracic echocardiography was conducted on the sedated rats using a General Electric Vivid 7 ultrasound with a 10-MHz intraoperative transducer (Tustin, California). Measurements of septal wall thickness during systole and diastole, posterior wall thickness during systole and diastole, LV end systolic and diastolic diameter, and FS were acquired by M-mode tracings of the LV obtained in a short axis view. From an apical view using a pulsed-wave Doppler, aortic and mitral blood flow echocardiographic images were obtained providing measurements of maximal flow velocity for both aortic and mitral valves. All echocardiographic measurements were averaged from three consecutive cardiac cycles. All M-mode and Doppler measurements were made in accordance with guidelines established by the American Society of Echocardiography.

Ex Vivo Cardiac Function

Ex vivo cardiac function was analyzed following echocardiography via an isolated working-heart model (ADInstruments, Colorado Springs, Colorado). Each animal was anesthetized using an intraperitoneal injection of heparinized (500 U) sodium pentobarbital (50 mg/kg). Following an absent tail pinch reflex, the heart was excised and placed into ice cold Krebs-Henseleit buffer (in mM: 120 NaCl, 5.9 KCl, 2.5 $CaCl_2$, 1.2 MgCl, 25 $NaHCO_3$, 17

glucose, and 0.5 ethylenediaminetetraacetic acid [EDTA]) aerated with 95% O_2 – 5% CO_2. Hearts were quickly cannulated by the ascending aorta and subjected to retrograde perfusion until all blood was cleared from the coronary vasculature. The pulmonary vein was then cannulated, and blood flow was redirected from the aorta to the left atrium to initiate the working-heart model. Once stabilized, a microtip catheter pressure transducer (Millar Inc., Houston, Texas) was placed into the LV via the apex for determination of LV developed pressure (LVDP), LV maximal rate of ventricular pressure development (+dP/dt), and LV rate of pressure decline (-dP/dt). Preload was set at 10 cmH_2O and was maintained for all subsequent functional measurements. Afterload was progressively increased every three to five minutes during data collection from 85 cmH_2O, to 100 cmH_2O, and to 115 cmH_2O. After data were collected at the three different afterloads, afterload was adjusted to 100 cmH_2O, and hearts were paced at 240 beats per minute using electrodes attached to the cannula. The LV functional data were collected and analyzed using a PowerLab data acquisition system (ADInstruments). Once data were collected, hearts were trimmed of connective tissue and fat and weighed. The LVs were then isolated, flash frozen in liquid N_2, and stored at –80°C until subsequent biochemical analyses.

Biochemical Analysis

Lipid Peroxidation

A commercially available assay kit (Bioxytech MDA-586, Oxis Research, Foster City, California) was used to measure malondialdehyde + 4-hydroxyalkenal (MDA + 4-HAE) as an indicator of cellular lipid peroxidation.

Tissues were homogenized in a radioimmunoprecipitation buffer for the assay. A 200 µL aliquot of each sample was added to a microcentrifuge tube followed by 650 µL of N-methyl-2-phenylindole in acetonitrile and briefly vortexed. Next, 150 µL of methanesulfonic acid was added, vortexed, and incubated at 45°C for 60 minutes. Samples were then centrifuged at 10,000 *g* for 10 minutes. The resulting supernatant was transferred to a cuvette and absorbency measured using a spectrophotometer at 586 nm. The MDA + 4-HAE was estimated from a standard curve. All samples were assayed in duplicate, and any samples varying more than 5% were reassessed.

Myosin Heavy Chain Analysis

Sample preparation was adapted from the method of Thomason, Baldwin, and Herrick (1986). Approximately 100 mg of LV tissue was minced in a 2 mL glass tissue homogenizer with surgical scissors. A 1:10 weight/volume ratio of homogenization buffer (250 mM sucrose, 100 mM KCl, 5 mM EDTA, and 20 mM Tris-Base, 4°C, pH 6.8) was added to the homogenizer. The sample was homogenized approximately five minutes using a ScilogexD160 homogenizer (Rocky Hill, Connecticut). Homogenates were centrifuged at 1,000 *g* for 10 minutes at 4°C and pellets collected and resuspended, with the same volume as the homogenization buffer, in a washing buffer (175 mM KCl, 0.5% Triton X-100, 2 mM EDTA, and 20 mM Tris-Base, 4°C, pH 6.8). Samples were centrifuged again at 1,000 *g* for 10 minutes at 4°C. The pellets were collected, resuspended with the same volume as the previous washing buffer, and centrifuged at 1,000 *g* for 10

minutes at 4°C. The pellets were resuspended in the final resuspension buffer (150 mM KCl, and 20 mM Tris-Base, pH 7.0) at 1/12 of the previous volume.

Total protein concentration was determined using the Bradford protein assay method (Bradford, 1976). Samples were then diluted to 2 mg/mL with the final resuspension buffer. Twenty-five µL of each sample was further diluted to 0.125 mg/mL with 2x Laemmli sample buffer (20% glycerol, 16% 1M Tris [pH 6.8], 4% sodium dodecylsulfate [SDS], 1% β-mercaptoethanol, and 0.2% bromophenol blue). To denature the proteins, samples were boiled for two minutes and then placed on ice for another 10 minutes. Following the 10 minutes on ice, samples were loaded onto polyacrylamide gels.

A SDS polyacrylamide gel electrophoresis was used to determine MHC isoform expression. The MHC gel composition used the modified method of Reiser and Kline (1998) from the original method of Talmadge and Roy (1993). A 0.75 mm Snap-A-Gel cassette (Jule, Inc., Milford, Connecticut) was used to cast polyacrylamide gels. The gel polymerization of separating gels (5% of 100% glycerol, 8% acrylamide-N,N'-methylene, 4% acrylamide-bis [50:1], 0.2 M Tris-Base [pH 8.8], 0.1 M glycine, and 0.4% of 10% SDS) was initiated by adding 0.05% *N,N,N′,N′*- tetramethylethylenediamine and 0.1% ammonium persulfate (10%). After initiating the gel polymerization, the separating gel solution was pipetted into cassettes to approximately 1 cm below the height of the bottom of comb. N-butonol was then pipetted to the top of the gel in order to flatten the separating gel during polymerization. Cassettes were then placed in the refrigerator for at least 60 minutes to allow the gel to polymerize.

Following polymerization of the separating gel, n-butanol was removed and cassettes rinsed five times with milli-pure water. The polymerization of the stacking gel (5% of 100% glycerol, 4% acrylamide-bis [50:1], 70 mM Tris-Base [pH 6.7], 4 mM EDTA and 0.4% of 10% SDS) was initiated by adding 0.05% tetramethylethylenediamine and 0.1% ammonium persulfate (10%). The stacking gel solution was pipetted to the top of the separating gel and a 10-lane comb was inserted (Jule, Inc.) into the stacking gel. Cassettes were then placed in the refrigerator for at least 60 minutes to allow the stacking gel to polymerize.

Following polymerization of the stacking gel, combs were removed. Cassettes were placed into Novex Sure Lock cells (Invitrogen Corporation, Carlsbad, California). Cassettes were locked and then the middle chamber was filled with upper running buffer (0.1 M Tris, 150 mM glycine, and 0.1% SDS). Next, 7.5 µL of BenchMark™ protein standard (Invitrogen Corporation) was loaded in the first lane. Following the protein standard, 7 µL of the samples was loaded in the remaining lanes. Lower running buffer (50 mM Tris, 75 mM glycine, and 0.05% SDS) was poured into the outside chamber of the electrophoresis unit.

Electrophoresis was conducted at 100 V, 25 mA for approximately three hours or until the tracking dye was near the bottom of the gel. The gels were then stained with Simply Blue safe stain for 60 minutes. Gels were then placed in distilled water overnight. The following day, gels were removed and placed between two transparency sheets and scanned. The density of MHC

protein bands was analyzed using Image J densitometry software (National Institutes of Health, Bethesda, Maryland).

Statistical Analysis

Data were analyzed and presented using GraphPad Prism statistical software. All data are expressed as mean ± standard deviation ($M \pm SD$). A two-way analysis of variance (ANOVA) was used to determine significant difference due to the main effects (DOX and RT) and interaction. Upon observation of a significant difference, a Tukey post-hoc pair-wise comparison was conducted to evaluate any pair-wise comparisons. For all statistical analysis, significance is set at $\alpha = 0.05$.

CHAPTER IV

RESULTS

The purpose of this study was threefold: (a) to examine the effects of chronic RT prior to the administration of doxorubicin (DOX) on *in vivo* and *ex vivo* cardiac function in male Sprague Dawley rats, (b) to determine if any cardioprotective effects are a result of a reduction in lipid peroxidation, and (c) to determine if any cardioprotective effects are a result of a preservation of the cardiac MHC isoform distribution.

General Observations

General observations are presented in Table 4.1. Six animals died during the course of this experiment, making the total mortality rate 20% for DOX treated-animals. Resistance training cut mortality in half, with SED+DOX having a mortality rate of 27% (4/15); whereas, the mortality rate for RT+DOX was 13% (2/15). Animals in both SAL groups continued to gain body weight in the five days following injection; however, these gains were not significant. A 2-way ANOVA on final body weight revealed a drug effect, $F(1, 38) = 29.2$, $p < 0.0001$, with the DOX-treated animals' body weight significantly lower than the SAL-treated animals. Five days following injection, body weight decreased 14% for both DOX groups, with no statistically significant difference between DOX groups. A 2-way ANOVA on absolute heart mass revealed both a drug

effect, $F(1, 38) = 6.757$, $p = 0.0132$, and activity effect, $F(1, 38) = 11.33$, $p = 0.0018$. Absolute heart mass was significantly higher in the RT+SAL group compared to SED+DOX. Relative heart mass also showed a drug effect, $F(1, 38) = 5.448$, $p = 0.025$, and activity effect, $F(1, 38) = 18.87$, $p = 0.0001$. Both RT groups had greater heart mass when compared to SED+SAL. RT+DOX heart mass relative to body weight was also significantly higher than SED+DOX.

Table 4.1

Subject Demographics

		N	Injection body weight (g) *M* ± *SD*	Final body weight (g) *M* ± *SD*	Heart mass (g) *M* ± *SD*	Relative heart mass (%) *M* ± *SD*
SAL	SED	9	441 ± 37	446 ± 36	1.5 ± 0.18	0.35 ± 0.02
	RT	9	439 ± 57	442 ± 54	1.7 ± 0.17†	0.39 ± 0.03*
DOX	SED	11	447 ± 31	386 ± 26	1.4 ± 0.14	0.37 ± 0.03
	RT	13	446 ± 23	384 ± 22	1.6 ± 0.13	0.40 ± 0.04* †

Note. Data are represented as *M* ± *SD*. SED = sedentary; RT = resistance trained; SAL = saline placebo; DOX = doxorubicin. $p < 0.05$.
*$p < 0.05$ versus SED+SAL.
†$p < 0.05$ versus SED+DOX.

Echocardiography

Cardiac geometry (see Table 4.2) and blood flow velocities (see Table 4.3) were measured by echocardiogram. No statistically significant differences were seen between the groups for SWd, PWs, PWd, LVDs, and FS. Five days post injection, a 2-way ANOVA on SWs revealed a drug effect, $F(1, 38) = 13.39$, $p = 0.0008$. A *post hoc* analysis determined that SED+DOX SWs was significantly thinner ($p < 0.05$) compared to both SAL groups. A drug effect, $F(1, 38) = 14.45$, $p = 0.0005$, was also observed on LVDd. A *post hoc* analysis determined that both SAL groups LVDd was significantly greater ($p < 0.05$) than RT+DOX. Echocardiography Doppler images were used to analyze maximum blood flow through the aortic valve (A-Vmax) and mitral valve (M-Vmax). A 2-way ANOVA on the A-Vmax revealed a drug effect, $F(1, 38) = 79.25$, $p < 0.0001$, activity effect, $F(1, 38) = 15.03$, $p = 0.0004$, and an interaction, $F(1, 38) = 13.42$, $p = 0.0008$. The M-Vmax also had a drug effect, $F(1, 37) = 82.44$, $p < 0.0001$, activity effect, $F(1, 37) = 7.002$, $p = 0.0119$, and interaction, $F(1, 37) = 13.32$, $p = 0.008$. Significant decreases in A-Vmax and M-Vmax occurred with both DOX groups when compared to both SAL groups. However, RT+DOX A-Vmax and M-Vmax was significantly faster ($p < 0.05$) when compared to SED+DOX.

Table 4.2

Echocardiogram Derived Cardiac Geometry

	SED+SAL		SED+DOX		RT+SAL		RT+DOX	
	M ± *SD*	*N*	*M* ± *SD*	*N*	*M* ± *SD*	*N*	*M* ± *SD*	*N*
SWs (mm)	4.02 ± 0.22*	9	3.02 ± 0.56	11	4.17 ± 0.39*	9	3.79 ± 0.43	13
SWd (mm)	2.25 ± 0.24	9	2.10 ± 0.40	10	2.41 ± 0.28	9	2.34 ± 0.35	13
PWs (mm)	3.79 ± 0.27	9	3.51 ± 0.63	11	3.94 ± 0.25	9	3.63 ± 0.89	13
PWd (mm)	2.49 ± 0.31	9	2.33 ± 0.38	11	2.58 ± 0.30	9	2.35 ± 0.28	13
LVDs (mm)	2.29 ± 0.32	8	2.48 ± 1.02	11	2.24 ± 0.24	9	1.93 ± 0.62	13
LVDd (mm)	5.93 ± 0.29†	9	4.94 ± 1.11	11	5.44 ± 0.57†	9	4.33 ± 1.08	13
FS (%)	61.12 ± 5.21	8	51.06 ± 14.1	11	58.69 ± 5.68	9	58.28 ± 9.22	13

Note. Data are represented as *M* ± *SD*. SED = sedentary; RT = resistance trained; SAL = saline placebo; DOX = doxorubicin; SWs = septal wall thickness during systole; SWd = septal wall thickness during diastole; PWs = posterior wall thickness during systole; PWd = posterior wall thickness during diastole; LVDs = left ventricular end systolic diameter; LVDd = left ventricular end diastolic diameter; FS = fractional shortening.
*$p < 0.05$ versus SED+DOX.
†$p < 0.05$ versus RT+DOX.

Table 4.3

Echocardiogram Derived Blood Flow Velocity

	SED+SAL		SED+DOX		RT+SAL		RT+DOX	
	M ± *SD*	*N*	*M* ± *SD*	*N*	*M* ± *SD*	*N*	*M* ± *SD*	*N*
M-Vmax	98 ± 11*†	8	39 ± 9	11	93 ± 17*†	9	68 ± 10*	13
A-Vmax	74 ± 15*†	9	31 ± 10	11	75 ± 10*†	9	57 ± 10*	13

Note. Data are represented as *M* ± *SD*. SED = sedentary; RT = resistance trained; SAL = saline placebo; DOX = doxorubicin; M-Vmax = maximum blood flow through mitral valve; A-Vmax = maximum blood flow through aortic valve.
*$p < 0.05$ versus SED+DOX.
†$p < 0.05$ versus RT+DOX.

Isolated Perfused Working Heart

Following echocardiographic measurements, the cardiac variables ESP, EDP, LVDP, +dP/dt, -dP/dt, and heart rate were analyzed using an isolated working-heart apparatus. Afterload was set at three different heights (85, 100, and 115 cmH_2O) during the analysis. No statistically significant differences in heart rate and EDP were observed between the four groups at any of the afterloads. Cardiac function at the three different afterloads is displayed in Figure 4.1. At an afterload of 85 cmH_2O, an interaction was observed for ESP, LVDP, and +dP/dt. A *post hoc* analysis revealed that both SAL groups and RT+DOX had significantly higher ($p < 0.05$) ESP, LVDP, and +dP/dt when compared to SED+DOX. Although RT+DOX was significantly higher than SED+DOX in ESP (34%) and LVDP (37%), RT+DOX ESP and LVDP were significantly lower than both SAL groups.

At an afterload of 100 cmH_2O, an interaction was observed for ESP, LVDP, and +dP/dt. A *post hoc* analysis revealed that both SAL groups and RT+DOX had significantly higher ESP, LVDP, and +dP/dt when compared to SED+DOX. The SED+DOX –dP/dt was also significantly less when compared to SED+SAL. Similar to an afterload of 85 cmH_2O, RT+DOX was significantly higher than SED+DOX in ESP (44%) and LVDP (46%); however, the group was significantly lower when compared to both SAL groups. The RT+DOX +dP/dt was also significantly lower when compared to SED+SAL.

At an afterload of 115 cmH_2O, an interaction was observed for ESP, LVDP, and +dP/dt. A *post hoc* analysis revealed that both SAL groups and RT+DOX had significantly higher ESP, LVDP, +dP/dt, and –dP/dt when compared to SED+DOX. Although RT+DOX was significantly higher than SED+DOX in ESP (44%), LVDP (46%), and +dP/dt (15%), RT+DOX ESP, LVDP, and +dP/dt were significantly lower when compared to both SAL groups.

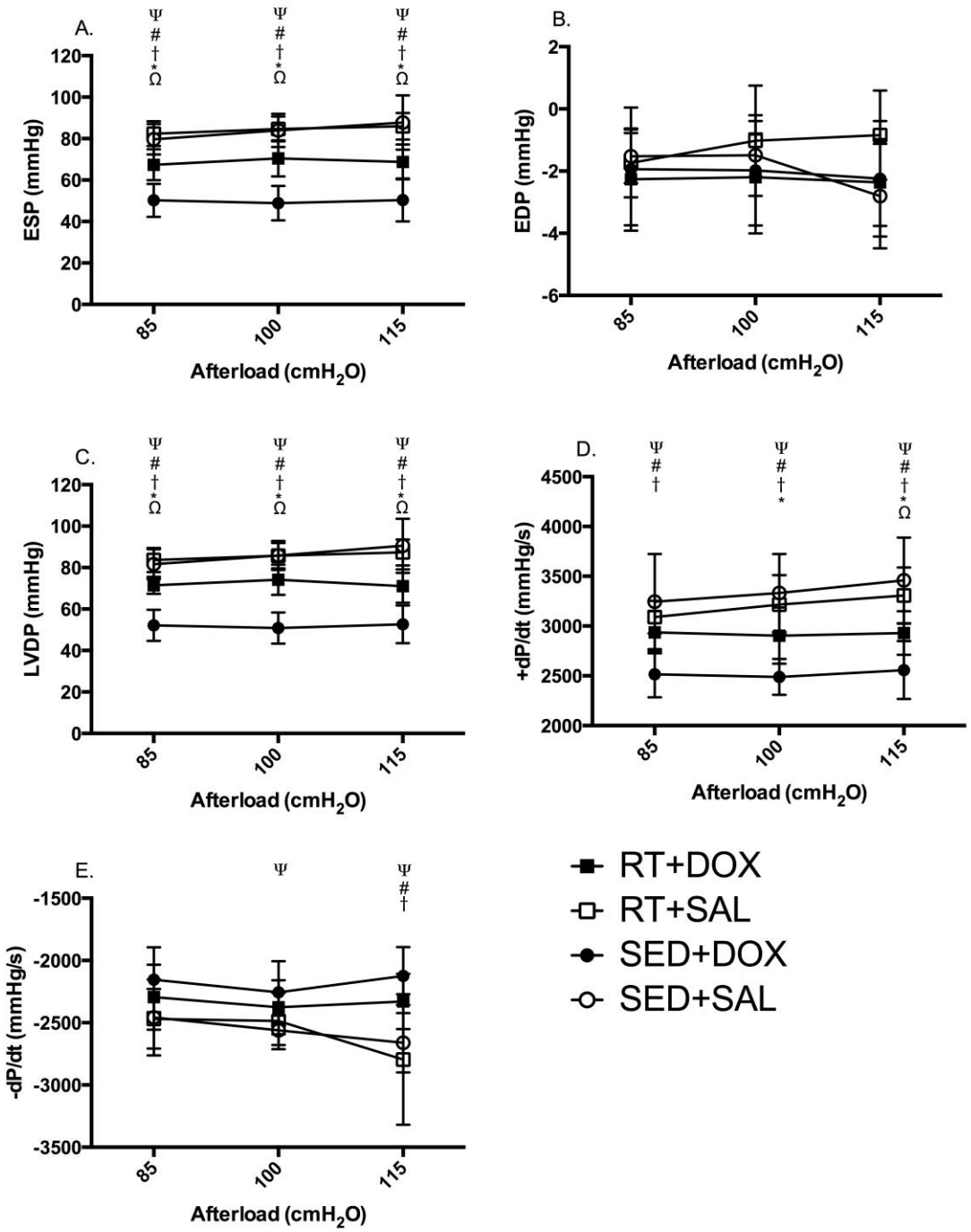


Figure 4.1. Unpaced *ex vivo* cardiac function. (A) end systolic pressure, (B) end diastolic pressure (C) left ventricular developed pressure, (D) maximal rate of developed pressure, and (E) maximal rate of pressure decline. Values are *M* ± *SD.* SED = sedentary; RT = resistance trained; SAL = saline placebo; DOX = doxorubicin.
Ψ significant differences between SED+SAL and SED+DOX
significant differences between RT+SAL and SED+DOX
† significant differences between RT+DOX and SED+DOX
* significant differences between SED+SAL and RT+DOX
Ω significant difference between RT+SAL and RT+DOX

Paced cardiac function at an afterload of 100 cmH_2O is displayed in Figure 4.2. Following the collection of unpaced data, the afterload was placed at 100 cmH_2O and LV pressure was measured at a standardized pace of 240 beats/min. An interaction was observed for ESP, LVDP, +dP/dt, and -dP/dt. Both SAL groups and RT+DOX had significantly higher ESP, LVDP, +dP/dt, and –dP/dt when compared to SED+DOX. No significant differences were seen between RT+DOX and SAL groups for all variables.

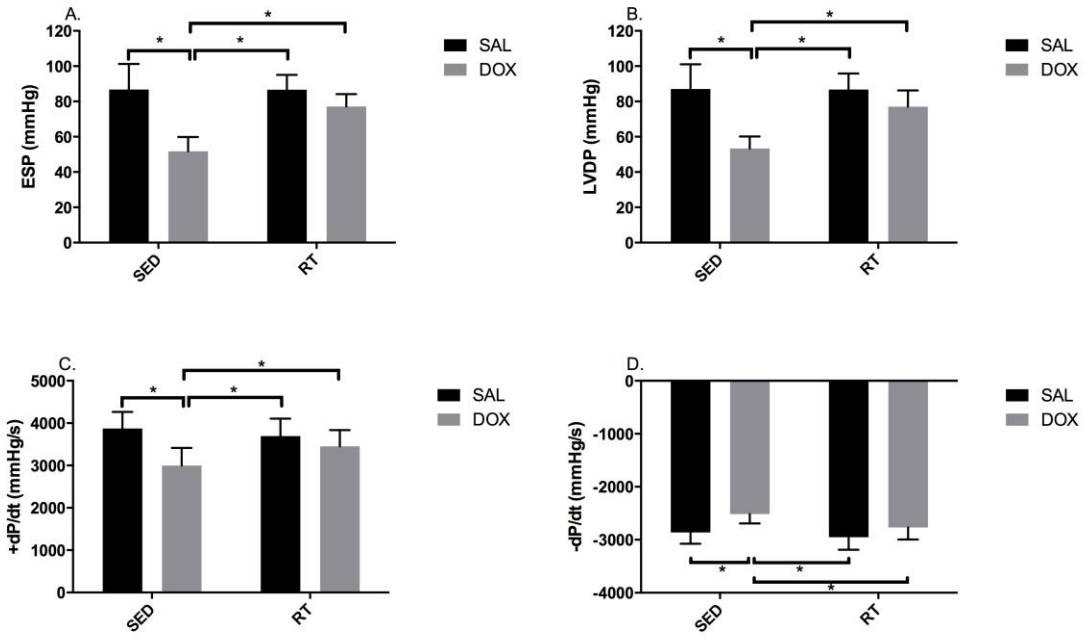


Figure 4.2. Paced *ex vivo* cardiac function. (A) end systolic pressure, (B) left ventricular developed pressure, (C) maximal rate of developed pressure, and (D) maximal rate of pressure decline. Values are *M* ± *SD*.
*significantly different than SED+DOX.

As afterload increases, the normal physiological response is an increase in LV pressure. Although not significant, increases (~ 5%) were

observed for both SAL groups in ESP, LVDP, and +dP/dt as the afterload increased from 85 cmH_2O to 115 cmH_2O. Both DOX groups showed little (< 2%) to no change in ESP, LVDP, +dP/dt, and –dP/dt as the afterload was increased from 85 cmH_2O to 115 cmH_2O.

Biochemical Analysis

All data from biochemical analyses are presented in Table 4.4 and Figures 4.3 and 4.4. To provide an index of oxidative stress in the myocardium, MDA + 4-HAE levels were analyzed. A 2-way ANOVA on MDA + 4-HAE levels revealed a drug effect, $F(1, 38) = 20.01$, $p < 0.0001$. A *post hoc* analysis was performed to examine group differences. No significant difference was seen within both DOX-treated and SAL-treated animals, however RT+DOX MDA + 4-HAE levels were lower than SED+DOX.

Table 4.4

Biochemical Analysis

	SED+SAL	SED+DOX	RT+SAL	RT+DOX
	M ± *SD*	*M* ± *SD*	*M* ± *SD*	*M* ± *SD*
MDA + 4-HAE (pmol/mg)	679 ± 94*†	896 ± 194	673 ± 105*†	829 ± 109
β-MHC (%)	9 ± 3*†	36 ± 12	17 ± 8*	25 ± 9*

Note. Data are represented as *M* ± *SD*. SED = sedentary; RT = resistance trained; SAL = saline placebo; DOX = doxorubicin; MDA = malondialdehyde; HAE = hydroxyalkenal; MHC = myosin heavy chain.
*$p < 0.05$ versus SED+DOX.
†$p < 0.05$ versus RT+DOX.

The MHC isoform distribution was examined to determine if an isoform shift played a protective role in DOX-induced cardiac dysfunction. A 2-way ANOVA on percentage of β-MHC revealed a drug effect, $F(1, 38) = 39.83$, $p < 0.001$. Additionally, an interaction effect was observed, $F(1, 38) = 11.74$, $p = 0.0015$. A *post hoc* analysis was performed to examine group differences. SED+DOX observed a significant increase in the percentage of β-MHC expression when compared to both SAL groups. The RT+DOX relative β-MHC expression was significantly higher than SED+SAL but not RT+SAL. The RT+DOX percentage of β-MHC was significantly lower than SED+DOX, indicating a preservation of the cardiac MHC isoform distribution as a result of RT.

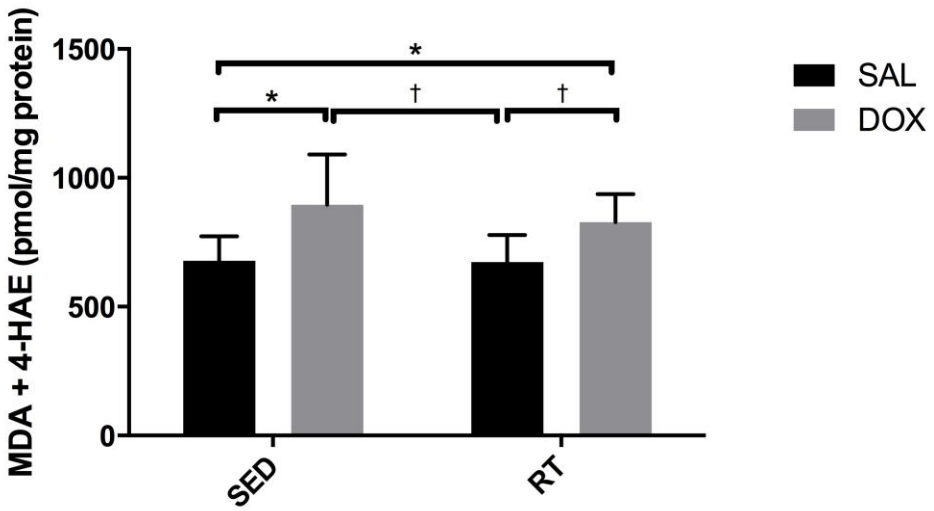
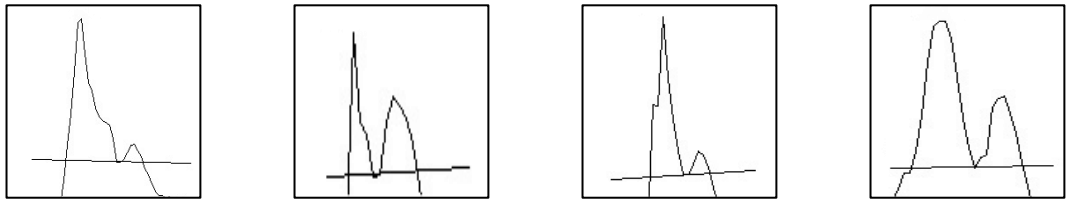


Figure 4.3. Myocardial lipid peroxidation in left ventricular homogenates. SED = sedentary; RT = resistance trained; SAL = saline placebo; DOX = doxorubicin. Values are *M* ± *SD*. MDA = malondialdehyde; HAE = hydroxyalkenal.
*significantly different than SED+SAL
†significantly different than RT+DOX.

A.



SED+SAL SED+DOX RT+SAL RT+DOX

B.

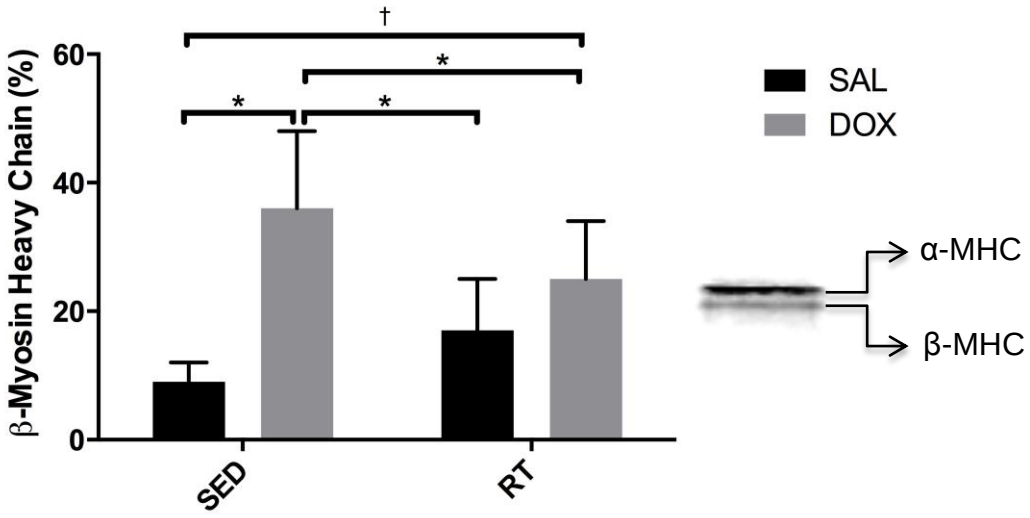


Figure 4.4. β-myosin heavy chain expression in left ventricular homogenates. Panel A is a representative scan, via ImageJ, from each group. Panel B is the β-myosin heavy chain expression in the left ventricle. SED = sedentary; RT = resistance trained; SAL = saline placebo; DOX = doxorubicin; MHC = myosin heavy chain. Values are *M* ± *SD*.
*significantly different than SED+DOX.
†significantly different than RT+DOX.

CHAPTER V

MANUSCRIPT

Introduction

Cancer is one of the leading causes of morbidity and mortality throughout the world. In 2015 it is estimated that there will be more than 1.6 million new cases of cancer diagnosed in the United States (Siegel, Miller, & Jemal, 2015). In addition, it is estimated that more than 580,000 people will succumb to the disease in 2015, thus statistically making cancer the second most common cause of death in the United States. Increased survival rates for cancer patients are due, in part, to progressive methods of prevention, detection, and treatment options available to patients. Increased survival rates have led to a greater emphasis on improving overall quality of life in cancer survivors, with a particular focus on reducing or attenuating treatment-related side effects.

A Food and Drug Administration approved chemotherapeutic agent successful in the treatment of a variety of cancers is doxorubicin (DOX), clinically known as Adriamycin®. Clinicians began prescribing DOX to cancer patients as a chemotherapy treatment in 1974. Since then, it has been used to treat a variety of malignancies, both solid and hematological (Weiss, 1992). While substantially effective at treating cancer, the implementation of DOX into

routine cancer treatment regimens has been limited due to its known cardiotoxicity. This well-characterized, dose-dependent cardiotoxicity can be manifested as cardiac dysrhythmias, contractile dysfunction, or even congestive heart failure (Steinherz, Steinherz, Tan, Heller, & Murphy, 1991). Studies have indicated that DOX treatment will significantly reduce systolic and diastolic function in an isolated perfused heart. Mechanistic actions of DOX that have been reported to induce cardiotoxicity include interference with deoxyribonucleic acid (DNA) strand separation during replication (Bachur et al., 1992), induction of apoptosis (Wonders, Hydock, Greufe, Schneider, & Hayward, 2009), and excess generation of reactive oxygen species (ROS) (Minotti, Menna, Salvatorelli, Cairo, & Gianni, 2004).

Other studies have examined one of the central mechanisms of DOX-induced cardiotoxicity, oxidative stress, by measuring lipid peroxidation. These studies have determined that DOX treatment leads to significant increases in lipid peroxidation, which initiates a cascade of events causing diminished cardiac function. The increase in lipid peroxidation has been linked to a change in myosin heavy chain (MHC) isoform distribution (Aragno et al., 2006). The MHC isoform distribution plays an essential role in cardiac contractile properties. An alteration in MHC isoform distribution is reported to cause diminished contractile velocity (Ramamurthy, Hook, Jones, & Larsson, 2001).

Aside from examining ways to prevent, diagnose, and treat cancer, researchers have diligently investigated interventions aimed at the reduction or attenuation of burdensome side effects commonly accompanying cancer

treatment. Purported to counteract many of the transient and chronic effects associated with cancer treatments, the role of regular exercise in the prevention and treatment of cancer has been the focus of much clinical research over the past 15 years.

Studies have suggested that exercise preconditioning may reduce DOX-induced cardiotoxcity following treatment (Hydock, Lien, Jensen, Schneider, & Hayward, 2011; Hydock, Lien, Schneider, & Hayward, 2008; Jensen, Lien, Hydock, Schneider, & Hayward, 2013). However, not fully understood are the mechanisms involved with the induction of exercise-mediated cardioprotection. Exercise-induced cardioprotection against DOX toxicity may be explained by upregulation of heat shock proteins (HSP) (Chicco, Schneider, & Hayward, 2005), down-egulation of pro-apoptotic pathways (Wonders et al., 2009), increased antioxidant enzymes (Ascensão et al., 2005; Ashrafi, Roshan, & Mahjoub, 2012; Chicco, Hydock, Schneider, & Hayward, 2006; Kanter, Hamlin, Unverferth, Davis, & Merola, 1985), decreased lipid peroxidation (Marques-Alexio et al., 2014; Wonders, Hydock, Schneider, & Hayward, 2008), decreased cardiac DOX accumulation (Jensen et al., 2013), preservation of MHC composition (Hydock et al., 2011), and/or increasing the expression of multi-drug resistance protein (Krause et al., 2007; Parry & Hayward, in press).

Although a substantial amount of research has focused on the effects of endurance training, little attention has been placed on the effects of resistance training (RT) on DOX-induced cardiotoxicity. Cancer rehabilitation programs have begun to incorporate RT due to the fact that this type of training can

result in significant improvements in muscular strength, aerobic capacity, and quality of life (Schwartz & Winters-Stone, 2009; Segal et al., 2003; Winters-Stone et al., 2012). Additionally, RT has been utilized as a rehabilitative therapy for heart failure, which may accompany DOX treatment, to counteract damaging peripheral side effects and improve quality of life (Alves, Nunes, Stefani, & Dal Lago, 2014). To our knowledge, no studies have been conducted which examine the effects of RT on DOX-induced cardiotoxicity. Therefore, the purpose of this study was threefold: (a) to examine the effects of chronic RT prior to the administration of DOX on *in vivo* and *ex vivo* cardiac function in male Sprague Dawley rats, (b) to determine if any cardioprotective effects are a result of a reduction in lipid peroxidation, and (c) to determine if any cardioprotective effects are a result of a preservation of the cardiac MHC isoform distribution.

Methods

Experimental Design

The exercise modality implemented in this study mimicked resistance exercise training and consisted of a raised-caged model, which forces animals to assume an erect bipedal stance while feeding and drinking. Following 12 weeks of training, a bolus intraperitoneal 12.5 mg/kg DOX injection was administered 24 hours after trained animals had been placed into non-elevated cages. Control animals received a bolus intraperitoneal injection of 0.9% saline (SAL). Rats were sacrificed five days after DOX treatment, and cardiac function was analyzed both *in vivo* and *ex vivo*. Following *ex vivo*

cardiac function assessment, the left ventricular (LV) was isolated and flash frozen in liquid nitrogen for biochemical analysis.

Subjects

Ten-week-old male Sprague Dawley rats (*N* = 48) were randomly assigned to sedentary (SED) (*n* = 24) or RT (*n* = 24) groups in a temperature-controlled facility with a 12:12-hour light-dark cycle. Rats were provided standard rat chow and water *ad libitum*. All protocols used for the study were approved by the University of Northern Colorado Institutional Animal Care and Use Committee and was in compliance with the Animal Welfare Act guidelines (see Appendix).

Exercise Training

Animals that were randomly assigned to the RT group were placed in cages that allow for a progressive elevation of food and water, encouraging the animals to rise to a bipedal stance each time they eat and drink. Cage height was raised to specified heights using specially designed plastic spacers that sat between the standard cage and the standard cage lid. Placement of the spacers between the cage and the lid raises the food and water to the desired height. This RT model has been shown to increase hind limb muscle mass and increased tibial cortical bone (Yao, Jee, Chen, Li, & Frost, 2001). On day one of the training protocol, cage height was raised from the standard 20.32 cm to a height of 28 cm. Cage height remained at 28 cm for one week. Following the first week, the height was raised by 2.5 cm. From there, every third day cages were raised 2.5 cm until they reached the final height of 35.5 cm. This height was maintained for an additional 10 weeks for a total of 12

weeks of training. Food and body weight were recorded daily, and adequate water consumption was confirmed for the first three weeks of training to ensure animals were able to reach both food and water. Following the first three weeks of training, animals had their food measured and water monitored three days per week, and body weight was measured weekly.

At the completion of the 12-week training period, all plastic spacers were removed so that that all food and water returned to the standard cage height. This was done to ensure that there were no effects of acute exercise on the observed dependent variables. All animals remained in these standard cages for 24 hours prior to DOX treatment. Following the 24-hour period, each animal was randomly assigned to one of four groups: SED+SAL (n = 9), SED+DOX (n = 15), RT+SAL (n = 9), and RT+DOX (n = 15). The SAL groups received a bolus intraperitoneal 0.9% SAL injection, and the DOX groups received a bolus intraperitoneal 12.5 mg/kg DOX injection.

Various exercise modalities have been used to mimic RT. These include tower climbing (Notomi et al., 2001), an erected bipedal stance exercise with a weighted vest (Westerlind et al., 1998), and an erected bipedal stance whole-body exercise (Yao et al., 2000). Consistent findings have observed that an exercise modality that mimics RT can cause adaptation to bone, skeletal muscle, and cardiac muscle (Barauna, Rosa, Irigoyen, & de Oliveira, 2007; Duncan, Williams, & Lynch, 1998; Notomi et al., 2001). There is no standard or set protocol for laboratory animal weight-bearing exercise modalities. The range of total repetitions and total resistance varies greatly between studies.

The RT model has been used by others (Mo et al., 2002; Rosa, Firth, Blair, Vickers, & Morel, 2011; Yao et al., 2000) and pilot studies in our laboratory. Recently in our laboratory, the RT model has been associated with positive functional adaptations in skeletal muscle, which has also been observed following resistance protocols discussed previously. These data, along with the pilot study, suggest that the exercise modality may be an effective model for exercise-induce cardiac adaptations.

Assessment of Cardiac Function

In Vivo Cardiac Function

In vivo cardiac function was analyzed five days after DOX treatment via echocardiography. Animals were sedated with ketamine (40 mg/kg, intraperitoneal), and the anterior and left lateral thoracic regions were shaved. Transthoracic echocardiography was conducted on the sedated rats using a General Electric Vivid 7 ultrasound with a 10-MHz intraoperative transducer (Tustin, California). Measurements of septal wall thickness during systole (SWs) and septal wall thickness during diastole (SWd), posterior wall thickness during systole (PWs) and posterior wall thickness during diastole (PWd), LV end systolic diameter (LVSd) and LV end diastolic diameter (LVDd), and fractional shortening (FS) were acquired by M-mode tracings of the LV obtained in a short axis view. From an apical view using a pulsed-wave Doppler, aortic and mitral blood flow echocardiographic images were obtained providing measurements of maximal flow velocity for both aortic and mitral valves. All echocardiographic measurements were averaged from three consecutive cardiac cycles. All M-mode and Doppler measurements were

made in accordance with guidelines established by the American Society of Echocardiography.

Ex Vivo Cardiac Function

Ex vivo cardiac function was analyzed following echocardiography via an isolated working-heart model (ADInstruments, Colorado Springs, Colorado). Each animal was anesthetized using an intraperitoneal injection of heparinized (500 U) sodium pentobarbital (50 mg/kg). Following an absent tail pinch reflex, the heart was excised and placed into ice cold Krebs-Henseleit buffer (in mM: 120 NaCl, 5.9 KCl, 2.5 $CaCl_2$, 1.2 MgCl, 25 $NaHCO_3$, 17 glucose, and 0.5 EDTA [ethylenediaminetetraacetic acid]) aerated with 95% O_2 – 5% CO_2. Hearts were quickly cannulated by the ascending aorta and subjected to retrograde perfusion until all blood was cleared from the coronary vasculature. The pulmonary vein was then cannulated, and blood flow was redirected from the aorta to the left atrium to initiate the working-heart model. Once stabilized, a microtip catheter pressure transducer (Millar Inc., Houston, Texas) was placed into the LV via the apex for determination of LV developed pressure (LVDP), LV maximum rate of developed pressure (+dP/dt), and minimum rate of developed pressure (-dP/dt). Preload was set at 10 cmH_2O and was maintained for all subsequent functional measurements. Afterload was progressively increased every three to five minutes during data collection from 85 cmH_2O, to 100 cmH_2O, and to 115 cmH_2O. After data were collected at the three different afterloads, afterload was adjusted to 100 cmH_2O, and hearts were paced at 240 beats per minute using electrodes attached to the cannula. The LV functional data were collected and analyzed using a

PowerLab data acquisition system (ADInstruments). Once data were collected, hearts were trimmed of connective tissue and fat and weighed. The LVs were then isolated, flash frozen in liquid N_2, and stored at – 80°C until subsequent biochemical analyses.

Biochemical Analysis

Lipid Peroxidation

A commercially available assay kit (Bioxytech MDA-586, Oxis Research, Foster City, California) was used to measure malondialdehyde + 4-hydroxyalkenal (MDA + 4-HAE) as an indicator of cellular lipid peroxidation. Tissues were homogenized in a radioimmunoprecipitation buffer for the assay. A 200 µL aliquot of each sample was added to a microcentrifuge tube followed by 650 µL of N-methyl-2-phenylindole in acetonitrile and briefly vortexed. Next, 150 µL of methanesulfonic acid was added, vortexed, and incubated at 45°C for 60 minutes. Samples were then centrifuged at 10,000 *g* for 10 minutes. The resulting supernatant was transferred to a cuvette and absorbency measured using a spectrophotometer at 586 nm. The MDA + 4-HAE was estimated from a standard curve. All samples were assayed in duplicate, and any samples varying more than 5% were reassessed.

Myosin Heavy Chain Analysis

Sample preparation was adapted from the method of Thomason, Baldwin, and Herrick (1986). Approximately 100 mg of LV tissue was minced in a 2 mL glass tissue homogenizer with surgical scissors. A 1:10 weight/volume ratio of homogenization buffer (250 mM sucrose, 100 mM KCl, 5 mM EDTA, and 20 mM Tris-Base, 4°C, pH 6.8) was added to the

homogenizer. The sample was homogenized approximately five minutes using ScilogexD160 homogenizer (Rocky Hill, Connecticut). Homogenates were centrifuged at 1,000 *g* for 10 minutes at 4°C and pellets collected and resuspended, with the same volume as homogenization buffer, in washing buffer (175 mM KCl, 0.5% Triton X-100, 2 mM EDTA and 20 mM Tris-Base, 4°C, pH 6.8). Samples were centrifuged again at 1,000*g* for 10 minutes at 4°C. The pellets were collected, resuspended with the same volume as previous washing buffer, and centrifuged at 1,000 *g* for 10 minutes at 4°C. The pellets were resuspended in the final resuspension buffer (150 mM KCl, and 20 mM Tris-Base, pH 7.0) at 1/12 of the previous volume.

Total protein concentration was determined using the Bradford protein assay method (Bradford, 1976). Samples were then diluted to 2 mg/mL with the final resuspension buffer. Twenty-five µL of each sample was further diluted to 0.125 mg/mL with 2x Laemmli sample buffer (20% glycerol, 16% 1M Tris [pH 6.8], 4% sodium dodecylsulfate [SDS], 1% β-mercaptoethanol, and 0.2% bromophenol blue). To denature the proteins, samples were boiled for two minutes and then placed on ice for another 10 minutes. Following the 10 minutes on ice, samples were loaded onto polyacrylamide gels.

The SDS polyacrylamide gel electrophoresis was used to determine MHC isoform expression. The MHC gel composition used the modified method of Reiser and Kline (1998) from the original method of Talmadge and Roy (1993). A 0.75 mm Snap-A-Gel cassette (Jule, Inc., Milford, Connecticut) was used to cast polyacrylamide gels. The gel polymerization of separating gels (5% of 100% glycerol, 8% acrylamide-N,N'-methylene, 4% acrylamide-bis

[50:1], 0.2 M Tris-Base [pH 8.8], 0.1 M glycine, and 0.4% of 10% SDS) was initiated by adding 0.05% *N,N,N′,N′*- tetramethylethylenediamine and 0.1% ammonium persulfate (10%). After initiating the gel polymerization, the separating gel solution was pipetted into cassettes to approximately 1 cm below the height of the bottom of comb. N-butonol was then pipetted to the top of the gel in order to flatten the separating gel during polymerization. Cassettes were then placed in the refrigerator for at least 60 minutes to allow the gel to polymerize.

Following polymerization of the separating gel, n-butanol was removed and cassettes rinsed five times with milli-pure water. The polymerization of the stacking gel (5% of 100% glycerol, 4% acrylamide-bis [50:1], 70 mM Tris-Base [pH 6.7], 4 mM EDTA and 0.4% of 10% SDS) was initiated by adding 0.05% tetramethylethylenediamine and 0.1% ammonium persulfate (10%). The stacking gel solution was pipetted to the top of the separating gel and a 10-lane comb was inserted (Jule, Inc.) into the stacking gel. Cassettes were then placed in the refrigerator for at least 60 minutes to allow the stacking gel to polymerize.

Following polymerization of the stacking gel, combs were removed. Cassettes were placed into Novex Sure Lock cells (Invitrogen Corporation, Carlsbad, California). Cassettes were locked and then the middle chamber was filled with upper running buffer (0.1 M Tris, 150 mM glycine, and 0.1% SDS). Next, 7.5 µL of BenchMark™ protein standard (Invitrogen Corporation) was loaded in the first lane. Following the protein standard, 7 µL of samples was loaded in the remaining lanes. Lower running buffer (50 mM Tris, 75 mM

glycine, and 0.05% SDS) was poured into the outside chamber of the electrophoresis unit.

Electrophoresis was conducted at 100 V, 25 mA for approximately three hours or until the tracking dye was near the bottom of the gel. The gels were then stained with Simply Blue safe stain for 60 minutes. Gels were then placed in distilled water overnight. The following day, gels were removed and placed between two transparency sheets and scanned. Of the two bands on the gel, the slower migrating band was the α-MHC isoform and the faster was the β-MHC isoform. The density of MHC protein bands was analyzed using ImageJ densitometry software (National Institutes of Health, Bethesda, Maryland).

To quantify protein bands, the scanned gel was opened in ImageJ and converted to grayscale. The brightness of the image was adjusted to isolate the protein bands. Both bands were selected and then plotted to present a graphical depiction of band intensity. The graphical depiction of band intensity showed two peaks. The area under the two peaks was then marked off and selected. After selecting each peak, values for the area under each band peak appeared in a window labeled “Results.” The first value represented the α-MHC isoform and the second value represented the β-MHC isoform. Both values were added together and the percentage of β-MHC was calculated.

Statistical Analysis

Data were analyzed and presented using GraphPad Prism statistical software. All data are expressed as mean ± standard deviation (*M* ± *SD*). A two-way analysis of variance (ANOVA) was used to determine significant

difference due to the main effects (DOX and RT) and interaction. Upon observation of a significant difference, a Tukey post-hoc pair-wise comparison was conducted to evaluate all pair-wise comparisons, which would identify differences between groups. For all statistical analysis, significance is set at $\alpha = 0.05$.

Results

The purpose of this study was threefold: (a) to examine the effects of chronic RT prior to the administration of DOX on *in vivo* and *ex vivo* cardiac function in male Sprague Dawley rats, (b) to determine if any cardioprotective effects are a result of a reduction in lipid peroxidation, and (c) to determine if any cardioprotective effects are a result of a preservation of the cardiac MHC isoform distribution.

General Observations

General observations are presented in Table 5.1. Six animals died during the course of this experiment, making the total mortality rate 20% for DOX treated-animals. The RT cut mortality in half, with SED+DOX having a mortality rate of 27% (4/15); whereas, the mortality rate for RT+DOX was 13% (2/15). Animals in both SAL groups continued to gain body weight in the five days following injection; however, these gains were not significant. A 2-way ANOVA on final body weight revealed a drug effect, $F(1, 38) = 29.2$, $p < 0.0001$, with DOX-treated animals body weight significantly lower than SAL-treated animals. Five days following injection, body weight decreased 14% for both DOX groups, with no statistically significant difference between DOX groups. A 2-way ANOVA on absolute heart mass revealed both a drug effect,

$F(1,38) = 6.757$, $p = 0.0132$, and activity effect, $F(1, 38) = 11.33$, $p = 0.0018$. Absolute heart mass was significantly higher in the RT+SAL group compared to SED+DOX. Relative heart mass also showed a drug effect, $F(1, 38) = 5.448$, $p = 0.025$, and activity effect, $F(1, 38) = 18.87$, $p = 0.0001$. Both RT groups had greater heart mass when compared to SED+SAL. The RT+DOX heart mass relative to body weight was also significantly higher than SED+DOX.

Table 5.1

Subject Demographics

		N	Injection body weight (g) *M ± SD*	Final body weight (g) *M ± SD*	Heart mass (g) *M ± SD*	Relative heart mass (%) *M ± SD*
SAL	SED	9	441 ± 37	446 ± 36	1.5 ± 0.18	0.35 ± 0.02
	RT	9	439 ± 57	442 ± 54	1.7 ± 0.17†	0.39 ± 0.03*
DOX	SED	11	447 ± 31	386 ± 26	1.4 ± 0.14	0.37 ± 0.03
	RT	13	446 ± 23	384 ± 22	1.6 ± 0.13	0.40 ± 0.04* †

Note. Data are represented as *M ± SD*. SED = sedentary; RT = resistance trained; SAL = saline placebo; DOX = doxorubicin. $p < 0.05$.
*$p < 0.05$ versus SED+SAL.
†$p < 0.05$ versus SED+DOX.

Echocardiography

Cardiac geometry (see Table 5.2) and blood flow velocities (see Table 5.3) were measured by echocardiogram. No statistically significant differences were seen between groups for SWd, PWs, PWd, LVDs, and FS. Five days post injection, a 2-way ANOVA on SWs revealed a drug effect, $F(1, 38) = 13.39$, $p = 0.0008$. A *post hoc* analysis determined that SED+DOX SWs was significantly thinner ($p < 0.05$) compared to both SED groups. A drug effect, $F(1, 38) = 14.45$, $p = 0.0005$, was also observed on LVDd. A *post hoc* analysis determined that SED+SAL LVDd was significantly greater ($p < 0.05$) than RT+DOX. Echocardiography Doppler images were used to analyze maximum blood flow through the aortic (A-Vmax) and mitral valves (M-Vmax). A 2-way ANOVA on A-Vmax revealed a drug effect, $F(1, 38) = 79.25$, $p < 0.0001$, activity effect, $F(1, 38) = 15.03$, $p = 0.0004$, and interaction, $F(1, 38) = 13.42$, $p = 0.0008$. The M-Vmax also had a drug effect, $F(1, 37) = 82.44$, $p < 0.0001$, activity effect, $F(1, 37) = 7.002$, $p = 0.0119$, and interaction, $F(1, 37) = 13.32$, $p = 0.008$. Significant decreases in A-Vmax and M-Vmax occurred with both DOX groups when compared to both SAL groups. However, RT+DOX A-Vmax and M-Vmax was significantly faster ($p < 0.05$) when compared to SED+DOX.

Table 5.2

Echocardiogram Derived Cardiac Geometry

	SED+SAL		SED+DOX		RT+SAL		RT+DOX	
	M ± *SD*	*N*	*M* ± *SD*	*N*	*M* ± *SD*	*N*	*M* ± *SD*	*N*
SWs (mm)	4.02 ± 0.22*	9	3.02 ± 0.56	11	4.17 ± 0.39*	9	3.79 ± 0.43	13
SWd (mm)	2.25 ± 0.24	9	2.10 ± 0.40	10	2.41 ± 0.28	9	2.34 ± 0.35	13
PWs (mm)	3.79 ± 0.27	9	3.51 ± 0.63	11	3.94 ± 0.25	9	3.63 ± 0.89	13
PWd (mm)	2.49 ± 0.31	9	2.33 ± 0.38	11	2.58 ± 0.30	9	2.35 ± 0.28	13
LVDs (mm)	2.29 ± 0.32	8	2.48 ± 1.02	11	2.24 ± 0.24	9	1.93 ± 0.62	13
LVDd (mm)	5.93 ± 0.29†	9	4.94 ± 1.11	11	5.44 ± 0.57†	9	4.33 ± 1.08	13
FS (%)	61.12 ± 5.21	8	51.06 ± 14.1	11	58.69 ± 5.68	9	58.28 ± 9.22	13

Note. Data are represented as *M* ± *SD*. SED = sedentary; RT = resistance trained; SAL = saline placebo; DOX = doxorubicin; SWs = septal wall thickness during systole; SWd = septal wall thickness during diastole; PWs = posterior wall thickness during systole; PWd = posterior wall thickness during diastole; LVDs = left ventricular end systolic diameter; LVDd = left ventricular end diastolic diameter; FS = fractional shortening.
*$p < 0.05$ versus SED+DOX.
†$p < 0.05$ versus RT+DOX.

Table 5.3

Echocardiogram Derived Blood Flow Velocity

	SED+SAL		SED+DOX		RT+SAL		RT+DOX	
	M ± *SD*	*N*	*M* ± *SD*	*N*	*M* ± *SD*	*N*	*M* ± *SD*	*N*
M-Vmax	98 ± 11*†	8	39 ± 9	11	93 ± 17*†	9	68 ± 10*	13
A-V max	74 ± 15*†	9	31 ± 10	11	75 ± 10*†	9	57 ± 10*	13

Note. Data are represented as *M* ± *SD*. SED = sedentary; RT = resistance trained; SAL = saline placebo; DOX = doxorubicin; M-Vmax = maximum blood flow through mitral valve; A-Vmax = maximum blood flow through aortic valve.
*$p < 0.05$ versus SED+DOX.
†$p < 0.05$ versus RT+DOX.

Isolated Perfused Working Heart

Following echocardiographic measurements, the cardiac variables ESP, EDP, LVDP, +dP/dt, -dP/dt, and heart rate were analyzed using an isolated working-heart apparatus. Afterload was set at three different heights (85, 100, 115 cmH_2O) during the analysis. No statistically significant differences in heart rate and EDP were observed between the four groups at any of the afterloads. Cardiac function at the three different afterloads is displayed in Figure 5.1. At an afterload of 85 cmH_2O, an interaction was observed for ESP, LVDP, and +dP/dt. A *post hoc* analysis revealed that both SAL groups and RT+DOX had significantly higher ($p < 0.05$) ESP, LVDP, and +dP/dt when compared to SED+DOX. Although RT+DOX was significantly higher than SED+DOX in ESP (34%) and LVDP (37%), RT+DOX ESP and LVDP were significantly lower than both SAL groups.

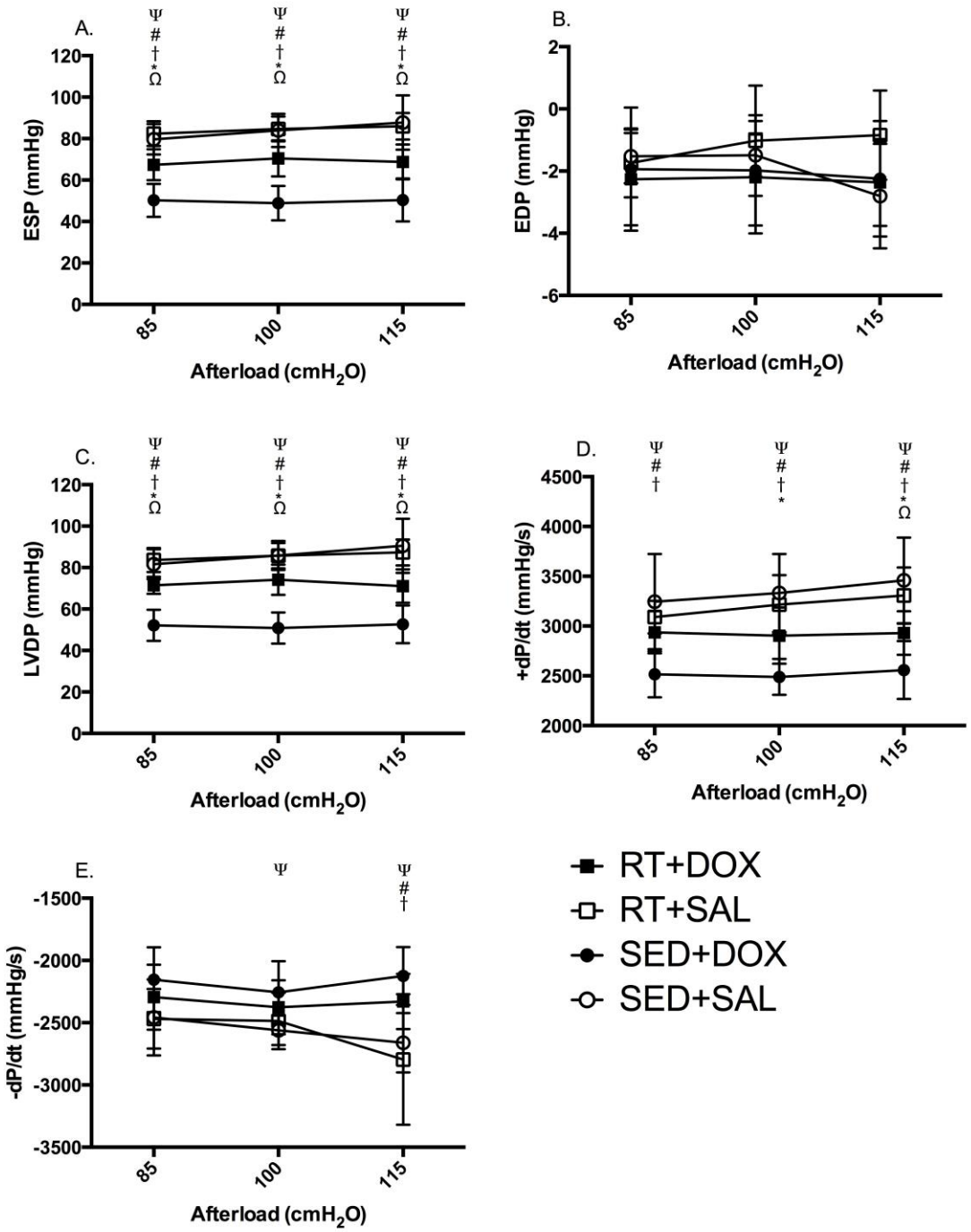


Figure 5.1. Unpaced *ex vivo* cardiac function. (A) end systolic pressure, (B) end diastolic pressure, (C) left ventricular developed pressure, (D) maximal rate of developed pressure, and (E) maximal rate of pressure decline. Values are *M* ± *SD*.
Ψ significant differences between SED+SAL and SED+DOX
significant differences between RT+SAL and SED+DOX
† significant differences between RT+DOX and SED+DOX
* significant differences between SED+SAL and RT+DOX
Ω significant difference between RT+SAL and RT+DOX

At an afterload of 100 cmH_2O, an interaction was observed for ESP, LVDP, and +dP/dt. A *post hoc* analysis revealed that both SAL groups and RT+DOX had significantly higher ESP, LVDP, and +dP/dt when compared to SED+DOX. The SED+DOX –dP/dt was also significantly less when compared to SED+SAL. Similar to an afterload of 85 cmH_2O, RT+DOX was significantly higher than SED+DOX in ESP (44%) and LVDP (46%); however, the group was significantly lower when compared to both SAL groups. The RT+DOX +dP/dt was also significantly lower when compared to SED+SAL.

At an afterload of 115 cmH_2O, an interaction was observed for ESP, LVDP, and +dP/dt. A *post hoc* analysis revealed that both SAL groups and RT+DOX had significantly higher ESP, LVDP, +dP/dt, and –dP/dt when compared to SED+DOX. Although RT+DOX was significantly higher than SED+DOX in ESP (44%), LVDP (46%), and +dP/dt (15%), RT+DOX ESP, LVDP, and +dP/dt were significantly lower when compared to both SAL groups.

Paced cardiac function at an afterload of 100 cmH_2O is displayed in Figure 5.2. Following the collection of unpaced data, the afterload was placed at 100 cmH_2O and LV pressure was measured at a standardized pace of 240 beats/min. An interaction was observed for ESP, LVDP, +dP/dt, and -dP/dt. Both SAL groups and RT+DOX had significantly higher ESP, LVDP, +dP/dt, and –dP/dt when compared to SED+DOX. No significant differences were seen between RT+DOX and SAL groups for all variables.

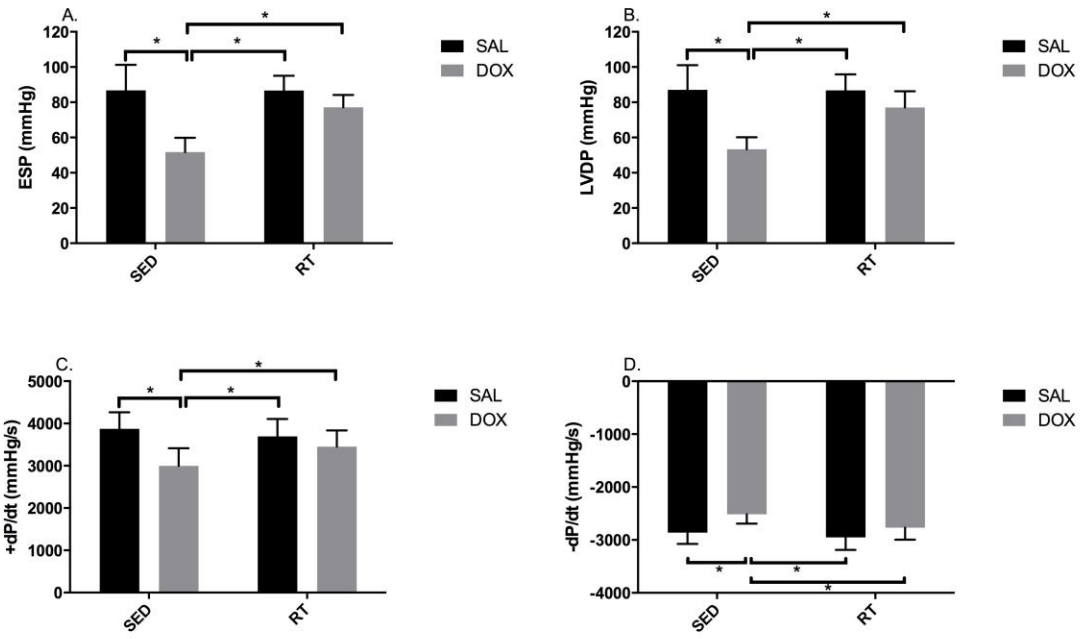


Figure 5.2. Paced *ex vivo* cardiac function. (A) end systolic pressure, (B) left ventricular developed pressure, (C) maximal rate of developed pressure, and (D) maximal rate of pressure decline. Values are *M* ± *SD*.
*significantly different than SED+DOX.

As afterload increases, the normal physiological response is an increase in LV pressure. Although not significant, increases (~ 5%) were observed for both SAL groups in ESP, LVDP, and +dP/dt as the afterload increased from 85 cmH_2O to 115 cmH_2O. Both DOX groups showed little (< 2%) to no change in ESP, LVDP, +dP/dt, and –dP/dt as the afterload was increased from 85 cmH_2O to 115 cmH_2O.

Biochemical Analysis

All data from biochemical analyses are presented in Table 5.4 and in Figures 5.3 and 5.4. To provide an index of oxidative stress-induced damage in the myocardium, MDA + 4-HAE levels were analyzed. A 2-way ANOVA on MDA + 4-HAE levels revealed a drug effect, $F(1, 38) = 20.01$, $p < 0.0001$. A

post hoc analysis was performed to examine group differences. No significant difference was seen within both DOX-treated and SAL-treated animals; however, RT+DOX MDA + 4-HAE levels were lower than SED+DOX.

The MHC isoform distribution was examined to determine if an isoform shift played a protective role in DOX-induced cardiac dysfunction. A 2-way ANOVA on percentage of β-MHC revealed a drug effect, $F(1, 38) = 39.83$, $p < 0.001$. Additionally, an interaction effect was observed, $F(1, 38) = 11.74$, $p = 0.0015$. A *post hoc* analysis was performed to examine group differences. The SED+DOX observed a significant increase in the percentage of β-MHC expression when compared to both SAL groups. The RT+DOX relative β-MHC expression was significantly higher than SED+SAL but not RT+SAL. The RT+DOX percentage of β-MHC was significantly lower than SED+DOX, indicating a preservation of the cardiac MHC isoform distribution as a result of RT.

Table 5.4

Biochemical Analysis

	SED+SAL	SED+DOX	RT+SAL	RT+DOX
	M ± *SD*	*M* ± *SD*	*M* ± *SD*	*M* ± *SD*
MDA + 4-HAE (pmol/mg)	679 ± 94*†	896 ± 194	673 ± 105*†	829 ± 109
β-MHC (%)	9 ± 3*†	36 ± 12	17 ± 8*	25 ± 9*

Note. Data are represented as *M* ± *SD*. SED = sedentary; RT = resistance trained; SAL = saline placebo; DOX = doxorubicin; MDA = malondialdehyde; HAE = hydroxyalkenal; MHC = myosin heavy chain.
*$p < 0.05$ versus SED+DOX.
†$p < 0.05$ versus RT+DOX.

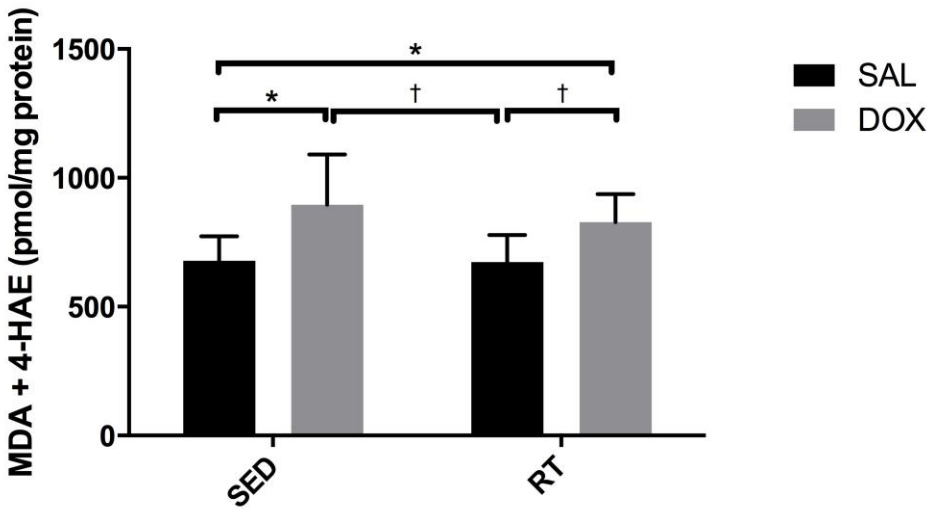


Figure 5.3. Myocardial lipid peroxidation in left ventricular homogenates. Values are *M* ± *SD*. MDA + 4-HAE = malondialdehyde + 4-hydroxyalkenal.
*significantly different than SED+SAL.
†significantly different than RT+SAL

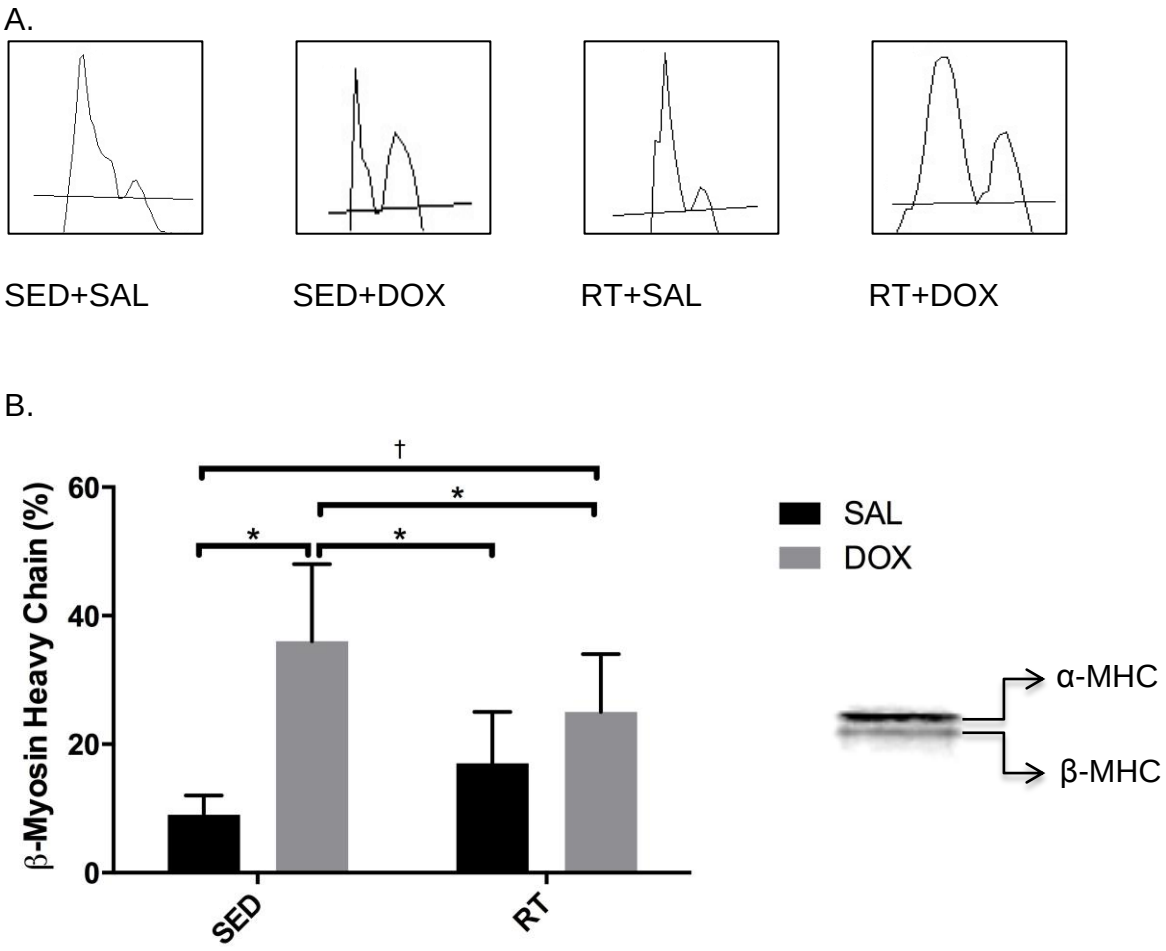


Figure 5.4. β-myosin heavy chain expression in left ventricular homogenates. Panel A is a representative scan, via ImageJ, from each group. Panel B is the β-myosin heavy chain expression in the left ventricle. Values are *M* ± *SD*.
*significantly different than SED+DOX.
†significantly different than RT+DOX.

Discussion

With increased survival rates for cancer patients, it has become imperative to examine ways of reducing or attenuating cancer treatment-related side effects. The chemotherapeutic agent DOX is one of the most effective chemotherapeutic agents ever developed; however, its implementation is limited due to its dose-dependent cardiotoxicity. Extensive

research has indicated that various types of exercise modalities can attenuate DOX-induced cardiotoxicity (Hydock et al., 2012; Hydock et al., 2008). Currently, cancer rehabilitation programs have begun to incorporate RT due to the fact that this type of training can result in significant improvements in muscular strength, aerobic capacity, and quality of life (Schwartz & Winters-Stone, 2009; Segal et al., 2003; Winters-Stone et al., 2012). The primary purpose of this study was to determine if chronic RT could attenuate the cardiac dysfunction induced by subsequent DOX treatment. In addition, the effect of DOX and exercise training on cardiac lipid peroxidation and relative MHC isoform distribution was examined to provide a potential explanation for any observed changes in cardiac function. Results indicate that DOX treatment caused cardiac dysfunction five days after its administration, prior chronic RT significantly attenuated this dysfunction, and the protective effects of this exercise modality were associated with a preservation of the MHC isoform distribution**.**

Doxorubicin-Induced Cardiac Dysfunction

The DOX treatment regimen used in the present study resulted in a significant impairment of cardiac function within five days of exposure, which is in agreement with previous investigations that examined cardiac function five days post DOX treatment (Hydock et al., 2011; Wonders et al., 2008). These signs include significant reductions in mitral and aortic blood flow velocities as well as diminished systolic and diastolic function. However, the DOX-induced alterations observed were significantly less in the RT+DOX group, indicating a cardioprotective effect of RT against DOX treatment.

Measuring ESP, LVDP, and +dP/dt can assess systolic function. In agreement with previous studies, DOX treatment led to significant decreases in systolic function (Chicco et al., 2006; Hydock et al., 2011). Several mechanisms have been suggested to explain the depression of LV systolic function that occurs following DOX treatment including cardiomyocyte apoptosis (Childs, Phaneuf, Dirks, Phillips, & Leeuwenburgh, 2002), impaired Ca^{2+} handling (Asayama et al., 1995; Boucek, Dodd, Atkinson, Oquist, & Olson., 1997), and deleterious MHC shifts (Hydock et al., 2011).

In the present study DOX-treated groups showed diminished diastolic function. Significant differences were not observed in EDP between the four groups; however, an increase in afterload did affect diastolic function in the form of diminished –dP/dt for SED+DOX. Although not significant, when increasing the afterload from 100 cmH_2O to 115 cmH_2O, SED+DOX –dP/dt decreased by 6%. This indicates that at higher afterloads, myocardial relaxation was impaired. Such an observation could have important ramifications for untrained cancer survivors who engage in infrequent exercise. Exposure to moderately elevated afterload-type activities such as high-intensity aerobic or resistance exercise may impair diastolic function during the activity. Several mechanisms could be involved in the impaired relaxation. Impairment may have been caused by diminished Ca^{2+} uptake via sarcoendoplasmic reticulum ATPase 2 (SERCA2) as well as a decrease SERCA2 mRNA expression, which have been observed in previous studies (Arai et al., 2000; Hydock et al., 2011; Zhang et al., 2012). Although the mechanisms are not fully understood, previous studies have suggested that

SERCA2 impairment could be a result of DOX-induced oxidative stress (Harris & Doroshow, 1985), which was in fact observed in the current study.

Doxorubicin-Induced Cardiotoxicity

Possible mechanistic actions that could be responsible for DOX-induced cardiac dysfunction include induction of apoptosis, excess generation of ROS, and unfavorable MHC shifts. One purpose of the current study was to examine oxidative stress, as measured by MDA + 4-HAE, following DOX treatment. DOX-treated groups showed a significant increase in oxidative stress compared to SAL, which is in agreement with previous studies (Chicco et al., 2006; Wonders et al., 2008). The myocardium is highly aerobic; therefore, it requires a constant supply of adenosine triphosphate ATP by oxidative phosphorylation. When DOX is administered it can lead to excess generation of ROS, mainly due to an increase in redox cycling at complex I in the electron transport chain (Marcillat, Zhang, & Davies, 1989). As a result there becomes an imbalance between the antioxidant and pro-oxidant forces within and outside the cell, which is referred to as oxidative stress. Increased oxidative stress may lead to a rise in intracellular Ca^{2+} due to impaired ryanodine receptor and SERCA2 function (Kim et al., 2006). Furthermore, this altered Ca^{2+} homeostasis creates a cascade of events that can induce apoptosis (Sokolove & Shinaberry, 1988).

Another purpose of the study was to determine if unfavorable shifts in the MHC isoform distribution occurred following DOX treatment. The DOX-treated animals showed a significant increase in the relative expression of the β-MHC isoform and a significant decrease in the relative expression of the α-

MHC isoform. As stated previous, DOX administration can lead to oxidative stress. This increased oxidative stress may also lead to an increased expression of β-MHC (Aragno et al., 2006). The expression of both α-MHC and β-MHC plays an imperative role in cardiac function (Miyata, Minobe, Bristow, & Leinwand, 2000). Cardiac α-MHC is the primary isoform in rat hearts (> 90%) and is associated with a greater contraction velocity and higher ATPase activity (Krenz et al., 2003). Studies have indicated that DOX treatment can alter MHC distribution causing decreased expression of the α-MHC isoform corresponding to an increase expression of the β-MHC isoform. This α to β shift significantly decreases cardiac contractility (Tardiff et al., 2000). In the present study, the cardiac dysfunction observed five days following DOX treatment might be a result of increased oxidative stress and altered MHC isoform distribution.

Exercise-Induced Cardioprotection

Despite the plethora of studies observing exercise-induced cardioprotection prior to DOX treatment, no studies have examined if RT prior to DOX treatment provides a cardioprotective effect. The RT model used in the current study protected against DOX-induced septal wall thinning, LV mass reduction, mitral and aortic blood flow decrements, and cardiac dysfunction.

From available evidence, it appears that multiple mechanisms may be simultaneously involved with the protective effects of chronic exercise against DOX-induced cardiotoxicity including, reduced DOX accumulation within the

cardiomyocyte (Jensen et al., 2013), attenuating unfavorable shifts in MHC (Hydock et al., 2011), and decreased oxidative stress (Chicco et al., 2005).

Since it has been proposed that the primary mechanism of DOX-induced cardiotoxicity involves increased oxidative stress, a purpose of the current study was to examine if the RT model used would attenuate DOX-induced oxidative stress. Contrary to the current study, Chicco et al. (2006) observed an attenuation of ethanol-induced cardiac lipid peroxidation following six weeks of RT. The difference in results between the two studies may be due to the intensity of the exercise movement or differing degrees of oxidative stress provided by DOX versus ethanol. In the previous study, animals performed exercise movements where the load lifted was started at 20% body weight and increased by 20% each week (Chicco et al., 2006), as compared to the current study in which animals exercised at a lower intensity with no external load. However, the results of the current study are in agreement with a previous study in which low intensity exercise did not attenuate DOX-induced oxidative stress although there was a preservation of cardiac function (Chicco et al., 2006).

Another purpose of the study was to determine if cardioprotection is the result of preservation in relative MHC distribution. Expression of the α-MHC isoform has been shown to increase following various types of exercise (Iemitsu et al., 2004; Jin et al., 2000). A possible mechanism involved in this increased expression of the α-MHC isoform may be an upregulation of myocardial thyroid hormone receptors. A study by Iemitsu et al. (2004) detected significant increases in myocardial thyroid hormone receptors α1,

correlating to an increase in α-MHC isoform expression following eight weeks of exercise. In the current study, RT+DOX observed significantly less alteration in MHC isoform distribution compared to SED+DOX, indicating an exercise effect. This is in agreement with a previous study indicating that exercise preconditioning preserved MHC isoform distribution following DOX treatment (Hydock et al., 2011). This suggests that the cardioprotection observed in this study may be a result of preservation of the MHC isoform distribution.

Although many studies observe consistent exercise-induced cardiac adaptations, characteristics of these adaptations differ. Endurance training results in eccentric hypertrophy that can lead to enlargement of the LV chamber and increased LV dimensions. The RT results in concentric hypertrophy, leading to a thickening of the LV wall and greater contractile force. This is due to the type of circulatory overload that occurs with RT (Pluim, Zwinderman, van der Laarse, & van der Wall, 2000). The present study using a RT model clearly demonstrated exercise-induced cardioprotection, similar to what has been seen with endurance training. However, the mechanisms surrounding the cardioprotection may differ. A central theme of endurance training attenuation of DOX-induced cardiotoxicity is significant decreases in oxidative stress. This was not observed in the present study. This suggests that alternative mechanisms may be involved with MHC isoform distribution as it relates to RT-induced cardioprotection.

Summary

This is the first study to provide evidence suggesting that RT may preserve cardiac function and prevent deleterious MHC shifts following DOX treatment. In the current study, DOX treatment resulted in impaired cardiac function, increased lipid peroxidation, and unfavorable shifts in the cardiac MHC isoform distribution. However, 12 weeks of RT preconditioning resulted in a preservation of cardiac function. The cardioprotective effects of exercise were associated with a preservation of the cardiac MHC isoform distribution. These findings suggest that chronic RT may be a beneficial counter measurement to DOX-induced cardiotoxicity. Incorporating resistance training into a rehabilitation program may influene an imporved quality of life for cancer survivors.

REFERENCES

Acehan, D., Jiang, X., Morgan, D. G., Heuser, J. E., Wang, X., & Akey, C. W. (2002). Three-dimensional structure of the apoptosome: Implications for assembly, procaspase-9 binding, and activation. *Molecular cell, 9*(2), 423-432.

Ali, M. K., Ewer, M. S., Gibbs, H. R., Swafford, J., & Graff, K. L. (1994). Late doxorubicin-associated cardiotoxicity in children. *Cancer, 74*(1), 182-188.

Alves, J. P., Nunes, R. B., Stefani, G. P., & Dal Lago, P. (2014). Resistance training improves hemodynamic function, collagen deposition and inflammatory profiles: Experimental model of heart failure. *PloS ONE, 9*(10), e110317.

Aragno, M., Mastrocola, R., Medana, C., Catalano, M. G., Vercellinatto, I., Danni, O., & Boccuzzi, G. (2006). Oxidative stress-dependent impairment of cardiac-specific transcription factors in experimental diabetes. *Endocrinology, 147*(12), 5967-5974.

Arai, M., Yoguchi, A., Takizawa, T., Yokoyama, T., Kanda, T., Kurabayashi, M., & Nagai, R. (2000). Mechanism of doxorubicin-induced inhibition of sarcoplasmic reticulum Ca2+-ATPase gene transcription. *Circulation Research, 86*(1), 8-14.

Asayama, J., Tanaka, T., Tatsumi, T., Nakagawa, C., Kobara, M., Matoba, S., . . . Nakagawa, M. (1995). Effect of doxorubicin on postrest contraction in isolated rat hearts. *Journal of Cardiovascular Pharmacology, 26*(5), 693-697.

Ascensão, A., Ferreira, R., Oliveira, P. J., & Magalhães, J. (2006). Effects of endurance training and acute doxorubicin treatment on rat heart mitochondrial alterations induced by in vitro anoxia-reoxygenation. *Cardiovascular Toxicology, 6*(3-4), 159-171.

Ascensão, A., Lumino-Oliveira, J., Machado, N. G., Ferreira, R., Gonçalves, I. O., & Moreira, A. C. (2011). Acute exercise protects against calcium-induced cardiac mitochondrial permeability transition pore opening in doxorubicin-treated rats. *Clinical science, 120*(1), 37-49.

Ascensão, A., Magalhães, J., Soares, J. M., Ferreira, R., Neuparth, M. J., Marques, F., . . . Duarte, J. A. (2005). Moderate endurance training prevents doxorubicin-induced in vivo mitochondriopathy and reduces the development of cardiac apoptosis. *American Journal of Physiology-Heart and Circulatory Physiology, 289*(2), H722-H731.

Ashrafi, J., Roshan, V. D., & Mahjoub, S. (2012). Cardioprotective effects of aerobic regular exercise against doxorubicin-induced oxidative stress in rat. *African Journal of Pharmacy and Pharmacology, 6*(31), 2380-2388.

Bachur, N. R., Yu, F., Johnson, R., Hickey, R., Wu, Y., & Malkas, L. (1992). Helicase inhibition by anthracycline anticancer agents. *Molecular Pharmacology, 41*(6), 993-998.

Balanehru, S., & Nagarajan, B. (1992). Intervention of adriamycin induced free radical damage. *Biochemistry International, 28*(4), 735-744.

Barauna, V. G., Rosa, K. T., Irigoyen, M. C., & de Oliveira, E. M. (2007). Effects of resistance training on ventricular function and hypertrophy in a rat model. *Clinical Medicine Research, 5*(2), 114-120.

Boucek Jr, R. J., Dodd, D. A., Atkinson, J. B., Oquist, N., & Olson, R. D. (1997). Contractile failure in chronic doxorubicin-induced cardiomyopathy. *Journal of molecular and cellular cardiology, 29*(10), 2631-2640.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1), 248-254.

Chicco, A. J., Hydock, D. S., Schneider, C. M., & Hayward, R. (2006). Low-intensity exercise training during doxorubicin treatment protects against cardiotoxicity. *Journal of Applied Physiology, 100*(2), 519-527. doi:10.1152/japplphysiol.00148.2005

Chicco, A. J., Schneider, C. M., & Hayward, R. (2005). Voluntary exercise protects against acute doxorubicin cardiotoxicity in the isolated perfused rat heart. *Journal of Cardiovascular Pharmacology, 4*(2), 182-189.

Childs, A. C., Phaneuf, S. L., Dirks, A. J., Phillips, T., & Leeuwenburgh, C. (2002). Doxorubicin treatment in vivo causes cytochrome C release and cardiomyocyte apoptosis, as well as increased mitochondrial efficiency, superoxide dismutase activity, and Bcl-2: Bax ratio. *Cancer Research, 62*(16), 4592-4598.

Colan, S. D., Sanders, S. P., & Borow, K. M. (1987). Physiologie hypertrophy: Effects on left ventricular systolic mechanics in athletes. *Journal of the American College of Cardiology, 9*(4), 776-783.

Corna, G., Galy, B., Hentze, M. W., & Cairo, G. (2006). IRP1-independent alterations of cardiac iron metabolism in doxorubicin-treated mice. *Journal of Molecular Medicine, 84*(7), 551-560.

Crompton, M. (1999). The mitochondrial permeability transition pore and its role in cell death. *Biochemical Journal, 341*(Pt. 2), 233-249.

Demirel, H. A., Powers, S, K., Caillaud, C., Coombes, J. S., Naito, H., Fletcher, L. .A, . . . Ji, L. L. (1998). Exercise training reduces myocardial lipid peroxidation following short-term ischemia-reperfusion*. Medicine and Science in Sports and Exercise, 30*(8), 1211-1216.

Di Marco, A., Gaetani, M., Orezzi, P., Scarpinato, B., Silvestrini, R., Soldati, M., . . . Valentini, L. (1964). 'Daunomycin', a new antibiotic of the rhodomycin group. *Nature, 201*, 706-707.

Duncan, N. D., Williams, D. A., & Lynch, G. S. (1998). Adaptations in rat skeletal muscle following long-term resistance exercise training. *European Journal of Applied Physiology and Occupational Physiology, 77*(4), 372-378.

Ferrans, V. (1978). Overview of cardiac pathology in relation to anthracycline cardiotoxicity. *Cancer Treatment Reports, 62*(6), 955-961.

Gibson, N. M., Quinn, C. J., Pfannenstiel, K. B., Bashore, A. C., Hydock, D. S., & Hayward, R. (2013). Exercise reduces myocardial doxorubicin accumulation by upregulating multidrug resistance protein expression. *Circulation, 128*(22 Suppl.), A9450.

Gibson, N. M., Quinn, C. J., Pfannenstiel, K. B., Hydock, D. S., & Hayward, R. (2013). Effects of age on multidrug resistance protein expression and doxorubicin accumulation in cardiac and skeletal muscle. *Xenobiotica, 44*(5), 472-479.

Goldfarb, A. H., Bloomer, R. J., & McKenzie, M. J. (2005). Combined antioxidant treatment effects on blood oxidative stress after eccentric exercise. *Medicine and Science in Sports and Exercise, 37*(2), 234-239.

Goormaghtigh, E., Chatelain, P., Caspers, J., & Ruysschaert, J. M. (1980). Evidence of a complex between adriamycin derivatives and cardiolipin: possible role in cardiotoxicity. *Biochemical Pharmacology, 29*(21), 3003-3010.

Goormaghtigh, E., Huart, P., Praet, M., Brasseur, R., & Ruysschaert, J. M. (1990). Structure of the adriamycin-cardiolipin complex: Role in mitochondrial toxicity. *Biophysical Chemistry, 35*(2–3), 247-257.

Harris, R. N., & Doroshow, J. H. (1985). Effect of doxorubicin-enhanced hydrogen peroxide and hydroxyl radical formation on calcium sequestration by cardiac sarcoplasmic reticulum. *Biochemical Biophysical Research Communications, 130*(2), 739-745.

Harris, M. B., & Starnes, J. W. (2001). Effects of body temperature during exercise training on myocardial adaptations. *American Journal of Physiology-Heart and Circulatory Physiology, 280*(5), H2271-H2280.

Harrison, J. F., Hollensworth, S. B., Spitz, D. R., Copeland, W. C., Wilson, G. L., & LeDoux, S. P. (2005). Oxidative stress-induced apoptosis in neurons correlates with mitochondrial DNA base excision repair pathway imbalance. *Nucleic Acids Research, 33*(14), 4660-4671.

Hayward, R., & Hydock, D. S. (2007). Doxorubicin cardiotoxicity in the rat: An in vivo characterization. *Journal of the American Association for Laboratory Animal Science, 46*(4), 20-32.

Hayward, R., Hydock, D., Gibson, N., Greufe, S., Bredahl, E., & Parry, T. (2013). Tissue retention of doxorubicin and its effects on cardiac, smooth, and skeletal muscle function. *Journal of Physiology and Biochemistry, 69*(2), 177-187.

Henkler, F., Brinkmann, J., & Luch, A. (2010). The role of oxidative stress in carcinogenesis induced by metals and xenobiotics. *Cancers, 2*(2), 376-396.

Herman, E., Mhatre, R., Lee, I., Vick, J., & Waravdekar, V. (1971). A comparison of the cardiovascular actions of daunomycin, adriamycin and N-acetyldaunomycin in hamsters and monkeys. *Pharmacology, 6*(4), 230-241.

Hilmer, S. N., Cogger, V. C., Muller, M., & Le Couteur, D. G. (2004). The hepatic pharmacokinetics of doxorubicin and liposomal doxorubicin. *Drug Metabolism and Disposition, 32*(8), 794-799.

Hrelia, S., Fiorentini, D., Maraldi, T., Angeloni, C., Bordoni, A., Biagi, P. L., & Hakim, G. (2002). Doxorubicin induces early lipid peroxidation associated with changes in glucose transport in cultured cardiomyocytes. *Biochimica et Biophysica Acta-Biomembranes, 1567*(1-2), 150-156.

Hydock, D. S., Lien, C. Y., Jensen, B. T., Parry, T. L., Schneider, C. M., & Hayward, R. (2012). Rehabilitative exercise in a rat model of doxorubicin cardiotoxicity. *Experimental Biology and Medicine, 237*(12), 1483-1492.

Hydock, D. S., Lien, C. Y., Jensen, B. T., Schneider, C. M., & Hayward, R. (2011). Exercise preconditioning provides long-term protection against early chronic doxorubicin cardiotoxicity. *Integrative Cancer Therapies, 10*(1), 47-57. doi:10.1177/1534735410392577

Hydock, D. S., Lien, C. Y., Schneider, C. M., & Hayward, R. (2008). Exercise preconditioning protects against doxorubicin-induced cardiac dysfunction. *Medicine and Science in Sports and Exercise, 40*(5), 808-817. doi:10.1249/MSS.0b013e318163744a

Hydock, D. S., Wonders, K. Y., Schneider, C. M., & Hayward R. (2005). Voluntary wheel running in rats receiving doxorubicin: Effects on cardiac myosin heavy chain. *Medicine and Science in Sports and Exercise, 37*(Suppl.), S439-S439.

Iemitsu, M., Miyauchi, T., Maeda, S., Tanabe, T., Takanashi, M., Matsuda, M., & Yamaguchi, I. (2004). Exercise training improves cardiac function-related gene levels through thyroid hormone receptor signaling in aged rats. American Journal of Physiology-Heart and Circulatory Physiology, 286(5), H1696-H1705.

Jensen, B. T., Lien, C. Y., Hydock, D. S., Schneider, C. M., & Hayward, R. (2013). Exercise mitigates cardiac doxorubicin accumulation and preserves function in the rat. *Journal of Cardiovascular Pharmacology, 62*(3), 263-269. doi:10.1097/FJC.0b013e3182982ce0

Jin, H., Yang, R., Li, W., Lu, H., Ryan, A. M., Ogasawara, A. K., . . . Paoni, N. F. (2000). Effects of exercise training on cardiac function, gene expression, and apoptosis in rats. *American Journal of Physiology-Heart and Circulatory Physiology, 279*(6), H2994-H3002.

Kanter, M., Hamlin, R., Unverferth, D., Davis, H., & Merola, A. (1985). Effect of exercise training on antioxidant enzymes and cardiotoxicity of doxorubicin. *Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology, 59*(4), 1298-1302.

Kavazis, A. N., Smuder, A. J., Min, K., Tümer, N., & Powers, S. K. (2010). Short-term exercise training protects against doxorubicin-induced cardiac mitochondrial damage independent of HSP72. *American Journal of Physiology-Heart and Circulatory Physiology, 299*(5), H1515-H1524.

Kim, S., Kim, S., Kim, B., Rah, S., Chung, S. M., Im, M., & Kim, U. (2006). Doxorubicin-induced reactive oxygen species generation and intracellular Ca^ 2^+ increase are reciprocally modulated in rat cardiomyocytes. *Experimental and Molecular Medicine, 38*(5), 535.

Krause, M. S., Oliveira, L. P., Silveira, E. M. S., Vianna, D. R., Rossato, J. S., Almeida, B. S., . . . de Bittencourt, P. I. H. (2007). MRP1/GS-X pump ATPase expression: is this the explanation for the cytoprotection of the heart against oxidative stress-induced redox imbalance in comparison to skeletal muscle cells? *Cell Biochemistry and Function, 25*(1), 23-32. doi:10.1027/cbf.1343

Krenz, M., Sanbe, A., Bouyer-Dalloz, F., Gulick, J., Klevitsky, R., Hewett, T. E., . . . Federico, A. (2003). Analysis of myosin heavy chain functionality in the heart. *Journal of Biological Chemistry, 278*(19), 17466-17474.

Lennon, S. L., Quindry, J. C., French, J. P, Kim, S., Mehta, J. L., & Powers, S. K. (2004). Exercise and myocardial tolerance to ischaemia-reperfusion. *Acta Physiologica Scandinavica, 182*(2), 161-169.

Levinger, I., Bronks, R., Cody, D. V., Linton, I., & Davie, A. (2005). Resistance training for chronic heart failure patients on beta blocker medications. *International Journal of Cardiology, 102*(3), 493-499.

Locke, M., Noble, E. G., Tanguay, R. M., Feild, M. R., Ianuzzo, S. E., & Ianuzzo, C. D. (1995). Activation of heat-shock transcription factor in rat heart after heat shock and exercise. *American Journal of Physiology–Cell Physiology, 37*(6), C1387.

Lowes, B. D., Minobe, W., Abraham, W. T., Rizeq, M. N., Bohlmeyer, T. J., Quaife, R. A., . . . Bristow, M. R. (1997). Changes in gene expression in the intact human heart. Downregulation of alpha-myosin heavy chain in hypertrophied, failing ventricular myocardium. *Journal of Clinical Investigation, 100*(9), 2315.

Lyu, Y. L., Kerrigan, J. E., Lin, C. P., Azarova, A. M., Tsai, Y. C., Ban, Y., & Liu, L. F. (2007). Topoisomerase IIβ—mediated DNA double-strand breaks: Implications in doxorubicin cardiotoxicity and prevention by dexrazoxane. *Cancer Research, 67*(18), 8839-8846.

Mantovani, G., Maccio, A., Madeddu, C., Mura, L., Massa, E., Gramignano, G., . . . Ferreli, L. (2003). Reactive oxygen species, antioxidant mechanisms, and serum cytokine levels in cancer patients: impact of an antioxidant treatment. *Journal of Environmental Pathology, Toxicology and Oncology, 22*(1), 17-28.

Marcillat, O., Zhang, Y., & Davies, K. (1989). Oxidative and non-oxidative mechanisms in the inactivation of cardiac mitochondrial electron transport chain components by doxorubicin. *Biochemistry Journal, 259*, 181-189.

Marques-Aleixo, I., Santos-Alves, E., Mariani, D., Rizo-Roca, D., Padrão, A. I., Rocha-Rodrigues, S., . . . Oliveira, P. J. (2014). Physical exercise prior and during treatment reduces sub-chronic doxorubicin-induced mitochondrial toxicity and oxidative stress. *Mitochondrion, 20,* 22-33.

McBride, J. M., Kraemer, W. J., Triplett-McBride, T., & Sebastianelli, W. (1998). Effect of resistance exercise on free radical production. *Medicine and Science in Sports and Exercise, 30*(1), 67-72.

Mccord, J. (1985). Oxygen-derived free radicals in postischemic tissue injury. *New England Journal of Medicine, 312*(3), 159-163.

Minotti, G., Menna, P., Salvatorelli, E., Cairo, G., & Gianni, L. (2004). Anthracyclines: Molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacological Reviews, 56*(2), 185-229.

Miyata, S., Minobe, W., Bristow, M. R., & Leinwand, L. A. (2000). Myosin heavy chain isoform expression in the failing and nonfailing human heart. *Circulation Research, 86*(4), 386-390.

Mo, A., Yao, W., Li, C., Tian, X., Su, M., Ling, Y., . . . Jee W. S. (2002). Bipedal stance exercise and prostaglandin E2 (PGE 2) and its synergistic effect in increasing bone mass and in lowering the PGE 2 dose required to prevent ovariectomized-induced cancellous bone loss in aged rats. *Bone, 31*(3), 402-406.

Narula, J., Haider, N., Virmani, R., DiSalvo, T. G., Kolodgie, F. D., Hajjar, R. J., . . . Khaw, B. A. (1996). Apoptosis in myocytes in end-stage heart failure. *New England Journal of Medicine, 335*(16), 1182-1189.

Noble, E. G., Moraska, A., Mazzeo, R. S., Roth, D. A., Olsson, M. C., Moore, R. L., & Fleshner M. (1999). Differential expression of stress proteins in rat myocardium after free wheel or treadmill run training. *Journal of Applied Physiology, 86*(5), 1696-1701.

Nohl, H., Gille, L., & Staniek, K. (1998). The exogenous NADH dehydrogenase of heart mitochondria is the key enzyme responsible for selective cardiotoxicity of anthracyclines. *Zeitschrift fur Naturforschung C-Journal of Biosciences, 53*(3), 279-285.

Nordberg, J., & Arnér, E. S. (2001). Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radical Biology and Medicine, 31*(11), 1287-1312.

Notomi, T., Okimoto, N., Okazaki, Y., Tanaka, Y., Nakamura, T., & Suzuki, M. (2001). Effects of tower climbing exercise on bone mass, strength, and turnover in growing rats. *Journal of Bone and Mineral Research, 16*(1), 166-174.

Palevo, G., Keteyian, S. J., Kang, M., & Caputo, J. L. (2009). Resistance exercise training improves heart function and physical fitness in stable patients with heart failure. *Journal of Cardiopulmonary Rehabilitation and Prevention, 29*(5), 294-298.

Parry T. L., & Hayward R. (in press). Exercise Training Does Not Affect Anthracycline Anti-Tumor Efficacy While Attenuating Cardiac Dysfunction. *American Journal of Physiology—Regulatory, Integrative and Comparative Physiology.*

Pluim, B. M., Zwinderman, A. H., van der Laarse, A., & van der Wall, E. E. (2000). The athlete's heart a meta-analysis of cardiac structure and function. *Circulation, 101*(3), 336-344.

Powers, S. K., Criswell, D., Lawler, J., Martin, D., Lieu, F.-K., Ji, L., & Herb, R. A. (1993). Rigorous exercise training increases superoxide dismutase activity in ventricular myocardium. *Group, 1*(2), 3.

Powers, S. K., & Demirel, H. (2001). Exercise, heat shock proteins, and myocardial protection from IR injury. *Medicine and Science in Sports and Exercise, 33*(3), 386-392.

Quintanilha, A. (1984). Effects of physical exercise and/or vitamin E on tissue oxidative metabolism. *Biochemical Society Transactions, 12*(3), 403-404.

Radi, R., Beckman, J. S., Bush, K. M., & Freeman, B. A. (1991). Peroxynitrite-induced membrane lipid peroxidation: The cytotoxic potential of superoxide and nitric oxide. *Archives of Biochemistry and Biophysics, 288*(2), 481-487.

Ramamurthy, B., Hook, P., Jones, A. D., & Larsson, L. (2001). Changes in myosin structure and function in response to glycation. *The FASEB* [Federation of American Societies for Experimental Biology] *Journal*, *15*(13), 2415-2422.

Reiser, P. J., & Kline, W. O. (1998). Electrophoretic separation and quantitation of cardiac myosin heavy chain isoforms in eight mammalian species. *American Journal of Physiology-Heart and Circulatory Physiology, 274*(3), H1048-H1053.

Rosa, B. V., Firth, E. C., Blair, H. T., Vickers, M. H., & Morel, P. C. (2011). Voluntary exercise in pregnant rats positively influences fetal growth without initiating a maternal physiological stress response. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 300*(5), R1134-R1141.

Salvesen, G. S., & Dixit, V. M. (1997). Caspases: Intracellular signaling by proteolysis. *Cell, 91*(4), 443-446.

Schimmel, K. J., Richel, D. J., van den Brink, R. B., & Guchelaar, H. J. (2004). Cardiotoxicity of cytotoxic drugs. *Cancer Treatment Reviews, 30*(2), 181-191.

Schwartz, R. G., McKenzie, W. B., Alexander, J., Sager, P., D'Souza, A., Manatunga, A., . . . Surkin, L. (1987). Congestive heart failure and left ventricular dysfunction complicating doxorubicin therapy: Seven-year experience using serial radionuclide angiocardiography. *The American Journal of Medicine, 82*(6), 1109-1118.

Schwartz, A. L., & Winters-Stone, K. (2009). Effects of a 12-month randomized controlled trial of aerobic or resistance exercise during and following cancer treatment in women. *The Physician and Sportsmedicine, 37*(3), 62-67.

Segal, R. J., Reid, R. D., Courneya, K. S., Malone, S. C., Parliament, M. B., Scott, C. G., . . . D'Angelo, M. E. S. (2003). Resistance exercise in men receiving androgen deprivation therapy for prostate cancer. *Journal of Clinical Oncology, 21*(9), 1653-1659.

Shapiro, C. L., Hardenbergh, P. H., Gelman, R., Blanks, D., Hauptman, P., Recht, A., . . . Henderson, I. C. (1998). Cardiac effects of adjuvant doxorubicin and radiation therapy in breast cancer patients. *Journal of Clinical Oncology, 16*(11), 3493-3501.

Siegel, R. L., Miller, K. D., & Jemal, A. (2015). Cancer statistics, 2015. *CA: A Cancer Journal for Clinicians, 65*(1), 5-29.

Singal, P. K., & Iliskovic, N. (1998). Doxorubicin-induced cardiomyopathy. *New England Journal of Medicine, 339*(13), 900-905. doi:10.1056/NEJM199809243391307

Singal, P. K., Li, T., Kumar, D., Danelisen, I., & Iliskovic, N. (2000). Adriamycin-induced heart failure: Mechanisms and modulation. *Molecular and Cellular Biochemistry, 207*(1-2), 77-86.

Sokolove, P. M., & Shinaberry, R. G. (1988). Na sup+/sup-independent release of Ca sup 2+/sup from rat heart mitochondria: Induction by adriamycin aglycone. *Biochemical Pharmacology, 37*(5), 803-812.

Somani, S., Frank, S., & Rybak, L. (1995). Responses of antioxidant system to acute and trained exercise in rat heart subcellular fractions. *Pharmacology Biochemistry and Behavior, 51*(4), 627-634.

Soufi, F. G., Saber M. M., Ghiassie, R., & Alipour, M. (2011). Role of 12-week resistance training in preserving the heart against ischemia-reperfusion-induced injury. *Cardiology Journal, 18*(2), 140-145.

Steinherz, L. J., Steinherz, P. G., Tan, C. T., Heller, G., & Murphy, M. L. (1991). Cardiac toxicity 4 to 20 years after completing anthracycline therapy. *The Journal of the American Medical Association, 266*(12), 1672-1677.

Szabó, C., & Ohshima, H. (1997). DNA damage induced by peroxynitrite: Subsequent biological effects. *Nitric Oxide, 1*(5), 373-385.

Tait, S. W., & Green, D. R. (2010). Mitochondria and cell death: Outer membrane permeabilization and beyond. *Nature Reviews Molecular Cell Biology, 11*(9), 621-632.

Talmadge, R. J., & Roy, R. R. (1993). Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. *Journal of Applied Physiology, 75*(5), 2337-2340.

Tam, N. N., Nyska, A., Maronpot, R. R., Kissling, G., Lomnitski L., Suttie, A., . . . Ho, S. M. (2006). Differential attenuation of oxidative/nitrosative injuries in early prostatic neoplastic lesions in TRAMP mice by dietary antioxidants. *The Prostate, 66*(1), 57-69.

Tardiff, J. C., Hewett, T. E., Factor, S. M., Vikstrom, K. L., Robbins, J., & Leinwand, L. A. (2000). Expression of the β (slow)-isoform of MHC in the adult mouse heart causes dominant-negative functional effects. *American Journal of Physiology-Heart and Circulatory Physiology, 278*(2), H412-H419.

Taylor, R. P., Harris, M. B., & Starnes, J. W. (1999). Acute exercise can improve cardioprotection without increasing heat shock protein content. *American Journal of Physiology-Heart and Circulatory Physiology, 276*(3), H1098-H1102.

Tewey, K. M., Chen, G., Nelson, E., & Liu, L. (1984). Intercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. *Journal of Biological Chemistry, 259*(14), 9182-9187.

Thomason, D., Baldwin, K., & Herrick, R. (1986). Myosin isozyme distribution in rodent hindlimb skeletal muscle. *Journal of Applied Physiology, 60*(6), 1923-1931.

Vincent, K. R., Vincent, H. K., Braith, R. W., Lennon, S. L., & Lowenthal, D. T. (2002). Resistance exercise training attenuates exercise-induced lipid peroxidation in the elderly. *European Journal of Applied Physiology, 87*(4-5), 416-423.

Von Hoff, D. D., Layard, M. W., Basa, P., Davis, J. H. L., Von Hoff, A. L., Rozencweig, M., & Muggia, F. M. (1979). Risk factors for doxorubicin-induced congestive heart failure. *Annals of Internal Medicine, 91*(5), 710-717. doi:10.7326/0003-4819-91-5-710

Weiss, R. B. (1992). The anthracyclines: Will we ever find a better doxorubicin? *Seminars in Oncology, 19*(6), 670-686.

Westerlind, K. C., Fluckey, J. D., Gordon, S. E., Kraemer, W. J., Farrell, P. A., & Turner, R. T. (1998). Effect of resistance exercise training on cortical and cancellous bone in mature male rats. *Journal of Applied Physiology, 84*(2), 459-464.

Winters-Stone, K. M., Dobek, J., Bennett, J. A, Nail, L. M., Leo, M. C., & Schwartz, A. (2012). The effect of resistance training on muscle strength and physical function in older, postmenopausal breast cancer survivors: a randomized controlled trial. *Journal of Cancer Survivorship, 6*(2), 189-199.

Wonders, K. Y., Hydock, D. S., Greufe, S., Schneider, C. M., & Hayward, R. (2009). Endurance exercise training preserves cardiac function in rats receiving doxorubicin and the HER-2 inhibitor GW2974. *Cancer Chemotherapy and Pharmacology, 64*(6), 1105-1113. Retrieved from http://dx.doi.org/10.1007/s00280-009-0967-z

Wonders, K. Y., Hydock, D. S., Schneider, C. M., & Hayward, R. (2008). Acute exercise protects against doxorubicin cardiotoxicity. *Integrative Cancer Therapies, 7*(3), 147-154. doi:10.1177/1534735408322848

Yao, W., Jee, W., Chen, J., Li, C., & Frost, H. (2001). A novel method to “exercise” rats: Making rats rise to erect bipedal stance for feeding-raised cage model. *Journal of Musculoskeletal and Neuronal Interactions, 1*, 241-247.

Yao, W., Jee, W., Chen, J., Tam, C., Setterberg, R., & Frost, H. (2000). Erect bipedal stance exercise partially prevents orchidectomy-induced bone loss in the lumbar vertebrae of rats. *Bone, 27*(5), 667-675.

Young, R. C., Ozols, R. F., & Myers, C. E. (1981). The anthracycline antineoplastic drugs. *New England Journal of Medicine, 305*(3), 139.

Zhang, S., Liu, X., Bawa-Khalfe, T., Lu, L. S., Liu, L. F., & Yeh, E. T. (2012). Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nature Medicine, 18*(11), 1639-1642.

Zhang Y. J., Shi, J., Li, Y. J., & Wei, L. (2009). Cardiomyocyte death in doxorubicin-induced cardiotoxicity. *Archivum Immunologiae et Therapiae Experimentalis, 57*(6), 435-445.

APPENDIX

UNIVERSITY OF NORTHERN COLORADO
INSTITUTIONAL ANIMAL CARE
AND USE COMMITTEE

UNIVERSITY of NORTHERN COLORADO

IACUC Memorandum

To: Dr. Reid Hayward

From: Laura Martin, Director of Compliance and Operations

CC: IACUC Files

Date: 1/4/2012

Re: IACUC Protocol 1201CE-RH-R-15 Approval

The UNC IACUC has completed a final review of your protocol "Strategies to Alleviate the Negative Side-Effects of Cancer Treatments". The protocol review was based on the requirements of Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training; the Public Health Policy on Humane Care and Use of Laboratory Animals; and the USDA Animal Welfare Act and Regulations. Based on the review, the IACUC has determined that all review criteria have been adequately addressed. The PI/PD is approved to perform the experiments or procedures as described in the identified protocol as submitted to the Committee. This protocol has been assigned the following number 1201CE-RH-R-15.

The next annual review will be due before January 4, 2012.

Sincerely,

Laura Martin, Director of Compliance and Operations