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Oxidative stress alterations following an exercise intervention in cancer survivors

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UNIVERSITY OF NORTHERN COLORADO
Greeley, Colorado
The Graduate School

OXIDATIVE STRESS ALTERATIONS FOLLOWING
AN EXERCISE INTERVENTION IN
CANCER SURVIVORS

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

Christopher P. Repka

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

May, 2014

This Dissertation by: Christopher P. Repka

Entitled: *Oxidative Stress Alterations Following an Exercise Intervention in Cancer Survivors*

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

Accepted by the Doctoral Committee

Reid Hayward, Ph. D., Research Advisor

David Hydock, Ph. D., Committee Member

Trent Lalonde, Ph. D., Committee Member

Alana Cline, Ph. D., Faculty Representative

Date of Dissertation Defense _____

Accepted by the Graduate School

Linda L. Black, Ed.D.
Dean of the Graduate School and International Admissions

ABSTRACT

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Exercise interventions in cancer patients have been demonstrated to reduce cancer-treatment related side effects. Despite this, the underlying mechanisms associated with the protective aspects of exercise are generally uncharacterized, but treatment-associated oxidative stress is thought to play a role. The purpose of this study was to determine the effect of an exercise intervention on fatigue, cardiorespiratory fitness (CRF), muscular strength, and blood markers of DNA and protein oxidation compared to a non-exercising, cancer control group. **METHODOLOGY:** An initial fasting blood draw and assessments of muscular strength and CRF were administered to fifteen cancer patients within six weeks of completing radiation or chemotherapy treatment. Eight cancer patients participated in a 10-week exercise intervention (EX) while seven continued standard care (CON). Following the intervention, subjects completed another fasted blood draw and a reassessment of fatigue, strength and CRF. Changes in plasma protein carbonyls, 8-OHdG, and antioxidant status were compared between the exercise and control groups. Baseline markers of oxidative stress were compared between healthy individuals (NC) and cancer patients. A correlation analysis of changes in fitness

parameters and oxidative stress in cancer patients was conducted. **RESULTS:** Baseline total antioxidant capacity was significantly lower, and plasma protein carbonyls significantly higher in cancer patients compared to NC ($p < 0.05$). Mean total fatigue scores decreased significantly from 5.0 ± 2.2 to 2.6 ± 1.5 ($p < 0.05$) in EX, whereas changes in CON (4.7 ± 2.5 to 3.2 ± 2.4) were not significant. All fatigue subscales significantly decreased in EX, while only cognitive fatigue increased significantly in CON ($p < 0.05$). Antioxidant capacity increased and protein carbonyls decreased in EX ($p < 0.01$) but not CON. Improvements in composite arm (41%) and leg strength (34%), isometric handgrip strength (11%), and VO_{2peak} (16%) all significantly improved in the EX group ($p < 0.05$), while none of these parameters significantly changed in CON. No significant changes over time were found in 8-OHdG, but a group by time interaction effect was detected ($p < 0.05$). Baseline antioxidant capacity significantly correlated ($p < 0.05$) with total, affective, sensory, and cognitive fatigue. Increases in antioxidant capacity were correlated ($p < 0.05$) with reductions in affective, sensory, and cognitive fatigue. Although 8-OHdG was not correlated with any fatigue parameter at baseline, changes in total and affective fatigue exhibited significant correlations with changes in 8-OHdG over time. **CONCLUSIONS:** A whole-body exercise intervention is an effective method of increasing muscular strength, CRF and antioxidant capacity, while reducing markers of protein oxidation. An exercise intervention was an effective method of reducing fatigue in cancer patients following cessation of treatment, whereas standard care resulted in non-significant reductions in fatigue. Oxidative stress may be implicated in cancer-related fatigue, while improved antioxidant capacity following an exercise intervention may play a role in mitigating fatigue.

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CHAPTER I

INTRODUCTION

Cancer is a group of diseases characterized by uncontrolled growth and regulation of cells. The etiology of cancer is associated with both environmental (chemicals, radiation, smoking, diet, and infectious organisms) and inherent factors (inherited genetic mutations, hormones, and immune conditions). The development of cancer depends on an interaction between these factors, and requires the circumvention of various cellular checkpoints. When a cell receives a “hit,” a carcinogen alters a cell’s DNA, affecting its function and growth. Normally, the cellular reproductive processes are halted or terminated at the checkpoints to prevent cancer development or progression, but in the presence of a promoter, the genetically altered cell may proliferate and metastasize, resulting in cancer.

Although nearly 600,000 people are expected to die of cancer in the US this year, overall cancer mortality has drastically decreased in the previous several decades, due to improved detection and treatment methods (American Cancer Society, 2014). The National Cancer Institute estimates that 13.7 million Americans with a history of cancer were alive in 2012 and about 1,665,540 new cancer cases are expected to be diagnosed in 2014. In 2010, there were 171.76 cancer related deaths per 100,000 compared to a

214.95 per 100,000 rate in 1990, illustrating improved efficacy of cancer treatment methods (Howlader, 2012). Although this is an encouraging trend, the adverse side effects of surgery, radiation, chemotherapy, and other treatment modalities are associated with severe physiological side effects that may last for years. Typical side effects include muscle weakness, reduced cardiorespiratory fitness, peripheral neuropathy, immunosuppression, and general fatigue, resulting in an overall decrease in quality of life. While these conditions are multicausal and multifactorial, oxidative stress is thought to play a pivotal role in many pathological processes associated with cancer and its treatments (Fang, Seki, & Maeda, 2009; Robbins & Zhao, 2004; Zhao & Robbins, 2009).

Oxidative stress is a state in which reactive oxygen species (ROS) are produced at a rate that exceeds cellular adaptive and repair capacities, namely enzymatic and non-enzymatic antioxidants. Also known as free radicals, ROS can cause damage to tissues by stealing electrons from nearby molecules, creating new free radicals and propagating a chain reaction. This process causes damage to lipid membranes within any cell, in a process called lipid peroxidation. ROS can also damage other cellular components, including proteins and nucleic acids, resulting in skeletal and cardiac muscle dysfunction, and potentially DNA modification, respectively. The development and progression of many cancers are associated with oxidative stress-induced DNA modification, but cancer cells themselves can produce increased levels of ROS, perpetuating dysfunction and cancer growth (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006; Yeh et al., 2006). Interestingly, many modes of cancer treatment depend on extremely high doses of ROS as a mechanism of cancer killing, including radiation, many chemotherapy regimens, and novel strategies such as pro-oxidant therapy and photodynamic therapy. Unfortunately,

oxidative stress typically affects healthy tissue in addition to malignant tissue, resulting in both acute and chronic side effects.

The most commonly produced ROS are superoxide and hydroxyl radicals. Most free radical production in healthy individuals occurs in the mitochondria, due to a “leakage” of electrons from 1-2% of the oxygen in the electron transport chain (Boveris & Chance, 1973). This can form a superoxide radical ($O_2^{\bullet-}$), as the unstable intermediate semiquinone anion can directly transfer electrons to molecular oxygen instead of reducing to ubiquinone (Turrens, 1997). Although once considered merely a byproduct of oxidative phosphorylation, several alternate sources of ROS production have since been identified. Among these, the inducible enzymatic production of various ROS indicates that they play important roles in cellular function. NAD(P)H oxidase catalyzes the production of superoxide in phagocytes, which use free radicals to destroy pathogens, in what is known as a respiratory burst. Another enzyme, xanthine reductase, is expressed in two forms: xanthine reductase, which creates the antioxidant uric acid, and xanthine oxidase, a pro-oxidant enzyme (Vorbach, Harrison, & Capecchi, 2003). These enzymes are tightly regulated to ensure an appropriate cellular level of ROS, and to maintain redox balance, illustrating the importance of ROS creation in healthy individuals.

While oxidative stress can be damaging to every cell and system within the body, ROS are necessary for proper cellular functioning. Nitric oxide (NO) is a free radical, but has long been recognized as an important mediator of vasodilation. In recent years, other ROS, including superoxide (Buetler, Krauskopf, & Ruedg, 2004), have been recognized as important cellular signaling molecules. Reactive oxygen species have been

shown to play signaling roles in muscular contraction, adaptive responses to stress, including heat shock proteins and cytokines, and activation of the MAP-K pathway and p53 tumor suppressor protein (Emerling et al., 2005; Jackson, 2009a; Liu, Chen, & St Clair, 2008; Valko et al., 2007; Zhang & Gutterman, 2007). Additionally, ROS are thought to be involved in the chronic adaptive response to exercise (Powers, Duarte, Kavazis, & Talbert, 2009).

Because ROS production is a normal physiological process, an antioxidant defense system is in place to counteract normally produced free radicals. Antioxidants are molecules that inhibit free radical reactions, typically acting as a reducing agent by donating an electron and ceasing the oxidative chain reaction. Enzymatic antioxidants include superoxide dismutases, catalases, and glutathione peroxidases, and common endogenous non-enzymatic antioxidants include glutathione, albumin, ferritin, uric acid, bilirubin and pyruvate. Common dietary non-enzymatic antioxidants include vitamin C and vitamin E. Any disruption in antioxidant capacity of healthy cells can alter redox balance and health status. Cancer cells typically have reduced antioxidant enzyme activity, but in certain types of cancers and metastatic growth, antioxidant enzyme activity is augmented, conveying multidrug resistance to these cells (Oberley, 2002).

Due to the extremely transient nature of ROS, it is difficult to perform chemical analyses of ROS themselves. Rather, downstream products of oxidative stress in blood and tissues may be analyzed via biochemical analysis. Malondialdehyde (MDA), a stable end product of lipid peroxidation, is likely the most common marker of oxidative stress. Other markers of lipid peroxidation include 4-hydroxynonenal (4-HNE) and F₂ isoprostanes such as 8-iso-prostaglandin F_{2α} (8-isoprostane). 8-Hydroxy-

2'-deoxyguanosine (8-OHdG), a fairly ubiquitous byproduct of oxidative DNA damage, is the primary biomarker for DNA oxidation. Carbonyl derivatives of proline, lysine, arginine and threonine are the most common oxidation product of protein. Additionally, the ratio of reduced glutathione to oxidized glutathione (GSH:GSSG) is a commonly used determinant of oxidative stress (Serru et al., 2001).

In addition to generalized damage to body tissues, and the association between chronic oxidative stress and cancer, numerous chronic pathophysiological conditions may be linked to oxidative stress, including diabetes, hypertension, vascular disease, cachexia, neural dysfunction, chronic heart failure, and ischemia-reperfusion injury. Cancer chemotherapy and radiation therapy are known to elicit many of these pathologies as side effects, most notably cardiac dysfunction. Oxidative stress has been hypothesized to initiate or aggravate these medical conditions (Y. Chen, Jungsuwadee, Vore, Butterfield, & St Clair, 2007; Robbins & Zhao, 2004; J. Wang & Yi, 2008).

Some of the most effective cancer treatments also are the most damaging to normal tissue. Doxorubicin, for instance, is a highly effective antineoplastic agent used for various cancers, yet causes a dose-dependent cardiotoxicity associated with oxidative stress (Chaiswing et al., 2004; Kanter, Hamlin, Unverferth, Davis, & Merola, 1985).

While the primary antineoplastic mechanisms of DOX are inhibition of topoisomerase II and intercalation in DNA (Chen, Jungsuwadee, Butterfield, & St Clair, 2007), resulting in inhibition of DNA replication and RNA transcription, the induction of ROS may contribute to antitumor activity (Taatjes, Fenick, Gaudiano, & Koch, 1998). Doxorubicin cardiotoxicity causes morphological damage within the myocardium, often apparent when left ventricular ejection fraction (LVEF) falls below 45%, and may result in

congestive heart failure (Ng, Better, & Green, 2006). Several authors have identified oxidative stress as a primary mechanism of DOX cardiotoxicity (Y. Chen et al., 2007; Simunek et al., 2009). Other common chemotherapy drugs, including tamoxifen, etoposide, 5-fluorouracil, alkylating agents and platinum coordinating complexes are known to induce significant oxidative stress. Radiation, by nature, is characterized by ROS production and exerts its physiological effects, both beneficial and harmful, via this mechanism. Even surgery is capable of producing enough ROS as a side effect to facilitate increased growth of metastatic tumors (Hyoudou, Nishikawa, Kobayashi, Umeyama, et al., 2006).

The challenge for oncologists and the entire cancer care team is to maximize effectiveness of treatment while minimizing side effects. Reduction of oxidative stress in healthy tissues is a method which may reduce or eliminate many of the cancer treatment-associated side effects. Antioxidant therapy, while an attractive strategy, is currently not advocated by the American Cancer Society (D'Andrea, 2005) due to the potential for reduced treatment efficacy and relatively poor reduction of side effects with dietary antioxidant supplementation (Lawenda et al., 2008; Myers et al., 1983; Unverferth et al., 1983). Additionally, endogenous antioxidant enzymes are coordinately regulated, so that glutathione peroxidases and catalases are upregulated in concert with SOD to match the current level of ROS production (McCord, 2008). Exogenous administration of any one of these alone could result in aberrant redox regulation.

An increasingly popular rehabilitation method in cancer patients is prescriptive, whole-body exercise. Exercise has been shown to minimize many side effects of cancer treatment. Exercise during treatment is known to increase tolerance for high doses of

chemotherapy (Chicco, Schneider, & Hayward, 2006) and can reduce the damaging side effect of hormonal therapy (Hydock, Lien, Schneider, & Hayward, 2007). Furthermore, exercise following treatment may reduce the chance for cancer recurrence (Courneya, 2003). Exercise following surgery has been shown to be beneficial in increasing the natural killer (NK) cell cytotoxic activity (NKCA), (Na, Kim, Kim, Ha, & Sup Yoon, 2000) suggesting that exercise may play a role in cancer treatment, rather than simply rehabilitation.

Resistance and aerobic exercise during and following treatment have been found to be beneficial as a means to reduce self reported fatigue (Mock et al., 2001; Schneider, Hsieh, Sprod, Carter, & Hayward, 2007c; Schwartz, 2007), which is the most prevalent symptom in cancer survivors. Prescriptive exercise programs have successfully improved muscular strength (Courneya et al., 2007) and muscular endurance (Schneider, Hsieh, Sprod, Carter, & Hayward, 2007a) in cancer survivors. A similar effect on cardiorespiratory endurance has been noted in cancer survivors (Schneider et al., 2007c). Improvements in functional capacity in cancer patients are associated with increased self-reported quality of life (L. W. Jones et al., 2008; Schneider et al., 2007a; Thorsen et al., 2005).

While acute, high intensity exercise bouts generate ROS, it is well documented that exercise training increases antioxidant enzyme capacity in animals and healthy individuals (Finaud, Lac, & Filaire, 2006; Ji, 2002). The concept of hormesis describes the positive adaptation to a mild stressor, whereas a larger dosage of the same stressor would be damaging or fatal. The response to ROS is exemplary of hormesis; lower doses of ROS, such as during an acute exercise bout, trigger an adaptive response in which the

antioxidant enzymes are upregulated. High doses, like those caused by radiation and chemotherapy, can cause severe side effects. Although a single bout of exercise can transiently activate mitochondrial (manganese) superoxide dismutase (MnSOD) gene transcription, substantial MnSOD upregulation may require more chronic stimulation, such as a prescriptive exercise intervention (Hollander et al., 1999). The effectiveness of exercise may be due, in part, to the reduction of oxidative stress in normal cells, and therefore protection of healthy tissues without a protective effect within cancer cells. It appears that the antioxidant enzyme upregulation in response to exercise is compartmentalized, as unilateral resistance training stimulated an enzymatic response only in the trained leg (Parise, Phillips, Kaczor, & Tarnopolsky, 2005). This suggests that healthy, exercising tissue can be protected, without increased antioxidant defense within cancer cells.

While oxidative stress is recognized as a contributing factor in cancer and treatment-related decrements in health, there is little research addressing the effect of exercise on both functional parameters and markers of oxidative stress or antioxidant enzymes. A notable study from 1985 investigating the effect of exercise on cardiotoxicity and antioxidant enzymes in rats remains one of the most sophisticated studies supporting exercise as a strategy to reduce treatment-induced oxidative stress and subsequent side effects (Kanter et al., 1985). Swim training significantly reduced cardiotoxicity of doxorubicin and increased blood values for three antioxidant enzymes (glutathione peroxidase, superoxide dismutase, catalase), indicating a protective effect of exercise via improved antioxidant defense. To date, no similar study has been conducted in a human population of cancer survivors. It is therefore reasonable to examine the

effect of a prescriptive exercise intervention on oxidative stress markers in cancer survivors following treatment known to induce severe oxidative stress.

Statement of Purpose

The primary purpose of this study is to determine the effects of a 10-week prescribed aerobic and resistance exercise intervention on plasma markers of oxidative stress and antioxidant enzyme capacity in cancer survivors versus a non-exercising, cancer control group. Markers of oxidative stress to be measured are 8-OHdG, and reactive carbonyl derivatives. Trolox-equivalent antioxidant capacity (TEAC) will be measured as a universal marker of antioxidant capacity in the blood. Additionally, selected physiological variables, including peak VO_2 , muscular strength, and fatigue will be compared between groups. The relationship between oxidative stress and baseline physiological variables will be determined. Similarly, the relationship between the change in oxidative stress and changes in physiological variables will be determined. Baseline oxidative stress and antioxidant enzyme capacity in cancer patients will be compared to a group of healthy individuals.

Research Hypotheses

- H1 There will be a significant decrease in blood markers of oxidative stress in exercising subjects compared to standard care following the 10-week intervention.
- H2 There will be a significant improvement in total antioxidant capacity in exercising subjects compared to standard care following the 10-week intervention.
- H3 There will be a significant improvement in cardiopulmonary function in exercising subjects compared to standard care following the 10-week intervention.
- H4 There will be a significant improvement in muscular strength in exercising subjects compared to standard care following the 10-week intervention.
- H5 There will be a significant improvement in self-reported fatigue in exercising subjects compared to standard care following the 10-week intervention.
- H6 There will be significantly greater baseline levels of oxidative stress markers in cancer survivors than in healthy, age and gender-matched controls.
- H7 At baseline, oxidative stress will exhibit a negative correlation with cardiopulmonary function and muscular strength, and a positive correlation with fatigue. In contrast, antioxidant capacity will exhibit a positive correlation with cardiopulmonary function and strength, and a negative correlation with fatigue
- H8 Changes in oxidative stress will exhibit a negative correlation with changes in cardiopulmonary function and muscular strength, and a positive correlation with fatigue. Changes in antioxidant capacity will exhibit a positive correlation with changes in cardiopulmonary function and strength, and a negative correlation with fatigue.

Significance of the Study

All cancer treatments inevitably have side effects, and beyond altering quality of life, they often limit the aggressiveness of treatment. Radiation, chemotherapy, and even surgery result in production of (ROS) and ultimately oxidative stress. Cancer cells themselves are capable of producing pathological levels of ROS. Cancer patients suffer

from fatigue, cachexia, decrements in cardiorespiratory fitness, and neurological disorders, all which may be associated with increased oxidative stress. Additionally, increased oxidative stress from radiation treatment is associated with the development of second cancers (Bostrom & Soloway, 2007). Unfortunately, there has been little success in antioxidant supplementation as a means to reduce treatment-associated side effects without affecting treatment efficacy (Lawenda et al., 2008). Prescriptive exercise, on the other hand, has been shown to increase muscular endurance and strength and cardiorespiratory fitness, while reducing fatigue. Additionally, in animal models, exercise has been shown to reduce treatment-associated side effects, increase antioxidant enzyme activity, and decrease markers of oxidative stress (Ascensao et al., 2005a; Wonders & Reigle, 2009). While exercise training has been shown to increase antioxidant capacity in healthy individuals and some pathological conditions, such as Parkinson's disease (Bloomer et al., 2008; Finaud et al., 2006) and diabetes (Ristow et al., 2009), to date, no study has examined the effect of a prescriptive exercise intervention on markers of oxidative stress and antioxidant capacity in cancer survivors. Additionally, there is little data on the degree of chronic oxidative stress in cancer survivors post treatment.

Assumptions

This study is based upon the assumption that all subjects accurately report their previous exercise, smoking, and antioxidant supplementation habits. It is assumed that blood draws were taken while the subjects were fasted, and had not engaged in strenuous activity for the previous 72 hours. Subjects in the standard care group are assumed to have maintained baseline physical activity.

Limitations

All subjects were recruited in Greeley, Colorado and surrounding towns, and therefore the results may not be generalizable to the entire US population. Due to the self-selected nature of participation in this study, it is possible that psychological factors beyond the scope of this study independently affected fatigue values at baseline or fatigue changes over time. Diets were not controlled and food logs were not utilized to evaluate subjects' consumption of antioxidant-containing foods, therefore it is possible that whole foods containing variable antioxidant compositions affected plasma antioxidant capacity or oxidative stress at either baseline or follow-up. Because there were a variety of cancer types and treatments among the subjects in this study, it is possible that undetected prolonged physiological effects of treatment and cancer type may play a role in patient response to time out of treatment in the control group, or response to exercise in the intervention group. Finally, although exercise interventions were similar, individualization of exercise regimens for each cancer patient based upon specific needs may have affected the biochemical and fatigue responses to exercise and could potentially limit the generalizability of this study.

Definition of Terms

Antioxidant. A molecule that inhibits free radical reactions, typically by acting as a reducing agent by donating an electron and ceasing the oxidative chain reaction. Enzymatic antioxidants include superoxide dismutases, catalases, and glutathione peroxidases. Non-enzymatic antioxidants include glutathione, ascorbic acid (vitamin C), α -tocopherol (vitamin E), flavonoids, and carotenoids (Baskin & Salem, 1997).

Cachexia. A syndrome of progressive weight loss, anorexia, and persistent erosion of host body cell mass, often in response to a malignant growth (Laviano, Meguid, Preziosa, & Rossi Fanelli, 2007).

Cancer. Cancer is a group of diseases characterized by abnormal, uncontrolled growth and regulation of cells that will lead to tissue failure (American Cancer Society, 2014).

Cancer Related Fatigue. An overwhelming, whole-body tiredness, unrelated to activity or exertion, that makes a person feel drained and produces a strong desire to lie down and sleep (Schneider, Dennehy, & Carter, 2003).

Carcinogen. A chemical, biological, or physical agent capable of permanently, directly, and irreversibly changing the molecular structure of a cell. With prolonged or continuous exposure, this predisposes the cell to transformation, and ultimately cancer development (Langhorne, Fulton, & Otto, 2007).

Chemotherapy. Treatment with at least one drug (Langhorne et al., 2007) employed as the primary means of anti-cancer therapy, to limit the spread of localized tumors, particularly in late stage cancers, or as adjuvant therapy to shrink tumors prior to radiation or surgery and destroy microscopic metastases following tumor removal (Chu & DeVita 2005).

Lipid Peroxidation. Oxidative degradation of lipids whereby free radicals "steal" electrons from the lipids in cell membranes, resulting in cellular damage (Baskin & Salem, 1997).

Hormesis. A beneficial physiological response or adaptation to a mildly damaging stimulus (Ji, Maria-Carmen, & Jose, 2006).

Oxidative Phosphorylation. The coupling of the phosphorylation of ATP to oxidation, in the electron transport chain of the mitochondrial membrane (Brooks, Fahey, & Baldwin, 2005).

Oxidative stress. A state in which the production of ROS exceeds the capacity of the enzymatic and non-enzymatic antioxidant system, resulting in a disturbance in redox balance and cellular damage (Valko et al., 2007).

Radiation Therapy. The use of high energy irradiation to destroy malignant cancer cells. There are two general types of radiation: brachytherapy (internal) and teletherapy (external). Cancer cells are destroyed both by the radiation directly and the free radicals it produces (Hellman, 2005).

Reactive Oxygen and Nitrogen Species (ROS). Extremely unstable molecules containing an unpaired valence electron, also known as free radicals, which can cause damage to tissues by stealing electrons from nearby molecules (Halliwell & Gutteridge, 2007). ROS also act as important signaling molecules (Valko et al., 2007).

Superoxide. The most commonly produced free radical. It is an oxygen with an additional electron (Halliwell & Gutteridge, 2007). Most free radical production in healthy individuals occurs in the mitochondria, due to a "leakage" of electrons from 1-2% of the oxygen in oxidative phosphorylation (Boveris & Chance, 1973), which can form a superoxide radical ($O_2^{\bullet-}$).

CHAPTER II

REVIEW OF LITERATURE

Introduction

The primary purpose of this study was to determine the effects of a 10-week prescribed aerobic and resistance exercise intervention on plasma markers of oxidative stress and antioxidant enzyme capacity in cancer survivors versus a non-exercising, cancer control group. Markers of oxidative stress measured were 8-OHdG and reactive carbonyl derivatives. Trolox-equivalent antioxidant capacity was measured as a universal marker of antioxidant capacity in the blood. Additionally, selected physiological variables, including VO₂ peak, muscular strength, and fatigue were compared between groups. The relationship between oxidative stress and baseline physiological variables was determined. Similarly, the relationship between the change in oxidative stress and changes in physiological variables was determined. Baseline oxidative stress and antioxidant enzyme capacity in cancer patients was compared to a group of healthy individuals. The following literature review will include carcinogenesis, cancer treatments and their side effects, the effect that exercise has on cancer patients recovering from cancer treatment, and the role that oxidative stress plays in all of these processes.

Overview of Cancer

Cancer Incidence

Cancer is characterized by abnormal cell growth, resulting from a failure in the normal growth and repair processes in the cell. As of January 2012, 13.7 million cancer survivors were living in America, and more than 1.6 million new cases of cancer are expected to have been diagnosed in 2013, making cancer the second leading cause of death in the United States, next to cardiovascular disease (American Cancer Society, 2014). Fortunately, advancements in cancer detection and treatment methods have markedly improved the survival rate for most cancer types. A consequence of reduced mortality is an increase in the number of people living with the damaging side effects of cancer treatments, including surgery, radiation, and chemotherapy.

Normal Cell Growth and Division

The normal cell cycle involves two distinct phases, called *interphase* and *mitosis* (*M phase*) (Voet, Voet, & Pratt, 2008). During interphase, no cell division occurs, but normal growth and metabolism take place, whereas cell division occurs during mitosis. Interphase, which comprises the majority of the cell cycle, is divided into three events: *synthesis* (S phase), and two *gap phases* (G_1 and G_2). Additionally, during the G_0 phase, or postmitotic stage, the cell is quiescent, meaning that the cell is not actively dividing or preparing to divide. Depending on the type of cell and the current needs of the organism, the length of the G_0 phase varies, and may be permanent in cells that do not divide, including mature cardiac muscle and nerve cells.

Cells proceed through the cell cycle in response to numerous external stimuli, including growth factors and cellular space (Burke, 1996). Cell growth is also regulated

by a series of checkpoints that will arrest the cell cycle if certain conditions are not met (Voet et al., 2008). One checkpoint halts progression into mitosis until all cellular DNA has been replicated. Other checkpoints in G_1 and S arrests the cell cycle in response to damaged DNA to allow time for DNA repair. If the appropriate cellular conditions are not met after a period of time at a checkpoint, the cell will undergo apoptosis. These checkpoints are crucial to the preservation of an organism's DNA and thus the prevention of mutations, including those resulting in cancer development.

The cell can transition from the G_0 phase to the G_1 phase in response to external signals, such as apoptosis of an identical cell. During the G_1 phase, cells undergo rapid protein synthesis in preparation for ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) synthesis (Burke, 1996). Once initiated, the G_1 phase lasts approximately 12 to 14 hours before proceeding to the S phase (Langhorne et al., 2007). During the S phase, which typically lasts 7-20 hours, RNA and DNA are formed, as well as histones, the basic protein of chromatin. Two strands of the newly formed DNA, the body's genetic code, wrap themselves around the histones, forming a double helix structure. The strands form bonds to one another between their nucleotide bases in specific pairs; the purines, adenine (A) and guanine (G), bind respectively to the pyrimidines, thymine (T) and cytosine (C).

The G_2 phase follows the S phase, and lasts 1-12 hours (Burke, 1996). The cell further prepares itself for mitosis by continued RNA synthesis, and development of organelles and the mitotic spindle apparatus, where chromosomes are condensed. Additionally, proofreading and repair is performed by enzymes, DNA ligase and the DNA polymerases, which remove and replace nucleotides on the complementary strand

that are inappropriately matched with the original DNA strand (Guyton & Hall, 2000).

Mitosis (M phase) is the period of actual cell division, and is further divided into 4 phases: prophase, metaphase, anaphase, and telophase. During mitosis, the cell is divided into 2 daughter cells containing the same number and type of chromosomes as the original parent cell.

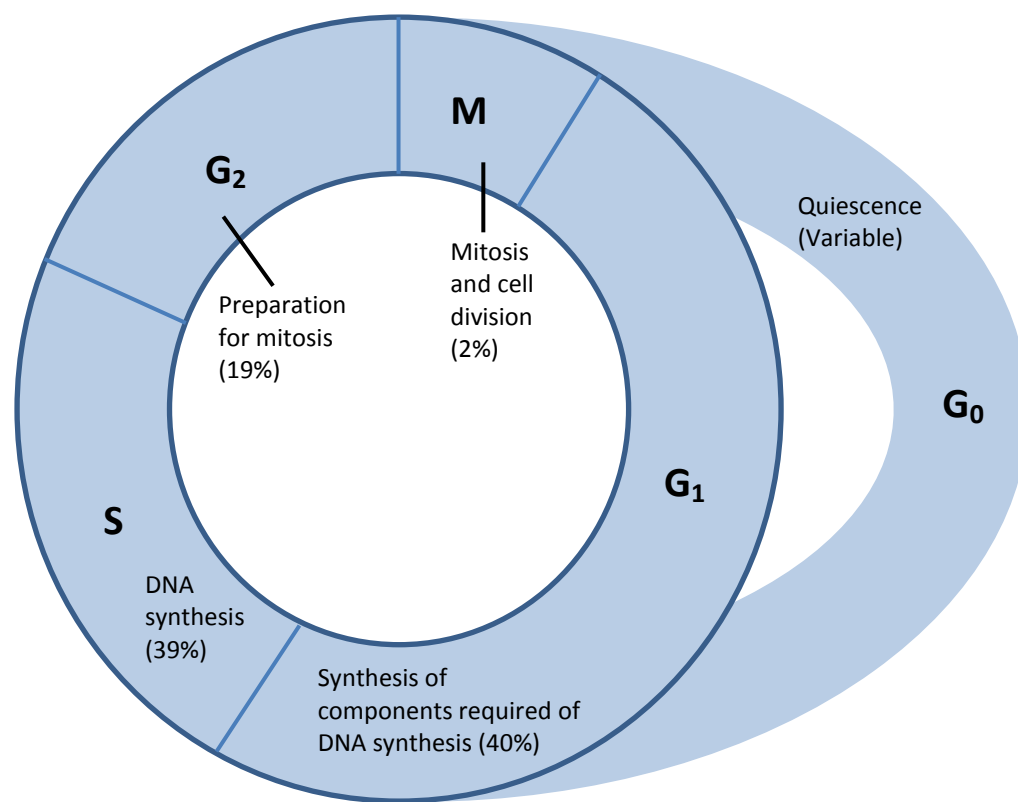


Figure 1. The cell cycle. Adapted from *Biochemistry*, By D. Voet, J.G. Voet, and C.W. Pratt, 2006. p. 1054. Copyright 2006 by John Wiley and Sons, Inc.

Cells within the late G₁ and early S phases are more susceptible to carcinogenesis (Ullrich, 2005). When a cell becomes cancerous, the cell cycle is completed more quickly, as the G₁ and G₂ phases are shortened, with a greater relative proportion of the

cell cycle engaged in cell proliferation (Voet et al., 2008). Rapidly proliferating cells are more sensitive to radiation and chemotherapy, as many therapies are most effective during the M phase, making cancer cells more susceptible to these treatments (Ullrich, 2005).

Carcinogenesis

Cancer cells are characterized by certain characteristics, including loss of proliferative control and capacity to differentiate, altered biochemical properties, chromosomal instability, and capacity to metastasize. Loss of proliferative control is the primary characteristic shared by all cancer cells. Normally, cell division is initiated by numerous factors, including *cyclins* and growth factors, and inhibited by others, such as lack of cellular space (contact inhibition) (Burke, 1996; Langhorne et al., 2007; Schneider et al., 2003). In a malignant cell, cell division continues even when the cellular environment does not favor proliferation, resulting in uncontrolled growth. Loss of capacity to differentiate indicates that cells do not attain the specific structural and functional characteristics of the tissue from which they are derived, as determined by their genetic coding (Langhorne et al., 2007). The degree of differentiation (well-differentiated, poorly-differentiated, or undifferentiated) is an important clinical indication of cancer progression. Altered biochemical properties allow for continued loss of proliferative control and differentiation, in addition to increased rates of anaerobic glycolysis, loss of cellular adhesiveness, and production of tumor-associated antigens and metabolic by-products, including hormone-like substances. Some of these metabolic alterations allow for some degree of biological and therapeutic targeting of cancer cells. Chromosomal instability is implicated in initiating carcinogenesis, but can also cause

continued cellular mutation, resulting in variable subpopulations of neoplasms within a given tumor, some of which may be particularly resistant to cancer therapy (Langhorne et al., 2007).

While the exact cause of most cancers is still undetermined (Pfeifer, 2007), there are several known processes and events which may need to act in concert in order to initiate carcinogenesis. The hypothesis that more than one insult, or “hit,” to a cell’s genes cause cancer is called the “multiple hit theory” (Schneider et al., 2003). Because the human body has numerous, redundant protective mechanisms against genetic alteration, more than one hit is necessary to overwhelm this elaborate defense system. Protective mechanisms include tumor suppressor genes (also known as antioncogenes), which, in the presence of DNA alteration, can inhibit growth, prevent adhesion of metastatic cells, repair DNA, or induce apoptosis, depending on the gene and biological circumstances (Langhorne et al., 2007). Possible insults to a cell include radiation (including solar), endogenous substances, (e.g., tobacco smoke, certain plastics, laboratory chemicals), lifestyle (e.g., obesity, alcohol consumption, stress level, lack of exercise, poor diet), and viruses (e.g., Human papilloma virus, Epstein-Barr, hepatitis B) (Schneider et al., 2003; Ullrich, 2005). Any material known to stimulate the development of cancer is known as a carcinogen. Carcinogens can cause genetic mutations in several ways, including DNA replication errors, elimination, insertion, or substitution of one of the components of the DNA strand.

Carcinogenesis is often described as occurring in two distinct events: *initiation* and *promotion*. Initiation is the mutational change that occurs when a carcinogen damages DNA by altering a given gene. If the normal repair processes are in place, no

cancer occurs. Otherwise, the cell may become permanently damaged, ultimately resulting in cellular death, or may develop into cancer in the presence of a cocarcinogen. Promotion describes the interaction of the cocarcinogens, or promoting agents, with the altered gene, resulting in damage to the proliferating mechanism of the cell (Langhorne et al., 2007). The introduction of an activator, followed by the introduction of a promoter, is necessary for the development of a tumor. A promoter does not mutate DNA or cause cancer itself, but rather interacts with the initiator to allow carcinogenesis to proceed. See figure 2. A final stage, *progression*, is sometimes described as the process of progressive advancement of the size and severity of the neoplasm.

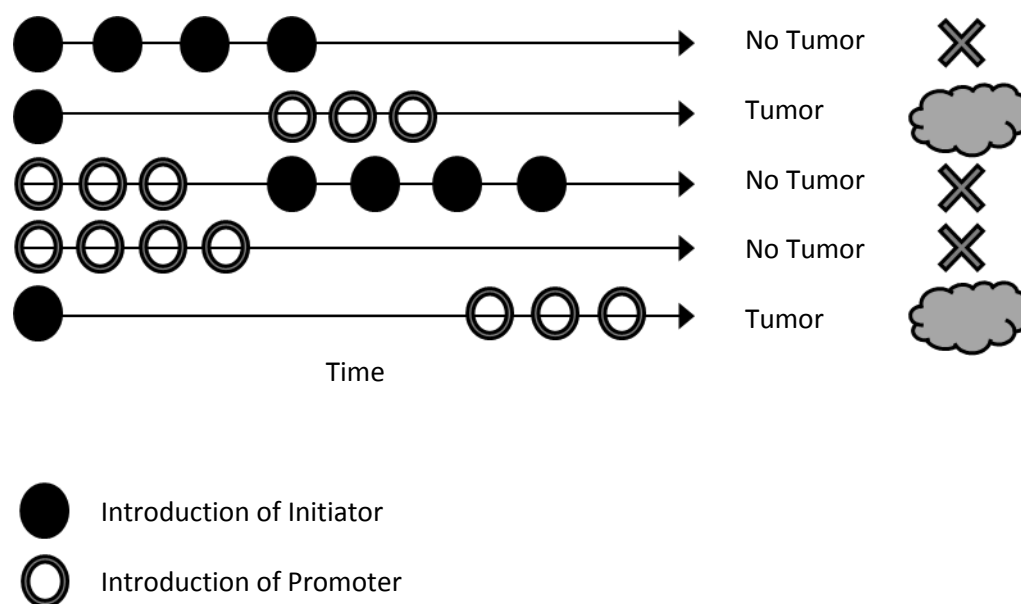


Figure 2. Initiation and Promotion. Adapted from *Oncology Nursing 5th Edition*, by M. Langhorne, 2007, p. 10.

Cancer Types

Although every type of cell in the body is capable of becoming cancerous, there are five broad classes of cancer. *Carcinomas* are solid tumors originating in epithelial cells, particularly in secretory tissues such as breast, prostate and pancreas (Schneider et al., 2003). Between 85% and 90% of all human cancers are carcinomas, making it by far the most common cancer. *Melanomas* are cancers originating in melanocytes, pigment-producing cells. These occur primarily in skin cells, but may occur elsewhere, including mucosal tissue and within the eye. *Sarcomas* are cancers that develop in bone, muscle, cartilage, fat or connective tissue and are fairly rare, accounting for less than 2% of all cancers. *Leukemia* is a cancer of blood-synthesizing organs, primarily the bone marrow, which results in abnormal leukocyte production. This type of cancer is diffuse, rather than a solid tumor, and therefore requires different treatment strategies than other types of cancer. *Lymphomas* are cancers of lymphoid tissue, and are broadly classified as Hodgkin's or Non-Hodgkin's, according to cell type, degree of differentiation, growth patterns, and type of reaction elicited by tumor cells (Langhorne et al., 2007). Although lymphoma originates in the lymphatic system and is classified as *hematological malignancy*, it presents as a solid tumor.

Cancer Stages and Grades

Most solid tumors are classified in severity using the TNM staging system. This system classifies tumors according to tumor size, the degree of spread to regional lymph nodes, and the presence or absence of distant metastases. Generally, stage I tumors are limited to the site of origin, stage II cancers have spread to local tissues, and stage III tumors have spread to lymph nodes and have extensive primary lesions and fixation to

deeper structures (Schneider et al., 2003). A cancer is considered to be stage IV when distinct metastases are present, regardless of other criteria. Refer to Table 1 on page 23. Cancer grading is another method of evaluating the severity of a cancer. The microscopic appearance of the cancer cells, as opposed to the tumor as a whole, is considered, and differentiation is the primary indicator of cancer grade.

Metastasis is the spread of cancer beyond the site of origin through veins, arteries and the lymphatic system. The ability to metastasize, the process in which cancer spreads from the tissue of origin to distant sites, is a trait unique to cancer and is another indicator of disease severity (Stetler-Stevenson & Kleiner, 2005). For metastasis to occur, cancer cells detach from a primary tumor due to increased motility, enter the circulatory system, and aggregate with blood borne elements (including other metastases), protecting them from destruction (Langhorne et al., 2007). The cells must then leave the circulation and penetrate the distant tissue (extravasation). Angiogenesis in the distant tissue provides blood to the metastatic lesion, supporting growth and malignancy. Less than 1% of metastases survive this process, and the longer they are in the circulatory system, the more likely they will be destroyed. Metastases can invade any type of tissue, regardless of the origin of cancer, but are common in local lymph nodes, lungs, bones, liver, and brain (National Cancer Institute, 2013). Metastatic spread of cancer is typically indicative of a more aggressive cancer, even compared to direct spread of cancer, which penetrate and destroy adjoining tissues. Often, metastases are microscopic and undetectable until further progression (Stetler-Stevenson & Kleiner, 2005). Because of the potential existence of tiny, undetected metastases, chemotherapy is often included in

a treatment regimen, even if the primary cancer has apparently been cleanly removed in entirety with surgery (DeVita, 2005).

Table 1

General Staging of the TNM System

Tumor stage	Tumor size	Lymph node status	Metastasis
I	< 2cm	None	None
II	2-5 cm	No, or on same side	None
III	> 5 cm	Yes, on same side	None
IV	Does not matter	Does not matter	Yes

Adapted from *Exercise and Cancer Recovery*, by Schneider et al., 2003, p. 87.

Cancer Treatment

Surgery

In addition to removal of cancerous tissue as a cancer treatment, surgery can be used to prevent or detect cancer. Certain precancerous conditions, such as moles and other skin anomalies, have a high risk of developing into malignant tissue. Removal of this tissue can therefore prevent the onset of cancer. Similarly, in individuals with a significant genetic risk of developing a certain type of cancer, as indicated by family history or genetic markers, may warrant prophylactic surgery to reduce the risk of cancer (DeVita, 2005). There are several surgical methods that may be employed to obtain tissue for the purpose of histological examination. Depending on the type of biopsy, varying degrees of tissue removal are required for analysis to determine if the tissue is cancerous, and to diagnose the stage of the cancer. Surgery, including open biopsies of the lungs or liver, may also be used to diagnose opportunistic infections following treatment-induced immune suppression (DeVita, 2005; Schneider et al., 2003).

Surgery may be used alone as cancer treatment, or in combination with adjuvant therapies, such as chemotherapy and radiation. The decision to include adjuvant therapy is largely determined by the extent of the primary lesion. When the tumor is confined to the site of origin, surgery alone is typically the preferred method (Rosenberg, 2005). The side effects of surgery alone are less damaging, and removal of the tumor can often permanently cure the patient of cancer. Because approximately 70% of patients presenting solid tumors already have micrometastases beyond the primary site, adjuvant therapy is often incorporated (DeVita, 2005). Beyond removal of the primary tumor mass, surgery may be used to remove metastatic tissue or tissue that is a risk of becoming metastatic (such as local lymph nodes), for reconstructive purposes, or for palliative purposes, such as removal of a tumor mass to improve comfort. The initial health of the patient is another determinant in cancer treatment (DeVita, 2005; Schneider et al., 2003). Very frail or elderly individuals may decide to only have surgery to remove the primary tumor burden to reduce the toxic side effects of radiation and chemotherapy, even if the cancer has already metastasized.

Radiation

Although long-term exposure to high energy irradiation can be carcinogenic, radiation therapy uses high energy irradiation to destroy malignant cancer cells. The objective of radiotherapy is to sterilize cancer cells so that they are incapable of unlimited proliferation (Hellman, 2005). There are two general types of radiation: brachytherapy (internal) and teletherapy (external). With both types, the target area is small, and therefore radiation is typically not used to treat metastatic cancers (Schneider et al., 2003). In brachytherapy, radioactive isotopes are placed within the body, near the tumor.

Placement of the implant is important in brachytherapy, because the dose of radiation is related to the inverse square of the distance from the radiation source (Hellman, 2005). Therefore, any cancerous tissue outside of a given radius may not be adequately affected by the radiation dose. In teletherapy, beams of radiation from outside the body are aimed at the target tissue. Dosage is determined both by intensity of the beam and exposure time.

Radiation-associated damage to biological systems spans a time scale of ~25 orders of magnitude (Bensasson, Land, & Truscott, 1993). In the physical stage (10^{-18} to 10^{-12} seconds), the radiation is actually absorbed (direct effect of radiation). In the chemical stage, (10^{-12} to 1 second), highly reactive excited states and reactive oxygen species are formed. In the biological stage (seconds to decades), the biochemical and cellular consequences of radiation are seen throughout the entire body. It was previously thought that the antitumor effects of radiation are due entirely to the direct effect of radiation on cell membranes and DNA, but it is now recognized that the production of free radicals is important as well (Bensasson et al., 1993). ROS react with tumor cell DNA, causing single or double strand breaks. The presence of cellular oxygen is important for the formation of ROS. Because tumors may be more poorly perfused than normal tissue, it is possible that radiation may damage healthy cells more than cancer cells. To improve ROS production within the tumor, hyperbaria in conjunction with radiotherapy has successfully improved clinical effectiveness (Kirk, Wingate, & Watson, 1976), but because this method is cumbersome, it is rarely used anymore. The formation of other cytotoxic agents following radiation may also facilitate the antitumor effect of radiation. These include numerous cytokines, such as tumor necrosis factor- α (TNF- α)

(Ralph et al., 2002), interleukins-1 and -6 (Hallahan, Haimovitz-Friedman, Kufe, Fuks, & Weichselbaum, 1993), and cell adhesion molecules (Hallahan, Kuchibhotla, & Wyble, 1996).

There are several possible effects of radiation on both normal and cancer cells: cellular death (either apoptosis or necrosis), accelerated senescence, terminal differentiation, or no or minor alterations in their divisional process (DeVita, 2005). Cells are most likely to be destroyed while proliferating, particularly in the M and G₂ phases, therefore making cancer cells more vulnerable to radiation than normal cells. Even so, normal cells are considerably damaged by radiation therapy. Local side effects of radiation therapy include pain, blistering, reduce range of motion, cell membrane damage, necrosis, fibrosis, fistula formation, and ulcerations (Allavena, Conroy, Aletti, Bey, & Lederlin, 1992; DeVita, 2005; Schneider et al., 2003). Whole body side effects include nausea, pain, fatigue, diarrhea, lung fibrosis, anemia, and cardiomyopathy. Local effects are typically associated with the radiation dose itself as well as cytotoxic agents and ROS, while whole body effects are associated mostly with cytotoxic agents and ROS production.

Chemotherapy

The use of chemotherapy is common, as more than half of cancer survivors have undergone treatment with at least one drug (Langhorne et al., 2007). Chemotherapy can be employed as the primary means of anti-cancer therapy (neoadjuvant therapy) to limit the spread of localized tumors, particularly in late stage cancers, or as adjuvant therapy to shrink tumors prior to radiation or surgery and destroy microscopic metastases following tumor removal (Chu & Devita, 2005). Chemotherapy is also the primary method of

combating hematological and metastatic cancers. There are numerous types of chemotherapy, with varying anti-cancer mechanisms, but typically the drugs damage or disable cells with abnormal growth cycles. The primary classes of chemotherapy drugs are alkylating agents, antimetabolites, antitumor antibiotics, and alkaloids. Almost universally, there is collateral damage to healthy cells, but less significantly than the damage to cancer cells. As in radiation therapy, chemotherapy can itself induce future cancer development, known as second cancers. (Van Leeuwen & Travis, 2005)

Typically, a chemotherapy regimen consists of drug administration every 2-4 weeks for 3-6 months. Administration methods can be oral (pill, liquid, capsule), intravenous (single venipuncture, catheter, port), intraperitoneal, or via spinal taps (Schneider et al., 2003). One challenge to the success of chemotherapy is the ability of malignant cells to adapt to a given drug, thereby becoming “drug resistant.” To combat drug resistance, as well as to increase the overall antitumor effectiveness and minimizing the toxicity of the regimen, two or more different drugs are often administered concomitantly (Kramer, Zakher, & Kim, 1988). Typical side effects of chemotherapy are whole-body and include fatigue, cachexia, anorexia, and nausea, as well as cardiac, endothelial, pulmonary, gastrointestinal, hepatic, and neuroendocrine dysfunction (Schneider et al., 2003). Local side effects usually are related to port implantation and acute pain and cellular damage at the site of administration (Langhorne et al., 2007). Treatment-related side effects not only affect quality of life and physical capacity, but can be dose-limiting, decreasing overall effectiveness and reducing a cancer patient’s survival time. Oxidative stress-induced side effects, particularly associated with anthracycline therapy, will be discussed in depth later.

Hormonal Therapy and Targeted Therapy

Hormonal therapy is most commonly used in cancers affecting primary or secondary sexual organs, including breast, prostate, and endometrial carcinomas. These cancers can be dependent upon binding of sex hormones to their receptors (if present) in cancer cells to continue growth. Hormonal therapy retains cancer cells in quiescence (G_1 or G_0), preventing continued growth (Sing-Hung, 2007). A common treatment used in breast cancer survivors is the anti-estrogen Tamoxifen, which may be used as an adjuvant treatment for as many as five years. Tamoxifen is used to treat metastases and to prevent recurrence (Schneider et al., 2003), as well as to prevent the onset of breast cancer in post-menopausal women with genetic risk or precancerous conditions. Aromatase inhibitors such as Arimidex® similarly decrease circulating levels of estrogen, but act upon the enzyme aromatase in the adrenal glands, reducing the endogenous production of hormones (Langhorne et al., 2007). Anti-androgens are used to target prostate cancer using a similar mechanism as anti-estrogens. Luteinizing hormone releasing hormone (LHRH) agonists such as goserelin acetate bind to LHRH receptors in the pituitary gland and inhibit luteinizing hormone production, in turn reducing the endogenous production of testosterone in males and estrogen in women. In advanced cases of breast or prostate cancer, or in very old cancer patients, hormone therapy is used alone as a palliative treatment by slowing or temporarily halting the growth of cancer without actually eliminating the tumor mass. Despite typically resulting in fewer and less severe side effects than radiation and chemotherapy, hormonal therapy is not without consequence, as Tamoxifen may result in post-menopausal symptoms and thromboembolic events (Sing-Hung, 2007). Tamoxifen has also been shown to induce oxidative stress (Ferlini et

al., 1999; Nazarewicz et al., 2007), and, like radiation and chemotherapy, the induction of ROS by Tamoxifen likely plays a role in its antitumor effects.

Much like hormonal therapy, targeted therapy exploits specific characteristics of cancer cells, in this case mutant kinases, which can be used to identify and attack a tumor (Sawyers, 2004). A well-known drug using this strategy is Herceptin (Trastuzumab), a monoclonal antibody that targets ErbB-2 (HER-2), a tyrosine kinase receptor that is involved in the development of breast cancer. These therapies have a relatively high response rate when appropriately used, but as of yet, only a fraction of human cancers contain known kinase-domain mutations. Another proposed form of targeted therapy alters the redox balance within a cancer cell, either increasing oxidative stress by inhibition of antioxidant (AOX) enzymes or induction of ROS production, or decreasing oxidative stress, by activation of AOX enzymes or inhibition of ROS production (J. Wang & Yi, 2008). These strategies will be discussed in a later section.

Immunotherapy

An alternate method of cancer treatment involves the use of cancer vaccines to stimulate the immune system to destroy cancer cells. Because the molecular composition of many tumor-associated antigens have been identified, it is possible to specifically target cancer cells. The effect is similar to an autoimmune disease, in which the immune system recognizes particular protein fragments and attacks these “self-antigens” (Dudley et al., 2002). The primary advantage of this method is the significant reduction in side effects, if used alone. Additionally, because the immune system is designed to protect the entire body, as various leukocytes circulate in the circulatory and lymphatic systems, immunotherapy would theoretically be an ideal method for destroying metastases remote

from the primary lesion. Unfortunately, the objective response rate to immunotherapy has, to date, been very low (2.6%) (Rosenberg, Yang, & Restifo, 2004).

Reactive Oxygen Species and Oxidative Stress

Reactive oxygen (and nitrogen) species (ROS / RNS) are extremely unstable molecules containing an unpaired valence electron (Halliwell & Gutteridge, 2007). Also known as free radicals, ROS can cause damage to tissues by stealing electrons from nearby molecules, creating a new radical and propagating a chain reaction. Such a chain reaction in lipid membranes can damage any cell, in a process called lipid peroxidation, but damage also occurs to other cellular components, including proteins, causing skeletal and cardiac muscle dysfunction, and nucleic acids, potentially resulting in DNA modification.

ROS are formed in all organisms, including healthy humans. Although oxygen is required for aerobic metabolism, and therefore human life, it can be toxic when it becomes a radical. The most commonly produced ROS are superoxide ($O_2^{\cdot-}$) and hydroxyl radicals ($OH\cdot$) (Halliwell & Gutteridge, 2007). The dot (\cdot) indicates that a molecule is a radical, with an unpaired electron. Additionally, non-radical oxygen derivatives such as hydrogen peroxide (H_2O_2), singlet oxygen (1O_2), lipid hydroperoxides (LOOH), and various other hydroperoxides (ROOH), although not truly radicals, are included in the ROS classification (Berlett & Stadtman, 1997). Most free radical production in healthy individuals occurs in the mitochondria, due to a “leakage” of electrons from 1-2% of the oxygen in oxidative phosphorylation (Boveris & Chance, 1973), which can form $O_2^{\cdot-}$. Because this original value was determined using isolated mitochondria in the presence of a non-physiological level of oxygen, this value may be

an overestimation (Vollaard, Shearman, & Cooper, 2005). Superoxide production in this manner occurs in complex I, but primarily occurs in complex III of the electron transport chain, as the unstable intermediate semiquinone anion can directly transfer electrons to molecular oxygen instead of reducing to ubiquinone (coenzyme Q) (Agostinelli & Seiler, 2006; Turrens, 1997). Because this process is non-enzymatic, a higher flux of oxygen through the mitochondria typically results in greater $O_2^{\bullet -}$ production.

ROS production is an important element of biological defense against pathogens and toxins. Free radical production occurs in leukocytes, which create superoxide to destroy pathogens, in what is known as a respiratory burst. NAD(P)H oxidase catalyzes the reaction ($NADPH + 2O_2 \leftrightarrow NADP^+ + O_2^{\bullet -} + H^+$). A downstream reaction of the respiratory burst catalyzed by myeloperoxidase creates hypochlorous acid, a highly reactive oxidant, from H_2O_2 and a chloride ion (Babior, 2000). The cytochrome P450 system in the endoplasmic reticulum, which plays diverse roles metabolizing steroids, fat-soluble vitamins, fatty acids, eicosanoids, drugs, and carcinogens, is another source of ROS in healthy individuals via a NADPH mechanism (Symons & King, 2003).

Oxidative stress produced by the P450 system have been found to mediate the chemical toxicity of certain drugs, including alcohol and acetaminophen (Gonzalez, 2005)

Cyclooxygenase (COX) (Armstead, 2001) and xanthine oxygenase (XO) are further pro-oxidant enzymes known to produce superoxide in both healthy and diseased individuals. XO, like NAD(P)H, is expressed phagocytic cells, but also plays a role innate immune function as it is expressed on the epithelial surface (Vorbach et al., 2003). Xanthine oxidoreductase has two forms: xanthine reductase, which creates the antioxidant uric acid, and xanthine oxidase, a pro-oxidant enzyme. Xanthine reductase is

constitutively expressed, but is rapidly converted to xanthine oxidase (Vorbach et al., 2003). Similarly, COX-2 is not constitutively expressed, but is highly inducible (Chan, 2001). These tightly regulated pro-oxidant enzymes illustrate the importance of ROS creation in healthy individuals.

Superoxide can dismutate spontaneously or enzymatically, producing hydrogen peroxide. Alternately, superoxide can reduce ferric iron(III) to ferrous(II) ($\text{Fe}^{3+} + \text{O}_2^{\cdot-} \rightarrow \text{Fe}^{2+} + \text{O}_2$) (Halliwell & Gutteridge, 2007). In another reaction known as the Fenton reaction, ferrous iron(II) is oxidized by hydrogen peroxide, creating ferric iron(III), a hydroxyl anion, and a hydroxyl radical ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^{\cdot}$). The overall reaction ($\text{O}_2^{\cdot-} + \text{H}_2\text{O}_2 \rightarrow \text{OH}^- + \text{OH}^{\cdot} + \text{O}_2$), known as the Haber-Weiss reaction, therefore creates hydroxyl radicals from superoxide and hydrogen peroxide. Fortunately, in healthy individuals, ferrous iron is not easily oxidized, as it is bound tightly in organic complexes, but certain metabolic and disease states can make iron accumulate. Hydroxyl radicals are extremely biologically reactive and are particularly damaging because there is no enzymatic reaction that can eliminate OH^{\cdot} (Manda, Nechifor, & Neagu, 2009). Therefore, the only defenses against cellular damage from OH^{\cdot} are effective repair systems, or preventing their formation to begin with.

Another radical, nitric oxide (NO^{\cdot}), is produced primarily by NO synthase in endothelial cells (eNOS), but has recently been shown to be produced in mitochondria (Giulivi, Poderoso, & Boveris, 1998). Although NO^{\cdot} is more commonly known as a signaling molecule, it is capable of reacting with $\text{O}_2^{\cdot-}$ to form peroxynitrite (ONOO^-), another highly reactive oxidant. Peroxynitrite is more stable than either NO or superoxide, and is capable of further alteration, forming strong oxidants including

hydroxyl-like and nitrogen dioxide radicals (Kojda & Harrison, 1999). Beyond the damaging effects of peroxynitrite, inactivation of nitric oxide by superoxide is likely a contributor of endothelial dysfunction. In much of the literature, reactive oxygen and nitrogen species (RONS) are discussed as a single entity and in this manuscript will be referred to, generally, as “ROS” unless otherwise specified.

Antioxidant Defense

Because ROS are continuously formed in healthy individuals, an antioxidant defense system is in place to counteract normally produced free radicals. Antioxidants are molecules that inhibit free radical reactions, typically by acting as a reducing agent by donating an electron and ceasing the oxidative chain reaction. Enzymatic antioxidants, including superoxide dismutases (SOD), catalases, glutathione peroxidases, thioredoxins, and lactoperoxidases, are central to this system (Leffler, 1993). SOD, found in cytosol, mitochondria, and extracellular spaces, catalyzes the dismutation of superoxide into hydrogen peroxide and oxygen ($2 O_2^{\cdot -} + 2 H^+ \rightarrow H_2O_2 + O_2$) (Baskin & Salem, 1997). While hydrogen peroxide is significantly less destructive than superoxide, it is still a harmful toxin, which can be transformed into water by glutathione peroxidase ($2 H_2O_2 + 2 GSH \rightarrow GSSG + 2 H_2O$) or catalase ($2 H_2O_2 \rightarrow 2 H_2O + O_2$). If SOD is active, but not supported by catalase and glutathione peroxidase, the net result may be enhanced oxidative stress, as H_2O_2 is more reactive than $O_2^{\cdot -}$ (Manda et al., 2009). Non-enzymatic antioxidants such as ascorbic acid (vitamin C), α -tocopherol (vitamin E), flavonoids, carotenoids, glutathione (GSH) are an important element of the mammalian antioxidant system (Halliwell & Gutteridge, 2007). These antioxidants are typically exogenously derived and can interact with dangerous free radicals and create less reactive radicals.

There is evidence that some degree of exogenously-derived, non-enzymatic antioxidants are sequestered, and released during periods of increased ROS production (Mastaloudis, Leonard, & Traber, 2001). Endogenous non-enzymatic antioxidants include proteins like albumin and ferritin, and metabolites, including uric acid, bilirubin and pyruvate, can also effectively act as free radical buffers, preventing damage to more important molecules, namely cellular elements (Manda et al., 2009).

The antioxidant defense system is capable of modification, primarily in response to the production of some degree of oxidative stress. The concept of *hormesis* describes a beneficial physiological response or adaptation to a mildly damaging stimulus (Ji et al., 2006). The adaptive response to exercise is the most well-known example of hormesis, and it has been suggested that a similar such effect occurs in response to oxidative stress. Indeed, many of the beneficial adaptations that occur with chronic exercise may be partially attributed to the presence of ROS during and follow exercise, which may act as signaling molecules (Ji, 2008; Powers et al., 2009). The modulation of antioxidant defense in response to exercise training is a paradigmatic example of this hormetic response and will be discussed more in depth later. The human antioxidant defense system can respond to acute and chronic exposure to ROS in the absence of exercise as well. Acute (Sasaki, Akamatsu, & Horio, 1997) and chronic (Iizawa, Kato, Tagami, Akamatsu, & Niwa, 1994) UVB radiation exposure has been shown to increase SOD activity in human keratinocytes. Similarly, diabetic pancreatic β -cells can increase their antioxidant defense (overexpression of numerous antioxidant enzymes) compared to normal β -cells due to their chronically elevated ROS production (Lacraz et al., 2009).

Oxidative Stress and Markers of Oxidative Stress

When the generation of reactive oxygen and nitrogen species exceed cellular adaptive and repair capacities, it is known as oxidative stress (Valko 2007). This occurs when acute production of ROS and/or RNS is higher than normal, due to some physiological or environmental stress, when the antioxidant enzyme system is compromised, or both. If the antioxidant defense system is in place, ROS produced during physiological or pathophysiological processes are never given the chance to cause damage, and redox balance is maintained. Reductive stress is an alternate pathological state, in which antioxidant activity, either endogenous or exogenous, far outstrips ROS production, resulting in redox imbalance (Rajasekaran et al., 2007).

As previously mentioned, proteins, fats and nucleotides are all potential sites of oxidative damage. Due to the extremely transient nature of reactive oxygen species, it is difficult to perform a chemical analysis of ROS themselves. Rather, downstream products of oxidative stress in blood and tissues may be analyzed via biochemical analysis. An exception to this is hydrogen peroxide, which is radical-producing, but not a radical itself, making it stable enough to measure directly (Bloomer, 2008). Perhaps the most commonly used marker of oxidative stress is malondialdehyde (MDA), a stable end product of lipid peroxidation. Because lipid peroxidation occurs in lipid membranes, all cells are potentially targets of lipid peroxidation, making MDA a fairly good marker of total body oxidative stress. MDA accumulation in tissue or plasma can be measured directly, or with a thiobarbituric acid reactive substances (TBARS) assay, in which thiobarbituric acid reacts with MDA to produce a fluorescent product (Vasankari, Kujala, Heinonen, Kapanen, & Ahotupa, 1995). Other markers of lipid peroxidation include 4-

hydroxynonenal (4-HNE) and F₂ isoprostanes such as 8-iso-Prostaglandin F₂alpha (8-isoprostane). MDA itself is capable of further reactivity with nucleic acids, including DNA, which may be possibly mutagenic (Marnett, 1999). A more common pathway to DNA damage is by direct modification via superoxide and peroxynitrite. 8-Hydroxy-2'-deoxyguanosine (8-OHdG), a fairly ubiquitous byproduct of oxidative DNA damage, is the primary biomarker for DNA oxidation and can be assessed in urine, serum, cerebrospinal fluid, cells or tissues.

Carbonyl derivatives of proline, lysine, arginine and threonine are the most common oxidation products of protein, and can be assayed in plasma, serum, cell lysates or purified proteins (Paromov, Qui, Yang, Smith, & Stone, 2008). Similarly, advanced oxidation protein products (AOPP) are makers of protein oxidation created during oxidative stress through the reaction of plasma proteins with chlorinated oxidants such as hypochlorous acid (Marsche et al., 2009; Witko-Sarsat et al., 1996). Although AOPP are most commonly used clinically to screen for chronic diseases such as renal complications, diabetes mellitus, and atherosclerosis, they are adequate markers of general oxidative stress. 3-Nitrotyrosine, an end product of peroxynitrite reacting with tyrosine, while theoretically could be used as a marker of protein oxidation, is in reality used as a marker of peroxynitrite, and therefore nitrative damage.

A more direct measurement of ROS involves the use of florescent probes or spin traps, which directly capture intracellularly produced radicals and convert them into more stable molecules (Powers & Jackson, 2008). While this method allows for determination of ROS production in different tissues and cellular compartments, there are disadvantages beyond the complicated and invasive nature of the procedure. An increase in ROS

production alone does not indicate a state of oxidative stress, as an increased activity of the antioxidant system may maintain redox balance. Additionally, microprobes and spin traps may disturb the intracellular environment, altering ROS production (Halliwell & Gutteridge, 2007).

Another method of determining redox status in living systems is measurement of antioxidant enzyme activity, and non-enzymatic antioxidant concentrations. Decreased enzymatic antioxidant capacity indicates poor ability to handle an increased ROS load, but does not actually indicate the level of ROS production. Commonly measured antioxidant enzymes include superoxide dismutase, glutathione peroxidase, and catalase (Finaud et al., 2006). Alternately, a measured reduction in non-enzymatic antioxidants, such as glutathione, can indicate a chronic overproduction of ROS. In particular, ratio of reduced glutathione to oxidized glutathione (GSH:GSSG) is a commonly used determinant of oxidative stress (Serru et al., 2001). In normal, healthy individuals, the GSH/GSSG ratio is approximately 10:1, but during chronic oxidative stress, GSH is depleted by the action of glutathione peroxidase (Ji, Fu, & Mitchell, 1992; Serru et al., 2001). Total antioxidant capacity of a tissue or blood sample can be determined, which, while lacking specificity, may be a more practical assessment of an individual's redox status. Several methods of determining total antioxidant capacity, including the Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), and ferric reducing ability of plasma (FRAP) assays, compare the antioxidant power of a given sample (e.g., blood, tissue, food items) to a standard antioxidant, Trolox (Wang et al., 2004). This is often a practical method of determining redox status because the interrelationship between all enzymatic and non-enzymatic antioxidants, and various

support mechanisms such as transporters, is complex and complimentary, meaning that the expression of any single antioxidant may be misinterpreted. For example, the overexpression of superoxide dismutase without a similar rise in catalase expression has been shown to result in increased rather than decreased oxidative stress (McCord, 1993).

Reactive Oxygen Species as Signaling Molecules

For years, ROS production was thought to be simply an unavoidable, damaging by-product of working muscles during exercise. It had been long assumed that exercise acutely induces oxidative stress simply because of the significant (100 to 200-fold) increase in oxygen flux through the mitochondria, thereby increasing electron leak and superoxide formation (Clanton, Zuo, & Klawitter, 1999). This was seemingly supported by evidence of hyperoxia-induced ROS production, even in the absence of skeletal muscle contraction (Flandin, Donati, Barazzone-Argiroffo, & Muzzin, 2005). This notion was countered by the finding that hypoxia can also increase ROS production (Bailey, 2001; Waypa et al., 2010). High altitude exposure similarly induces oxidative stress, which may be implicated in acute mountain sickness (Bailey, Davies, Young, Hullin, & Seddon, 2001; Bailey et al., 2009; Dosek, Ohno, Acs, Taylor, & Radak, 2007). One explanation offered to explain these paradoxical effects was that the reduced mitochondrial oxygen saturation (pO_2) during exercise may cause ROS production (Bailey, 2001). Mitochondria may act as oxygen sensors by inducing ROS in response to low pO_2 , which in turn mediate transcriptional changes implicated in adaptation to hypoxia and aerobic exercise (Brunelle et al., 2005; Chandel et al., 1998; Chandel et al., 2000; Emerling et al., 2005).

It is now recognized that ROS production by contracting muscle plays an important role in the adaptive response to exercise (Jackson, 2005, 2009a; Powers et al., 2009). Nitric oxide (first called endothelium-derived relaxing factor) has long been recognized for its ability to vasodilate. When the NO-producing enzyme eNOS (endothelial nitric oxide synthase) is downregulated or eliminated, vascular dilation is prevented. (Fleming & Busse, 1999). Oxidative stress may actually mediate this, as superoxide reacts readily with NO, producing peroxynitrite. Only recently were alternate ROS identified as direct cellular signaling molecules. Low concentrations of ROS are important for cellular signaling (Zhang 2007). For instance, low levels of ROS are apparently crucial for normal force production, and a slight increase in ROS generation causes an increase in force production (Reid, 2001). When higher levels of ROS are present, a subsequent reduction in force production occurs, partially explaining the development of fatigue in response to submaximal contractions (Reid, Khawli, & Moody, 1993). Modification of contractile function may be due to ROS exposure to redox-sensitive, sulfhydryl groups of myofibrillar proteins, and phosphatases and kinases of multiple elements of the contractile system (Jackson, 2009a; Powers & Jackson, 2008), including SERCA (Smith & Reid, 2006), ryanodine receptors (Zima & Blatter, 2006), and myosin heavy chains (Powers et al., 1994).

Recently, the role of ROS as a mediator in numerous signaling pathways involved in growth, differentiation, proliferation and apoptosis has gained attention (Ji, 2008). Among these are the mitogen-activated protein kinase (MAP-K), nuclear factor (NF) κ B, activator protein 1 (AP-1) heat shock proteins, p53, and the p38 cascade. One primary result of ROS activation of the MAP-K and NF κ B pathways is increased expression of

antioxidant enzymes (Allen & Tresini, 2000), so that with chronic ROS exposure, antioxidant enzyme activity increases (Hammeren et al., 1992; Powers, Ji, & Leeuwenburgh, 1999; Witt, Reznick, Viguie, Starke-Reed, & Packer, 1992). ROS stimulated MAP-K activation can also acutely trigger glucose uptake in skeletal muscle, both during muscle contraction (Sandstrom et al., 2006), and independent of active contraction, (Chambers, Moylan, Smith, Goodyear, & Reid, 2009) indicating a metabolic signaling role of ROS. Because these signaling cascades are implicated in numerous acute and chronic responses to exercise, including muscular hypertrophy, angiogenesis, and vascular adaptation (Ji, 2008), it is possible that these too may be ROS dependent to some degree.

The precise redox balance of a healthy cell, in response to signaling cascades, exercise, and exogenous stimuli, has a potent and sometimes counterintuitive effect. For instance, a hormetic response to ROS in skeletal muscle adaptation is apparent, as ROS signaling likely contributes both to muscle fiber adaptation following contractile activity, as well as prolonged muscle disuse (Powers et al., 2009). This seemingly contradictory interaction is likely due to the time course and magnitude of ROS generation. Exercise is a relatively short (minutes to hours) and moderate stimulus for ROS production, facilitating cellular adaptation and protection against future insult, while chronic, high ROS production promotes cellular damage and death, as show in Table 2.

Table 2

ROS Dose and Potential Health Effects

ROS dose	Causes	Benefits	Detriments
Acute extreme ROS (oxidative stress)	Radiation, Chemotherapy	-Apoptosis/necrosis of cancer cells	-Apoptosis/necrosis of normal, healthy cells -Tissue damage
Chronic continuous ROS (oxidative stress)	Sedentary lifestyle, Poor diet, smoking, UV exposure, air pollution, Chronic disease, inflammation		-Carcinogenic -Induction of further chronic disease
Transient, moderate ROS (redox balance)	-Exercise -Caloric Restriction	-Cellular signaling -Cellular defense (respiratory burst) -Mediates hormetic changes associated with exercise (Including ↑ AOX enzymes, ↑ insulin sensitivity, muscular adaptations)	
Suboptimal ROS (reductive stress)	-Endogenous AOX imbalance -exogenous AOX overdose		-Mimics oxidative stress Reduced capacity for cellular defense and signaling - -Inhibition of cellular and systemic adaptation in response to stimuli (e.g. exercise)

Note: ROS production and degradation must be matched for optimal healthy and physiological functioning.

The complex interaction between the so-called “tumor suppressor protein,” p53, ROS, and antioxidant enzymes illustrates the intricate interrelationship between cellular signaling, oxidative stress, and cancer etiology, pathology, and prevention. (Y. Chen et al., 2007; Hussain et al., 2004; Liu et al., 2008; Tu et al., 2009). ROS act as both upstream mediators of p53 signaling, as well as downstream second messengers of p53-regulated apoptosis. Physiological levels of p53 help maintain cellular redox balance, and this may be one of the tumor-suppressing mechanisms of p53. Alternately, both hypo- and hyper-physiological levels of p53 result in ROS generation via antioxidant suppression and/or prooxidant activation (Liu et al., 2008). Although p53 is known to induce antioxidant enzymes, overexpression of p53 may increase cellular MnSOD and glutathione peroxidase (GPX), but not catalase, potentially resulting in inadequate removal of H₂O₂ (Hussain et al., 2004). In response to stressors, including oxidative stress, a small proportion (~2%) of cellular p53 is translocated to the mitochondria, signaling apoptosis (Marchenko, Zaika, & Moll, 2000). In this way, in response to mild oxidative stress, p53 protects the mitochondria genome (Liu et al., 2008). Similarly, in response to chemotherapeutic agents, ROS-activated p53 activity leads to apoptosis (Ueno et al., 1999). When the degree of oxidative stress is more severe, the DNA-binding activity of p53 is abolished. Because p53 is pivotal for most DNA-damage-induced apoptosis, and p53 mutations are found in 70% of human cancers, its dysregulation, either via ROS or other means, is implicated in cancer initiation (Brosh & Rotter, 2009). Apoptotic dysregulation can additionally make these cancer cells resistant to low dose radiation, as it depends on cancer apoptosis as its mechanism of action.

Like with p53, ROS have been shown to act both upstream and downstream of inflammatory cytokines. Tumor necrosis factor (TNF)- α , interleukin-1 β (IL-1 β), and interferon- γ (IFN- γ), elicit mitochondrial ROS production (Yang et al., 2007), yet ROS have been shown to induce production of macrophage inflammatory protein-2 (MIP-2), tumor necrosis factor- α (TNF- α) and interleukin-6, (IL-6) (Naha, Davoren, Lyng, & Byrne, 2010). Further demonstrating their diverse role in cellular signaling and maintenance, ROS are known to be the second messengers responsible for regulation of angiogenesis and tumor growth through vascular endothelial growth factor VEGF (C. Xia et al., 2007), and they act as the initial signal for heat shock protein (HSP) 72 induction, which in turn are molecular chaperones capable of cellular protection (Tang et al., 2007).

ROS Production with Acute and Chronic Exercise

Contractile activity of skeletal muscle has been known to produce ROS since 1978, when Dillard et al. demonstrated that lipid peroxidation occurs during exercise (Dillard, Litov, Savin, Dumelin, & Tappel, 1978). Since then, more than 300 articles have directly investigated the effect of acute exercise on oxidative stress (Fisher-Wellman & Bloomer, 2009). An early study reported that MDA increased in subjects subjected to a graded exercise test to exhaustion, but not in those who exercised at 70% of VO₂ max or less (Lovlin, Cottle, Pyke, Kavanagh, & Belcastro, 1987). This helped illustrate the dose-dependent nature of exercise-induced oxidative stress; a concept already hypothesized because of increased flux through the electron transport chain, and therefore increased electron leakage. Similarly, there was a significantly greater concentration of plasma protein carbonyls following 120 minutes of steady state exercise compared to 30 and 60 minutes of exercise at the same relative intensity (Bloomer,

Davis, Consitt, & Wideman, 2007). Single bouts of exercise have been shown to alter thiobarbituric acid reactive substances (TBARS), a marker of malondialdehyde and phospholipid peroxidation (Radak et al., 1999), reactive carbonyl derivatives, a marker of amino acid modification (Radak et al., 1998), and 8-hydroxydeoxyguanosine (8-OHdG), a marker of DNA mutation (Asami et al., 1998; Niess, Hartmann, Grunert-Fuchs, Poch, & Speit, 1996). Exercise can decrease the ratio of glutathione (GSH) to glutathione disulfide (GSSD), indicating antioxidant depletion in response to oxidative stress (Vollaard et al., 2005). As will be discussed later, transient, post-exercise oxidative stress is a potent stimulus for increasing antioxidant enzyme activity, and ultimately confers resistance to future ROS exposure.

Chronic exercise is also capable of measurably increasing markers of oxidative stress. With chronic exercise, overtraining seems to induce oxidative stress, whereas moderate exercise can reduce resting and post exercise ROS production (Finaud et al., 2006; Ji, 2002). Excessive aerobic exercise, such as 30km/day for 8 days (Okamura et al., 1997) and 10 hours/day for 30 days (Poulsen, Loft, & Vistisen, 1996) drastically increased urinary 8-OHdG levels, even to a greater extent than intense, acute bouts of exercise. Individuals with a compromised antioxidant defenses, such as cancer patients during and following treatment, have a much lower threshold for such exercise-induced, chronic oxidative stress.

While electron leak is clearly a primary source of ROS production, more recently, alternate mechanisms of exercise-induced ROS have been proposed, supporting the notion that ROS production is not simply a byproduct, but rather a tightly regulated signaling system. Isometric contraction is capable of generating ROS, despite a

negligible increase in mitochondrial oxygen flux (Alessio et al., 2000). More recently, it was shown that mechanical loading (stretch) of isolated myotubes induced ROS production, despite no change in oxygen flux (Chambers et al., 2009). The transverse tubule NADPH oxidase produces superoxide in response to depolarization, stimulating calcium release for muscle contraction (Espinosa et al., 2006; Hidalgo, Sanchez, Barrientos, & Aracena-Parks, 2006), indicating superoxide's vital signaling role in muscular function, and an alternate pathway for exercise induced ROS production. Similarly, NADPH oxidases of plasma membranes (Javesghani, Magder, Barreiro, Quinn, & Hussain, 2002), as well as cardiac (Cherednichenko et al., 2004) and skeletal (R. Xia, Webb, Gnall, Cutler, & Abramson, 2003) sarcoplasmic reticula may be implicated in exercise-induced ROS production. Furthermore, following intense exercise and muscular damage, phagocytic cells typically invade the area and contribute to the total exercise-associated ROS production (Malech & Gallin, 1987). ROS production in this manner can be significant enough to damage tissue that had been undamaged by the exercise dose (Zerba, Komorowski, & Faulkner, 1990). Another consideration when exercising outside is exposure to airborne pollutants that may directly or indirectly increase oxidative stress (O'Neill et al., 1995). Alternate mechanisms may exist, but have not been elucidated as of yet. For instance, the mitochondria from fast, type II muscle fibers of rats have a greater inherent propensity to produce ROS (Anderson & Neuffer, 2006). Interestingly, oxidative, type I muscle fibers still have higher antioxidant enzyme capacity (Ji et al., 1992).

Exercise Increases Antioxidant Capacity

Numerous studies indicate increased antioxidant enzyme activity (SOD, GPx, CAT) in skeletal and cardiac muscle tissues and blood following endurance training in animals and healthy humans (Finaud et al., 2006; Ji, 2002). While an acute bout of exercise can transiently activate MnSOD gene transcription, substantial MnSOD upregulation may require more chronic stimulation, or in other words, training (Hollander et al., 1999). Aerobic training, in particular, has elicited augmentation of antioxidant defense. Accumulation of 8-OHdG was significantly less in exercise trained (9wks swimming) rats than age matched controls (Radak et al., 1999). Similarly, F₂-isoprostane levels (a measure of lipid peroxidation) decreased 34% in premenopausal women following 15 weeks of aerobic exercise training (Schmitz et al., 2008). Surprisingly, there is also evidence for mobilization of non-enzymatic antioxidants (vitamins C and E) from body reserves during an exercise bout (Mastaloudis et al., 2001), but the plasticity of this mechanism in response to training has not been characterized.

Regardless of the mechanism, acute exercise is clearly a potent mediator of ROS formation. As discussed, these ROS are necessary signaling molecules, orchestrating changes in muscle function, glucose uptake, and endothelial function. While exhaustive exercise may result in transient oxidative stress, moderate levels of oxidative stress can have a hormetic effect on antioxidant enzymes (Ji et al., 2006), imparting individual tissues and the organism as a whole with a greater capacity to tolerate a serious oxidative insult, such as ischemia-reperfusion injury, radiation, or chemotherapy.

The Role of ROS in Aging and Diseases

Increased baseline oxidative stress in cancer patients may precipitate cancer-related anorexia/cachexia (Mantovani et al., 2004). Specifically, cachexia has been associated with elevated levels of muscle malondialdehyde, a marker of lipid peroxidation and total body oxidative stress (Laviano et al., 2007). The mechanism behind this may be an increase in protein degradation via the ubiquitin-proteasome pathway upregulation in response to cancer or treatment-associated oxidative stress. There is evidence indicating increased life span associated with calorie restriction (CR) in many organisms, including rodents (Barger, Walford, & Weindruch, 2003; Masoro, 1988) primates, and humans (Cruzen & Colman, 2009; Ristow & Zarse, 2010), possibly due to reduced free radical generation and increased antioxidant enzyme activity. Ristow & Zarse, (2010) suggest an effect of mitochondrial hormesis (mitohormesis) in response to both CR and exercise, in which exposure low level stress (ROS and otherwise) within the mitochondria increases resistance to stress ameliorates oxidative stress which may increase lifespan. In elderly rats (34 mo), a 50% loss of muscle mass occurred in hind limb muscles but this was countered by CR (Weindruch, 1995). Caloric restriction resulted in significantly increased GPX and CAT activity. Reactive oxygen species may also contribute to sarcopenia and muscle aging, likely due to the combined effect of premature cellular senescence, dysfunctional sarcoplasmic reticuli, and reduced regenerative capacity (Fulle et al., 2004). Increased serum protein carbonyls were associated with decreased grip strength in elderly women (mean age, 77.4 yrs), suggesting that oxidative damage to contractile proteins may directly reduce strength and therefore functional capacity (Howard et al., 2007).

Although a low level of ROS production (“redox tone”) may be crucial for contractile function at rest, increased ROS production results in oxidative stress and decreased contractile function. Whereas antioxidant exposure at *rest* results in net *reduction* in force development in response to electric stimulation (Reid et al., 1993), during fatiguing stimulations, treatment with the superoxide scavenger, Tiron, *augmented* force generation (Mohanraj, Merola, Wright, & Clanton, 1998). Oxidative stress can also alter skeletal muscle function by modifying ryanodine receptors and the calcium pump in SR membranes (Kagan, Ritov, Gorbunov, Menshikova, & Salama, 1998), specifically by oxidation of critical channel proteins in the presence of high hydrogen peroxide levels (Brotto & Nosek, 1996). Reactive oxygen species, therefore, may alter Ca^{++} movement in and out of the cell, resulting in some degree of excitation-contraction uncoupling with aging (Renganathan, Messi, & Delbono, 1997). Caloric restriction can preserve the mechanical properties of hind-limb skeletal muscle in elderly rats, and is associated with increased ryanodine receptor and dihydropyridine receptor activity (Mayhew, Renganathan, & Delbono, 1998), possibly due to reduced oxidative damage.

Beyond its interrelationship with cancer (which will be discussed in full later), oxidative stress has been implicated in the development or progression of numerous chronic diseases, including diabetes (Houstis, Rosen, & Lander, 2006; Kojda & Harrison, 1999; Rudich et al., 1998; Wu, Chiou, Chang, & Wu, 2004), hypertension (Kojda & Harrison, 1999), vascular disease (Kotur-Stevuljevic et al., 2007), cachexia (Mantovani et al., 2004; McClung, Judge, Powers, & Yan, 2010), neurodegeneration (Pope, Land, & Heales, 2008), cartilage degradation (Henrotin, Kurz, & Aigner, 2005), Parkinson’s disease (Bloomer et al., 2008; Serra et al., 2001), and chronic heart failure (Eleuteri et al.,

2009; Kojda & Harrison, 1999). Free radical damage is well recognized as a fundamental mechanism of ischemia-reperfusion injury (Chan, 2001; Omar et al., 1990; Powers et al., 1998). Some infectious diseases, such as influenza, *Helicobacter pylori*, and HIV, exert their cytotoxicity via oxidative stress (Mates & Sanchez-Jimenez, 1999). Additionally, generalized phenomena such as aging (Berlett & Stadtman, 1997; Finkel & Holbrook, 2000; Harman, 1956; Jackson, 2009b) fatigue, (Fulle et al., 2000; Richards, Roberts, McGregor, Dunstan, & Butt, 2000; Richards, Wang, & Jelinek, 2007), muscle weakness (Powers & Jackson, 2008; Smith & Reid, 2006) and muscle wasting (Moylan & Reid, 2007) are commonly attributed, at least in part, to oxidative stress.

Dietary and Other Exogenous Antioxidants

Dietary supplementation of antioxidants has been shown to prevent some of the health promoting effects of exercise. Although chronic, continuous ROS exposure is associated with insulin resistance (Houstis et al., 2006; Rudich et al., 1998), exercise training, is well known to improve insulin sensitivity (Duncan et al., 2003; James, Kraegen, & Chisholm, 1984). Ristow et al. (2009) demonstrated that the exercise-induced ROS may be essential to mediate these changes. Both trained and untrained groups improved indices of insulin sensitivity following exercise in the absence of antioxidant supplementation (vitamin C and vitamin E), whereas antioxidants prevented these improvements. Similarly, increased RNA expression of SOD and GPX was inhibited by dietary antioxidant supplementation. Again, this demonstrates the beneficial hormetic effect of repeated, transient ROS production due to exercise, and this may be abrogated by dietary antioxidants.

While exogenous SOD administration is an effective method of protection against ischemia-reperfusion injury, very high doses of SOD can actually exacerbate the damage (Omar et al., 1990). The apparent bell-shaped curve in the effectiveness of SOD in this case may be due to over-scavenging of superoxide, which may actually reduce the potential termination of lipid peroxidation by superoxide ($\text{LOO}^\cdot + \text{O}_2^{\cdot-} + \text{H}^+ \rightarrow \text{LOOH} + \text{O}_2$) (McCord, 1993). This concept is supported by research showing that SOD overproduction in transfected cells can mimic oxidative stress, by increased lipid peroxidation (Elroy-Stein, Bernstein, & Groner, 1986). Additionally, endogenous antioxidant enzymes are coordinately regulated, so that glutathione peroxidases and catalases are upregulated in concert with SOD to match the currently level of ROS production (McCord, 2008). Exogenous administration of any one of these alone will likely result in aberrant redox regulation.

Because many cancer therapies are thought to initiate side effects such as cardiotoxicity via oxidative stress, there have been numerous attempts to reduce such side effects via antioxidant treatment. For instance, pretreatment of mice with metallothionein, a free radical scavenging protein, attenuated doxorubicin-associated cardiotoxicity without reducing its antitumor action (Naganuma, Satoh, & Imura, 1988). Unfortunately, in humans, antioxidant therapy has failed to produce similarly positive results (Minotti, Menna, Salvatorelli, Cairo, & Gianni, 2004). Moreover, antioxidant supplementation has the potential to decrease oxidative damage to healthy tissues during or following cancer treatment, it may also protect cancer cells, decreasing the effectiveness of treatment. Lawenda et al., (2008) reviewed several clinical studies, and concluded that antioxidant treatment, although potentially capable of reducing treatment-

related side effects, should be avoided because of the potential for reduced treatment effectiveness. Most significant among these studies were two authored by Bairati et al., in which 540 cancer patients undergoing radiation were either given a placebo or antioxidants (α -tocopherol with or without β -carotene). Although they reported a 38% reduction in severe, acute side effect in the antioxidant group (Bairati, Meyer, Gelinas, Fortin, Nabid, Brochet, Mercier, Tetu, Harel, Abdous, et al., 2005), this was accompanied by a 29% and 56% reduction in the local tumor control rates for α -tocopherol and α -tocopherol plus β -carotene, respectively. (Bairati, Meyer, Gelinas, Fortin, Nabid, Brochet, Mercier, Tetu, Harel, Masse, et al., 2005). While some studies indicate that antioxidant supplementation can reduce risk of mortality and recurrence (Nechuta et al., 2011) and increase effectiveness of treatment (Chinery et al., 1997), and several authors suggest that dietary AOX supplementation during treatment should be avoided because of the contentious nature of the subject (Block, 2004; D'Andrea, 2005). A paper by Achuthan et al. (2011) indicated that in chemotherapy resistant cancers, a primary mechanism behind the failure of treatment regimens is low level of ROS associated with the hypoxic environment in the cell. This suggests that reduced ROS in tumorous tissues may support the survival of cancer cells.

Venkataraman et al. found that SOD overexpression inhibited the growth of androgen-independent prostate cancer cells (Venkataraman et al., 2005), but in the presence of a stressor (hyperthermia), SOD promoted cell survival (Venkataraman et al., 2004). This illustrates the intricacies of redox balance, whereas cells are protected by SOD in the presence of an environmental stressor, but are hindered by SOD in the absence of a stressor. The supplementation of antioxidants during chemotherapy and

radiation may similarly protect cancer cells from the environment stress of treatment (Conklin, 2000). There is recent evidence that a state of reductive stress, characterized by excessive levels of reducing equivalents, may be implicated in human disease, namely cardiomyopathy caused by mutations in the alpha B-crystallin gene of mice (Rajasekaran et al., 2007). It is possible that on overabundance of dietary antioxidants may similarly result in other unfavorable effects, including the development and progression of cancer (D. P. Jones & Go, 2010).

Cancer and Oxidative Stress

The interrelationship between cancer and oxidative stress is interesting, in that oxidative stress is implicated in both the etiology and pathology of cancer (Laviano et al., 2007; Loft & Poulsen, 1996; Toyokuni, Okamoto, Yodoi, & Hiai, 1995; C. Xia et al., 2007). Reactive oxygen species are implicated in initiation, promotion and progression of cancer, as well as inhibition of processes that can normally interrupt the cell cycle and repair damage within cancer cells or otherwise initiate apoptosis (Valko et al., 2006). The rise in ROS associated with breast cancer is common enough that a reduced GSH/GSSG ratio has been identified as an important biochemical parameter for detecting breast malignancy (Yeh et al., 2006). Further complicating things, both ROS and antioxidants have been proposed as a treatments for cancer in recent years (Renschler, 2004; Tandon, Sharma, Mahajan, & Bardi, 2005; Trachootham, Alexandre, & Huang, 2009; J. Wang & Yi, 2008). Alternately, oxidative stress is capable of interfering with cancer treatment (Shacter, Williams, Hinson, Senturker, & Lee, 2000), yet there is evidence that antioxidants may also interfere with cancer treatment (Lawenda et al.,

2008). Clearly, the normal physiological redox balance is delicate and complicated, and is dependent on cell type, as well as endogenous and environmental stressors.

Oxidative Stress

A cursory comparison of exogenous sources of ROS production with known carcinogens reveals striking parallels (e.g., chemotherapeutics, UV light, ionizing radiation, environmental toxins). It is therefore not surprising that soon after the discovery of ROS, Harman (1962) hypothesized that oxidative stress was implicated with not just aging, but cancer as well. An abundance of epidemiological evidence supports the notion that oxidative stress may promote cancer (Bjelakovic, Nikolova, Simonetti, & Gluud, 2008; Dai et al., 2009; Hopkins, Fedirko, Jones, Terry, & Bostick, 2010; Li et al., 2009; Loft & Poulsen, 1996; Matsui & Rai, 2008; Minelli, Bellezza, Conte, & Culig, 2009; Singh & Kulawiec, 2009; Valko et al., 2006; Zipprich et al., 2009). Additionally, secondary cancers caused by radiation therapy are likely associated with oxidative stress (Bostrom & Soloway, 2007). Several mechanisms confirming this interaction have been identified (Loft & Poulsen, 1996; Valko et al., 2006), and will be discussed in the proceeding section.

Mechanisms of ROS-Mediated Carcinogenesis

ROS may potentiate carcinogenesis at various steps in cancer initiation, promotion and progression. ROS are implicated in initiation by creating genomic instability. For instance, hydroxyl radicals can react with guanosine to form 8-hydroxydeoxyguanosine (8-OhdG), potentially resulting in G:C to T:A transversion point mutations (Loft & Poulsen, 1996; Wu et al., 2004). As discussed previously, DNA damage alone is not sufficient for cancer initiation. ROS-mediated signaling cascades

can potentiate survival of cancer cells via proliferation, angiogenesis, metastasis and resistance to apoptosis (Halliwell, 2007).

ROS promote cell proliferation primarily through activation of growth factor receptors and intracellular signaling pathways (J. Wang & Yi, 2008). Elevated ROS activate NF κ B and AP-1, both of which mediate tumor cell proliferation (Laurent et al., 2005). Additionally, ROS are capable of inhibiting protein tyrosine phosphatases, control elements of numerous growth factor receptors, including platelet-derived growth factor receptor (PDGFR), by oxidizing cysteine residues on their active site, ultimately abolishing their negative regulatory function (Chiarugi, 2005; Meng, Fukada, & Tonks, 2002). In other words, even in the presence of their ligands, these growth factors receptors are perpetually inactivated in the presence of sufficient ROS, resulting in unconstrained growth.

ROS can similarly interact with protein tyrosine kinases, which are activated by oxidation of their thiol groups, resulting in aberrant growth signaling (Chiarugi, 2005). Vascular endothelial growth factor (VEGF) can promote tumor angiogenesis, and NADPH oxidase (NOX)-associated H₂O₂ production has been shown to increase VEGF expression (Arbiser et al., 2002). Supporting the notion that ROS mediate angiogenesis via VEGF, Xia et al. (2007) demonstrated that NOX4 knockdown in ovarian cancer cells resulted in decreased expression of VEGF and tumor angiogenesis. The metastatic potential of several types of tumors is also associated with ROS level (Lim et al., 2005), possibly due to altered expression of integrins, improved metastatic invasion, and avoidance of anoikis, a type of programmed cell death associated with detachment from the extracellular matrix (J. Wang & Yi, 2008). The size of benign tumors has been

shown to be correlated with level of 8-OHdG, and therefore may trigger the transition from benign to malignant tumor (Loft & Poulsen, 1996).

The precise redox status of a cancer cell, depending on cell type and cancer stage, can alter the interaction between ROS and cancer treatment. Figure 3 (Valko et al., 2006) shows the continuum of ROS exposure in cancer development. Lee and Shacter (1999) found that H₂O₂ induced apoptosis in lymphoma within a very limited concentration range (~50µm), but induced necrosis instead at higher concentrations (~75-100 µm). Although the primary objective of cancer killing is achieved, necrosis invariably leads to inflammation and more significant side effects. Shacter et al. (2000) later found that including the antioxidants agents Desferal, Tempol, and dimethylsulfoxide in various chemotherapy regimens enhanced chemotherapy-induced apoptosis and phagocytosis of cancer cells, while reducing H₂O₂ associated ATP depletion, and subsequently cellular necrosis and/or pyknosis (nucleus degeneration). Recalling that these effects are in contrast to other studies, the disparity may be explained by variations in cancer type, treatment type, and type of antioxidant treatment.

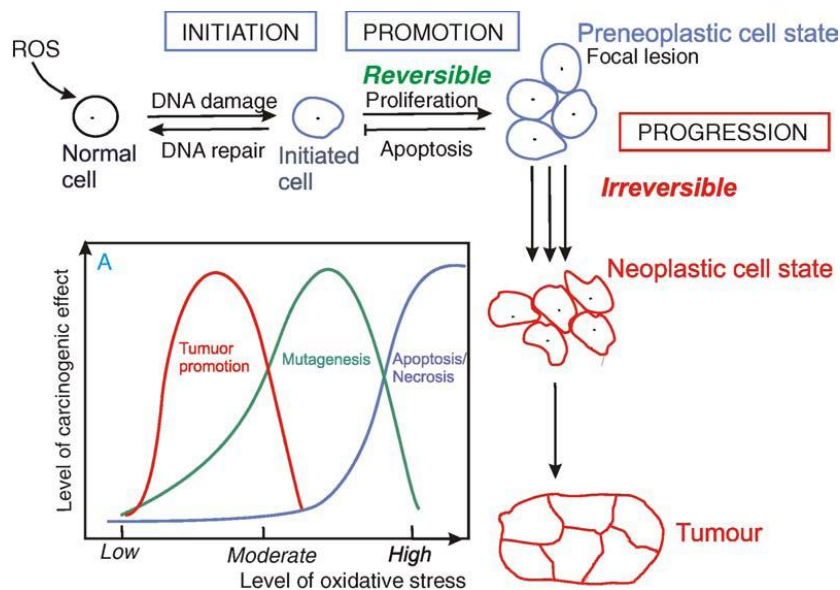


Figure 3. The level of free radicals at various stages of carcinogenic process. From “Free radicals, metals and antioxidants in oxidative stress-induced cancer” in *Chemico-Biological Interactions* by Valko et al., 2006, pg 20.

Antioxidant Enzymes in Cancer Cells

Cancer cells have altered antioxidant enzyme expression (Oberley, 2002; Oberley & Oberley, 1997; Trachootham et al., 2009). Depending on the type of tumor and the location of the cell, manganese superoxide dismutase (MnSOD, found in mitochondria) may be either increased or decreased, but enzyme activity is universally altered (Oberley, 2002). Most cancer cells exhibit reduced MnSOD, but metastatic prostate cells have been shown to have increased MnSOD imparting particular resistance to treatment. Similarly, cells at the invading edge of tumors often exhibited elevated levels of MnSOD. Because there are no longitudinal studies on oxidative stress and cancer, it is difficult to determine whether altered antioxidant enzyme expression is a cause, side effect, or comorbidity of cancer. As has been mentioned previously, oxidative damage to DNA is implicated in cancer development, and impaired antioxidant defenses may contribute to this initial redox imbalance.

Cancer Treatment Side Effects

Treatments for cancer such as surgery, chemo, radiation, and hormone therapies result in detrimental physical and psychological side effects. The most frequently reported symptom is cancer-related fatigue, which, according to the National Cancer Institute, occurs in 14 to 96% of cancer survivors who have been treated for cancer (National Cancer National Cancer Institute, 2013). Other side effects include immunosuppression, cardiomyopathy, neurological dysfunction, and reduced aerobic fitness and muscular strength. Loss of physical function and the ordeal of cancer treatment can be a psychological, social, and economic burden, resulting in a further reduction in quality of life and an increased level of depression

Immune System

Both radiation and chemotherapy are associated with hematological toxicities and cause myelosuppression by damaging the blood cell-producing machinery of the bone marrow and directly destroying mature, circulating leukocytes (Schneider et al., 2003). Leukopenia is a condition in which the total leukocyte count is reduced. Neutropenia describes an absolute decrease in the number of circulating neutrophils, and greatly increases the risk of infection. Lymphocytopenia results from low white blood cells production in the lymph system. Thrombocytopenia is a condition characterized by low levels of circulating platelets. All of these conditions result in immunosuppression, and can result from cancer therapy, or the disease itself. Extreme immunosuppression can become the limiting factor in treatment dosage, and increases the risk of prescribing exercise to cancer patients, as intense and even moderate exercise can also suppress the immune system (Langhorne et al., 2007; Schneider et al., 2003)

Cardiorespiratory Fitness

Data from the Rocky Mountain Cancer Rehabilitation Institute (RMCRI) illustrates the severely reduced cardiorespiratory fitness of cancer survivors. Average VO_2 peak values for the RMCRI cancer population were markedly lower than the general US population, with a median VO_2 value of 22 mL/kg/min, and a mean of 21.6 ± 6.3 mL/kg/min (unpublished data). When using norms for the general US population (Thomson, Gordon, & Pescatello, 2010), the mean value for all age and gender groups were classified as “very poor.” Similarly, individuals who had been treated with chemotherapy in childhood retained significantly reduced cardiorespiratory fitness, as evidenced by VO_2 max values that are often only 50% - 70% of age- and gender-matched controls (Johnson et al., 1997). Even in very fit cancer patients, radiation and chemotherapy can substantially reduce cardiorespiratory fitness. Schumacher et al. (2008) showed that a 37% decrease in total hemoglobin mass in an elite endurance athlete following cancer diagnosis and chemotherapy was associated with a 42% reduction in aerobic capacity. Additionally, Wiley (1998) demonstrated an average decrease in VO_2 max from 28.23 ± 5.54 mL/kg/min before surgery and chemotherapy, to 24.52 ± 6.13 mL/kg/min after both surgery and chemotherapy in ten women with stage II breast cancer. Primary causes of decrements in cardiorespiratory fitness include anemia (Barrett-Lee et al., 2006; Glaspy, 2001; Harrison et al., 2001), cardiotoxicity (Ascensao, Ferreira, Oliveira, & Magalhaes, 2006; Chaiswing et al., 2004; B. Chen, Peng, Pentassuglia, Lim, & Sawyer, 2007; Chicco, Hydock, Schneider, & Hayward, 2006; Hayward & Hydock, 2007; Hydock, Lien, Schneider, & Hayward, 2008; Kanter et al., 1985; Wonders, Hydock, Schneider, & Hayward, 2008), skeletal muscle degradation (Al-

Majid & Waters, 2008; Bonetto et al., 2009; Laviano et al., 2007), and mitochondrial and metabolic dysfunction (Kovacic, Pozos, Somanathan, Shangari, & O'Brien, 2005).

Cardiovascular System

Reduced cardiorespiratory fitness following cancer therapy is often demonstrated by the damage to systems supporting aerobic capacity, particularly in the animal model. The majority of cancer patients receiving chemotherapy (Barrett-Lee et al., 2006; Kitano et al., 2007) and radiation (Harrison et al., 2001) develop anemia. Numerous chemotherapy treatments, particularly antitumor anthracyclines such as doxorubicin, are characterized by a dose dependent cardiotoxicity (Simunek et al., 2009), resulting in altered myosin heavy chain expression, reduced left ventricular mass, relative wall thickness, fractional shortening, and mean and maximal flow velocity in rats (Chicco, Hydock, et al., 2006; Hayward & Hydock, 2007; Hydock, Lien, & Hayward, 2009). Doxorubicin cardiotoxicity-caused morphological damage within the myocardium is often apparent when left ventricular ejection fraction (LVEF) falls below 45%, and may result in congestive heart failure (Ng et al., 2006). In animal studies, doxorubicin has been shown to inactivate important mitochondrial enzymes, such as myocardial cytochrome c oxidase (Chandran et al., 2009). Further treatment-associated side effects, including additional mitochondrial (Miyagawa et al., 2010) and cardiac pathologies (Meinardi et al., 1999), cachexia (Samuels et al., 2000; Takahashi, Yasumoto, & Mai, 2005), and pulmonary and endothelial dysfunction, are well established in the clinical oncology and cancer rehabilitation literature (DeVita, 2005; Schneider et al., 2003). The individual or combined effects of any of these pathophysiological defects, in addition to the tumor burden itself, is capable of reducing aerobic capacity. Furthermore, the

majority of cancer survivors experience fatigue following cancer treatment (Kasper & Sarna, 2000; Smets, Garssen, Schuster-Uitterhoeve, & de Haes, 1993; Winningham, 2001), which often results in reduced physical activity and subsequent deconditioning. Animal models also indicate reduced physical activity with chemotherapy treatment. Hydock et al. found that voluntary wheel running in rats reduced significantly within two weeks of doxorubicin treatment, and was reduced to less than 50% of baseline by week 5 (Hydock, Wonders, Schneider, & Hayward, 2009).

Doxorubicin, in particular, has been extensively studied because it is a very effective antineoplastic agent, but is associated with severe, dose-dependent cardiotoxicity (Ascensao et al., 2006; B. Chen et al., 2007; Chicco, Hydock, et al., 2006; Hayward & Hydock, 2007; Hydock et al., 2008; Wonders et al., 2008). Oxidative stress is among the mechanisms attributed to the DOX-induced cardiomyopathy (Chaiswing et al., 2004; Kanter et al., 1985), in addition to apoptosis (Arola et al., 2000), metabolic alterations (Wakasugi et al., 1993), and cardiac myosin heavy chain shifts (Hydock, Wonders, et al., 2009). While the primary antineoplastic mechanisms of DOX are inhibition of topoisomerase II and intercalation in DNA, resulting in inhibition of DNA replication and RNA transcription, the induction of ROS may contribute to antitumor activity (Figure 4). Some authors even argue that “induction of oxidative stress is responsible for most if not all biological activity” of anthracyclines (Taatjes et al., 1998).

Antioxidant therapy has been investigated as a means to minimize doxorubicin cardiotoxicity, with mixed results. Grape seed proanthocyanidins, a dietary antioxidant supplement, have been shown to improve tumor response rates and mitigate DOX-induced myocardial oxidative stress in tumor-bearing mice (X. Y. Zhang, Li, Wu, & Gao,

2005). In contrast, patients receiving high doses of vitamin E or N-acetylcysteine failed to exhibit reduction of cardiomyopathy in clinical trials (Myers et al., 1983; Unverferth et al., 1983). The use of the iron chelator dexrazoxane (DRZ) has also been used as a method to reduce cardiotoxicity (Moss, 2007). While DRZ is able to reduce the incidence of contractile dysfunction, the possibility that it may reduce DOX efficacy has prevented its universal use.

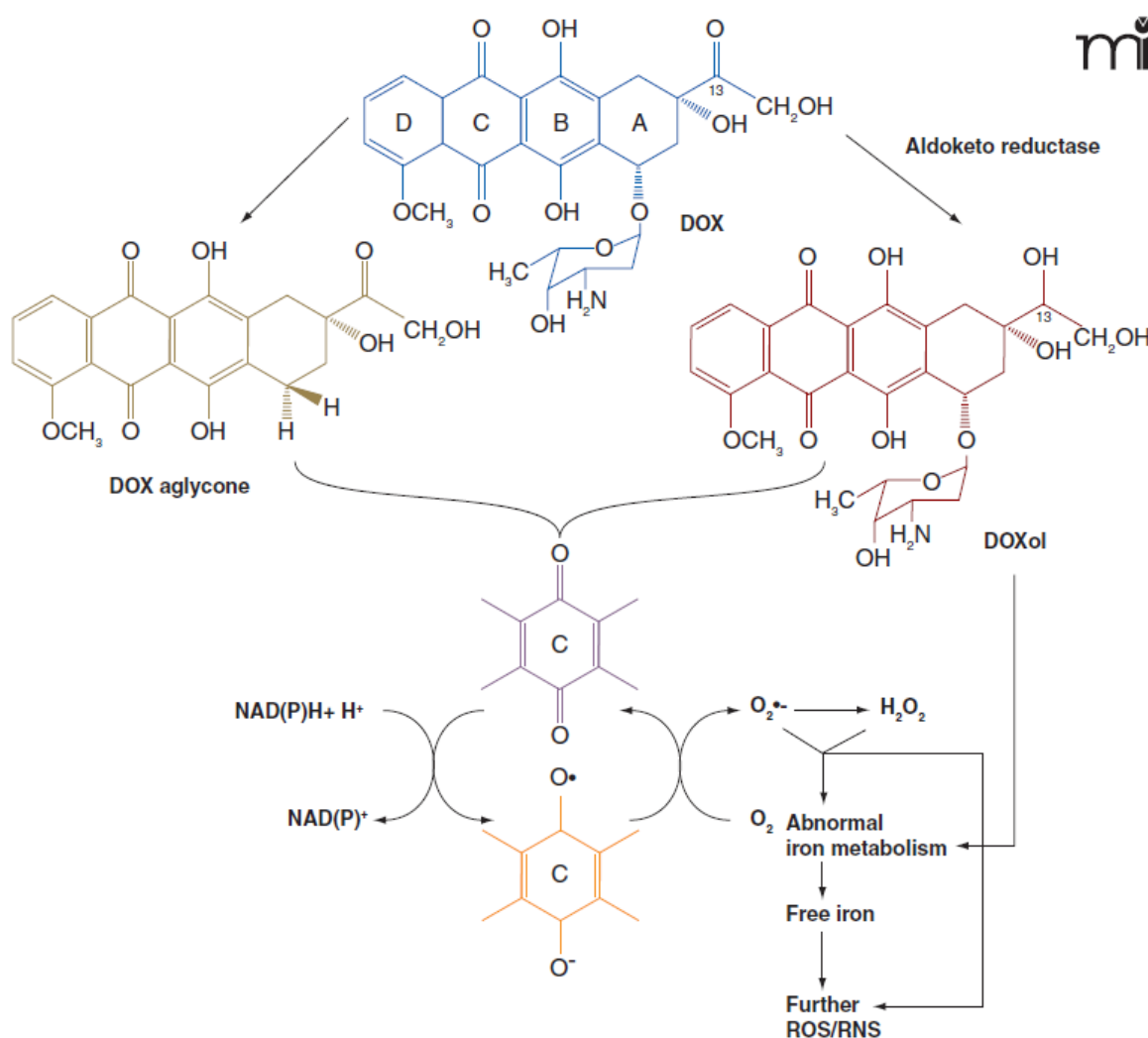


Figure 4. Doxorubicin-induced generation of ROS/RNS in the heart. From *Collateral damage in cancer chemotherapy: oxidative stress in nontargeted tissues* in Molecular Interventions, 7(3). By Chen, Y., Jungsuwadee, P., Vore, M., Butterfield, D. A., and St Clair, D. K. Copyright 2007, p. 147-156.

Fatigue

Fatigue associated with both cancer and cancer treatments has been well documented for decades (Piper, Lindsey, & Dodd, 1989; Questad, Malec, Harvey, Kienker, & Romsaas, 1982; Rieger, 1988; Smets et al., 1993). Fatigue is the most frequently reported symptom in cancer survivors and is similar in nature to chronic fatigue syndrome, as both are more chronic and debilitating than standard fatigue, causing individuals to alter their behavior and demeanor, but all patients experience cancer-related fatigue differently (de Jong, Courtens, Abu-Saad, & Schouten, 2002). Because fatigue is both a physiological and psychological condition, it is difficult to pinpoint a specific mechanism, but there are likely many. Some proposed factors contributing to fatigue are muscular wasting related to cachexia, sleep disturbances, psychological depression, (Piper, Lindsey, & Dodd, 1987), anemia (de Jong et al., 2002), increased metabolic byproducts (e.g., inflammatory cytokines) and metabolic imbalance due to increased energy requirement (e.g., due to tumor growth), decreased metabolic capacity (mitochondrial damage), and decreased substrate availability (due to anorexia, nausea, or vomiting) (Glaspy, 2001).

While there is a lack of evidence explicitly relating oxidative stress to cancer-related fatigue, most of the proposed mechanisms of fatigue can be driven by ROS and oxidative stress. Indirect evidence comes from Gramignano et al., who demonstrated that four weeks of L-carnitine administration successfully reduced both fatigue, and resulted in a non-significant decrease in oxidative stress in patients currently undergoing cancer treatment (Gramignano et al., 2006). Chronic fatigue syndrome, a very similar pathology, has been associated with oxidative stress (Jammes, Steinberg, Mambrini,

Bregeon, & Delliaux, 2005) and glutathione deficiency (Bounous & Molson, 1999). The hypothesized interaction between oxidative stress and fatigue warrants further investigation.

Muscular Strength

Decreased strength following cancer treatment is a significant factor in decreased quality of life in cancer patients (Courneya & Friedenreich, 1999; Extermann et al., 2006; Kasper & Sarna, 2000; Schneider et al., 2003). The primary mechanism of loss of muscular strength with cancer and cancer treatment appears to be related to cachexia and muscle wasting. Numerous studies link cancer and treatment-related ROS production with cachexia, among other potential mechanisms (Laviano et al., 2007; Mantovani et al., 2004). Oxidative protein damage has been shown to be associated with poor grip strength in healthy elderly women (Howard et al., 2007). The loss of muscle mass and cardiovascular fitness is compounded by significant decreases in physical activity following treatment. Studies have shown that muscular endurance declines after only two weeks of physical inactivity in healthy individuals (Haddad, Roy, Zhong, Edgerton, & Baldwin, 2003), and oxidative enzymatic activity decreases following 3 months of inactivity (Coyle et al., 1984). Fortunately, it has been demonstrated that resistance exercise can prevent or reverse losses in muscular strength (Schneider et al., 2007a) and muscle wasting. This exercise-induced protection against muscle wasting appears to be at least partially due to a reduced oxidative damage in the skeletal muscles of rats treated with DOX. Exercise was shown to diminish skeletal muscle protein oxidation and increase antioxidant enzymes, and subsequently reduce expression of autophagy genes

(Smuder, Kavazis, Min, & Powers, 2011a) and proteases such as caspase-3 (Smuder, Kavazis, Min, & Powers, 2011b).

Cancer Treatment-Related Side Effects and Oxidative Stress

Both cancer and its treatments induce considerable oxidative stress. Even the physiological stress of surgery is capable of producing enough ROS as a side effect to facilitate increased growth of metastatic tumors (Hyoudou, Nishikawa, Kobayashi, Umeyama, et al., 2006). Because treatment-associated oxidative stress is implicated in cancer-related fatigue (Fulle et al., 2000), cachexia (Laviano et al., 2007), cardiomyopathy (Ascensao et al., 2005b), and neuropathy (Kannarkat, Lasher, & Schiff, 2007), it often governs the tolerance limit of treatment. Treatment-associated oxidative stress also can result in second malignancies, also known as treatment-induced cancer (Bostrom & Soloway, 2007). Additionally, cancer cells have lower levels of MnSOD, GPx and CAT, but produce high levels of hydrogen peroxide (Oberley, 2002), resulting in greater oxidative stress in cancer patients even prior to cancer therapy.

Cancer treatments, particularly radiation and chemotherapy, are associated with significant, whole-body side effects, many of which are associated with oxidative stress. Whereas radiation therapy actually depends on free radical generation as a means of destroying cancer cells (Ahn et al., 2006), oxidative stress associated with chemotherapy is often a side effect. Doxorubicin (DOX), for instance, is a very effective anti-cancer agent, but is known for its cardiotoxic effects (Chaiswing et al., 2004; Y. Chen et al., 2007; Gilliam et al., 2009; Hayward & Hydock, 2007; Shacter et al., 2000; Simunek et al., 2009). Although DOX kills cancer cells through DNA inhibition and topoisomerase II inhibition, it damages heart, kidney and brain tissue through oxidative stress. Of the

Table 3

Chemotherapy drugs known to induce significant oxidative stress

Class (subclass)	Drug Name	Trade Name
Anti-neoplastic antibiotics		
(Anthracyclines)	bleomycin	Blenoxane
	mitomycin	Mitomycin-C, MTC
	Doxorubicin	Adriamycin
	epirubicin	Ellence
	daunorubicin	Daunomycin
Alkylating agents		
(Mustard gas derivatives)	mechlorethamine	chlormethine, mustine, Mustargen
	cytophosphane / cyclophosphamide	Endoxan, Cytoxan, Neosar, Procytox, Revimmune
(Alkyl sulfonate)	ifosfamide	Mitoxana, Ifex
	Uramustine	
	Melphalan	Alkeran
	chlorambucil	Leukeran
	Busulfan	Busulfex, Myleran
Platinum coordinating complexes		
	cisplatin	
	carboplatin	
	oxaliplatin	
	Nedaplatin	Aqupla
	satraplatin	
	triplatin tetranitrate	
Nitrosoureas		
	Carmustine	
	Lomustine	
	Streptozotocin	Streptozocin, STZ, Zanosar
Epipodophyllotoxins		
	etoposide	Eposin, Etopophos, Vepesid, VP-16
	teniposide	Vumon, VM-26
Camptothecins		
	topotecan	Hycamtin
	irinotecan	Camptosar, Campto
Other		
	fluorouracil (5-FU)	Adrucil, Carac, Efudex and Fluoroplex
	cytarabine	cytosine arabinoside or Ara-C
	tamoxifen	

132 FDA approved anticancer drugs, 56 are known to induce oxidative stress (Chen et al., 2007). Table 3 lists chemotherapy drugs known to induce substantial oxidative stress. While late effects of radiation treatment have been associated with chronic oxidative stress (Robbins & Zhao, 2004; Zhao & Robbins, 2009), there is little information regarding long-term effects of chemotherapy on oxidative stress.

Currently, there is no good characterization of the time-course of chronic oxidative stress status following the end of cancer chemotherapy or radiation treatment in the literature. Many papers evaluate oxidative stress while patients are undergoing treatment (Cetin et al., 2004; Conklin, 2000; Honda, Yamada, Tomonaga, Ichinose, & Kamihira, 2000; Sabitha & Shyamaladevi, 1999; Weijl et al., 1998) or within hours and days after treatment (Sangeetha, Das, Koratkar, & Suryaprabha, 1990), but rarely evaluate markers of oxidative stress or antioxidants more than a week after the end of treatment. In mice, extracellular MDA levels remained elevated one week after chemotherapy, and oxidized glutathione was significantly higher than controls 6 weeks after treatment (Abushamaa, Sporn, & Folz, 2002). Unfortunately, it is difficult to extrapolate these data to humans due to varying lifespans. Jonas et al. (Jonas et al., 2000) found that GSH/GSSG ratio continually decreased over the two weeks following the end of treatment, indicating either that treatment directly impairs the antioxidant system, or that chronic oxidative stress is capable of depleting the glutathione pool and diminish reductive capacity.

While some studies show increased oxidative stress in response to treatment (Abushamaa et al., 2002; Cetin et al., 2004; Faber, Coudray, Hida, Mousseau, & Favier, 1995; Sangeetha et al., 1990), others indicate a *lower* level of oxidative stress following

treatment compared to pretreatment, likely due to reduced tumor burden. For instance, Gadjeva et al. (Gadjeva, Dimov, & Georgieva, 2008) found that plasma MDA concentration dropped in melanoma patients following surgery. While chemotherapy subsequently increased oxidative stress compared to post-surgery levels, MDA levels were still significantly lower than pre-surgery values. This discrepancy is likely due to variations in treatment method and cancer type. Moreover, Erhola et al. (Erhola et al., 1997) found that the overall response to chemotherapy (“complete or partial remission”, versus “no change or progressive disease”) determined whether urinary 8-OHdG concentration increased or decreased. Therefore it appears that it is justifiable to further investigate the level of oxidative stress in patients in the weeks and months following cancer treatment.

Targeted delivery of SOD (Hyoudou et al., 2008) or catalase (Hyoudou, Nishikawa, Kobayashi, Umeyama, et al., 2006) to cancer cells have the potential to inhibit proliferation and metastasis, largely by decreasing the ROS-mediated growth signaling. At this time, no clinical trials have been conducted to confirm the effectiveness of these strategies. An alternate strategy to reduce ROS in cancer cells is inhibition of NADPH oxidase, which in turn reduces VEGF signaling, which may inhibit tumor growth. NADPH oxidase inhibition with minodronate (Kubo et al., 2006; Sato et al., 2006) and histamine (Agarwala & Sabbagh, 2001) have been demonstrated to be effective cancer treatment methods in clinical trials. In the case of ROS production following oncological surgery, administration of catalase successfully inhibited metastatic growth (Hyoudou, Nishikawa, Kobayashi, Kuramoto, et al., 2006).

Alternately, the use of targeted ROS production has been used as a cancer treatment, both as a single agent, employing ROS to destroy cancer cells, or as an adjuvant therapy to facilitate the therapeutic effects of radiation and chemotherapy (Fang et al., 2009; Manda et al., 2009; Trachootham et al., 2009; J. Wang & Yi, 2008). Because cancer cells often have chronically elevated oxidative stress due to increased ROS production, reduced antioxidant capacity, or both, exogenous ROS may be sufficient to trigger cellular death. Indeed, most cancer therapies increase ROS as a primary or secondary mechanism of antitumor activity. Procarbazine, a ROS-producing antineoplastic drug, has been used alone and in conjunction with radiation since the 1960s (Renschler, 2004). Even then, Berneis and colleagues recognized that ROS mediated the anticancer effects of the drug (Berneis, Kofler, Bollag, Kaiser, & Langemann, 1963). Radiation therapy has been used as a treatment for cancer for even longer, about 100 years, owing largely to Marie Curie, but the production of ROS by radiation was not recognized until 1948 (Stein & Weiss, 1948). Later, Harman identified the implication of free radicals in the ability of radiation to both treat and cause cancer (Harman, 1956, 1962).

Today, we have alternate methods of ROS induction to treat cancer. As previously mentioned, augmenting ROS induction with hyperbaria during radiation treatment can improve its efficacy. Another approach to this involves administration of radiation sensitizers, which include the drug classes tirapazamine, 2-nitroimidazoles, and indolequinones (Kovacic, 2007). A more recently developed anticancer modality is called photodynamic therapy (J. Wang & Yi, 2008). In this treatment, a drug (photosensitizer) is injected into the bloodstream, and is absorbed by all body cells, but

tends to stay in cancer cells longer. Approximately 24 to 72 hours later, the photosensitizer is activated by light, producing ROS that can destroy the cells containing the drug (Dolmans, Fukumura, & Jain, 2003). Inhibition of antioxidant enzyme defense within tumor cells to induce ROS has also been explored. B-phenylethyl isothiocyanate is a natural compound that depletes cellular GSH and inhibits the GPx enzyme, resulting in severe ROS accumulation in malignant cells (Trachootham et al., 2006). Methoxyestradiol, an anticancer agent in clinical trials, inhibits SOD and induces apoptosis in leukemia cells (J. Wang & Yi, 2008).

One particularly valuable use of pro-oxidant therapy may be the reduction of drug-resistance in tumors. A decreased reductive state, specifically increased glutathione redox potential, has been long recognized as a factor in acquired multidrug resistance (Kramer et al., 1988). The introduction of either a NADPH oxidase (NOX1) (Wartenberg et al., 2005) or a ROS-producing agent (emodin) (Pelicano, Carney, & Huang, 2004) into cancer cells can reduce multi-drug resistance via decreased expression of the multi-drug resistance transport and hypoxia-inducible factor 1 α .

Exercise Before, During, and Following Cancer Treatment

The beneficial effects of exercise on cardiopulmonary function, strength, and overall health status in healthy individuals has been well-documented for decades. The role of exercise to promote improved physical and mental health and rehabilitation in the cancer population during and following cancer treatments has only more recently become recognized as important. In the 1980s, Winningham et al. conducted some of the first studies investigating the effect of exercise on cancer treatment-related side effects (MacVicar, Winningham, & Nickel, 1989; Winningham, 1983; Winningham &

MacVicar, 1988; Winningham, MacVicar, Bondoc, Anderson, & Minton, 1989). In the more than 20 years since then, a growing body of evidence has supported the use of exercise as a means to rehabilitate individuals from cancer and treatment-related side effects.

Exercise during treatment is known to increase tolerance for higher doses of chemotherapy (Chicco, Schneider, et al., 2006), and exercise following treatment can reduce or reverse the damaging side effect of cancer treatments (Hydock et al., 2007) and even reduce the chance for cancer recurrence (Courneya, 2003). In particular, resistance and aerobic exercise have been found to be beneficial as a means to reduce fatigue, while improving quality of life (QOL), muscular strength and endurance, and cardiorespiratory endurance (Courneya et al., 2007; Schneider et al., 2007a, 2007c). Exercise following surgery has been shown to be beneficial in increasing the natural killer (NK) cell cytotoxic activity (NKCA) , suggesting that exercise may play a role in cancer treatment, rather than simply rehabilitation (Na et al., 2000). Like so many physiological and pathophysiological processes, NKCA are known be mediated (in this case, inhibited) by ROS, (Betten, Dahlgren, Mellqvist, Hermodsson, & Hellstrand, 2004) perhaps explaining the increase following exercise and the decrease associated with cancer (Mellqvist et al., 2000).

Cardiorespiratory Fitness

While it may seem intuitive that an aerobic exercise intervention improves cardiorespiratory fitness in cancer survivors, the reasons for the reduced fitness level in this population are complex and multifactorial, potentially altering the normal physiological alterations in response to exercise. Because exercise was not a commonly

prescribed intervention in cancer survivors until 15 years ago, there had previously been little evidence to support the exercise as a rehabilitation modality. There is now a very large body of evidence demonstrating the effectiveness of an exercise intervention for the improvement of cardiorespiratory fitness (Courneya et al., 2003; Courneya et al., 2007; Dimeo et al., 2004; Schneider et al., 2007c; Segal et al., 2009). Unpublished data from the Rocky Mountain Cancer Rehabilitation Institute indicates that improvement in cardiopulmonary fitness, and fatigue following a 3-month, multimodal exercise program did not differ between cancer types, regardless of baseline values. A recent study in leukemia patients currently receiving high-dose treatment indicated that an in-hospital exercise intervention significantly improved performance in a submaximal cycle ergometer test time to termination, from 8.9 ± 8.8 minutes at baseline to 17 ± 14.3 minutes post-intervention (Battaglini et al., 2009). In a 2011 meta-analysis investigating the effect of exercise on VO_2 peak, pooled data of all six studies (571 adult cancer patients; 344 exercise and 227 usual care control) indicated that exercise training significantly increased VO_2 peak ($+2.90 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), whereas usual care was associated with a decline in VO_2 peak ($-1.02 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) (L. W. Jones, Liang, et al., 2011).

There has been relatively scarce clinical confirmation of mechanisms of exercise-induced improvements in (or maintenance of) cardiorespiratory fitness in cancer patients during or following cancer treatment. In a study by Dimeo et al. (1997), cancer survivors who participated in an exercise program following high dose chemotherapy had higher aerobic capacity as well as increased hemoglobin concentration compared to non-exercisers. Animal studies indicate numerous potential mechanisms of improved or

maintained cardiorespiratory fitness, many of which include cardiac preservation via increased myocardial antioxidant status (Kanter et al., 1985; Pushpalatha, Nishanth, & Sathyavelu Reddy, 2007; Simunek et al., 2009; Wouters, Kremer, Miller, Herman, & Lipshultz, 2005). Other possible mechanisms include reduced inflammatory cytokines (Battaglini et al., 2009), increased or maintained muscle mass (Laviano et al., 2007) and mitochondrial hormesis (Ji et al., 2006; Ristow & Zarse, 2010).

Fatigue

Because cancer related fatigue is multifactorial, appropriate treatment for this condition is difficult to determine. Treating this fatigue as a psychological pathology alone, using pharmacological methods (Bruera, Roca, Cedaro, Carraro, & Chacon, 1985; Rozans, Dreisbach, Lertora, & Kahn, 2002) or counseling (Gramignano et al., 2006), has been generally ineffective. Alternately, exercise has been shown to have significant therapeutic benefit (Dimeo, 2001), and daytime inactivity may actually worsen fatigue symptoms (Berger & Farr, 1999). Various methods of prescribed exercise, including resistance and aerobic exercise, both supervised and at home, have been shown to improve fatigue symptoms in cancer patients following treatment (Courneya & Friedenreich, 1999; Courneya et al., 2007; F. Dimeo et al., 2003; F. Dimeo, Schwartz, Wesel, Voigt, & Thiel, 2008; F. C. Dimeo, 2001; Hsieh et al., 2008; Peddle, Au, & Courneya, 2008; Saylor & Smith, 2009; Schneider, Hsieh, Sprod, Carter, & Hayward, 2007b; Schneider et al., 2007c; Schwartz, 2007; Smets et al., 1993).

Studies have indicated that the effect of exercise on cancer-related fatigue may be associated with modulation of 5-HT neurotransmitter regulation, vagal afferent activation, muscle and ATP metabolism, hypothalamic–pituitary–adrenal axis function,

circadian rhythms, and/or cytokine regulation (Ryan et al., 2007). To date, there has been no research regarding the interaction between cancer related fatigue and oxidative stress. Because individuals with chronic fatigue disorders have an amplified oxidative stress response from an acute exercise bout (Jammes et al., 2005) it is reasonable to postulate that an exercise intervention capable of increasing antioxidant enzyme capacity may reduce postexertional fatigue following an exercise intervention.

Muscular Strength

As has been noted, cancer patients often suffer from muscle wasting disorders, or more generalized losses in muscular strength. A whole-body, 6-month exercise intervention was capable of significantly increasing muscular fitness for bench press (+47.6%), lat pull-down (+51.1%), leg press (+67.1%), shoulder press (+41.8%), and curl-up crunches (+32.5%) in cancer patients following the end of treatment (Schneider et al., 2007a). Remarkably, this exercise intervention also increased muscular fitness for lat pull-down (+83.6%) and leg press (49.7%) in cancer patients still undergoing radiation or chemotherapy. Similarly, Courneya et al., (2007) found that an isolated resistance exercise training (RET) program increased muscular strength in breast cancer patients receiving chemotherapy 25% to 35%. This finding was accompanied by an increased chemotherapy completion rate, from 84.1% in a usual care group, versus 89.8% in the RET group, as well as an increase from 65.9% (usual care) to 78.0% (RET) of patients who received at least 85% of their planned relative dose intensity. These findings indicate that exercise training can increase treatment tolerance as well as muscular strength, possibly due to reduced treatment toxicity related to oxidative damage.

Despite the recognition that oxidative damage plays a role in cancer treatment side effects, the literature describing these mechanisms often fail to suggest exercise as a means of attenuating this damage. Instead, antioxidant or pharmacological treatment is suggested. A noteworthy exception to this is paper by Peng et al. (2005), which acknowledged the biological and therapeutic benefits of exercise as a strategy of improving physiological function and quality of life in cancer survivors. The authors also mention the difficulties of attracting research funding for a therapy that is inherently free, and therefore is not a wise investment for private funding agencies or pharmaceutical companies. Simultaneously, cancer rehabilitation researchers espouse the use of exercise, but rarely investigate the underlying mechanisms for the efficacy of exercise. Therefore, further biochemical and genetic studies regarding the mechanisms underlying the effectiveness of exercise as a means to minimize side effects in cancer patients during and following treatment are warranted.

Counteracting Cancer Treatment-Associated Oxidative Stress With Exercise

Although it was in a non-cancer population, Parise et al. (2005) offer some insight for the prescription of exercise as an intervention to counteract oxidative stress. This study evaluated antioxidant enzyme activity in the vastus lateralis muscle of both legs of older males (71 ± 7 yrs) following unilateral resistance exercise training. There was a significant increase in catalase and CuZnSOD activity, as well as muscular strength in the subjects' training leg, but not in the untrained leg. This demonstrates the importance of total body exercise interventions in cancer survivors, as training effects and changes in antioxidant enzyme capacity are local. Additionally, it may be surmised that because of this localized change in antioxidant capacity, antioxidant enzymes should increase in

active tissue, but not in cancer cells. Krause et al. indicated that antioxidant enzymes may not be sufficient to protect cardiac cells against damage, as illustrated by a lack of effect by antioxidant therapy. Additional aspects of antioxidant defense, such as multidrug resistance-associated proteins (MRP) and MRP1 gene product (a GS-X pump ATPase), a physiological GSSG transporter, may play a role in total body antioxidant capacity (Krause et al., 2007). This illustrates that exercise, and multi-system, multidimensional, intervention, may be superior to pharmacological interventions as a method to reduce treatment-related side effects mediated by ROS.

Wonders et al., (2008) reported that doxorubicin treatment increased left ventricular MDA concentrations in sedentary rats, but rats that had performed an acute exercise bout 24 hours before receiving treatment had no increase in MDA . This accompanied an attenuation of doxorubicin cardiotoxicity, indicating a protective role of exercise, possibly via resistance to oxidative stress. Yamashita et al., (1999), found that a single bout of exercise resulted in time sensitive changes in infarct size in mice exposed to left ventricular artery occlusion. Exercise was most protective against infarction at 0.5 and 48 hours prior to LVA occlusion, which was mirrored by significant increases in MnSOD activity at those time points only. Furthermore, mice treated with *N*-2 mercaptopropionyl glycine (MPG), a synthetic analog of glutathione, completely eliminated the protective effect of exercise against infarction at both 0.5h and 48h as well as the increase MnSOD activity. The antioxidant activity of MPG prevented the moderate, transient oxidative stress normally produced by exercise, thereby interrupting the redox-sensitive signaling cascade, which would ultimately increase MnSOD via the hormesis. Both of these studies suggest that the protective mechanism of exercise against

chemotherapy induced damage (in these instances, cardiotoxicity) is at least partly due to upregulation of antioxidant enzymes following a transient, low level increase in oxidative stress (i.e., exercise). Unfortunately, there has been no research investigating the role of exercise (and subsequently increased antioxidant capacity and reduced chronic oxidative stress) *following* treatment as a mediator of reduced chronic side effects related to cancer treatment.

Perhaps the best evidence for improved antioxidant defense in cancer survivors is a 1985 study by Kanter et al. (1985). This group randomly assigned rats to exercise ($n = 53$) and nonexercise ($n = 52$) groups. After nine weeks of swim training, nine rats from each group were sacrificed and tissue and blood samples were taken. The remaining rats were then subdivided into swim trained, swim trained-DOX treated, sedentary, and sedentary-DOX treated. After an additional twelve weeks, tissue and blood samples were taken, and the rats were tested for CAT, SOD, and GPx. Swim training significantly reduced cardiotoxicity of doxorubicin and increased blood values for all three antioxidant enzymes (Table 4). Heart antioxidant levels of DOX treated swimmers were lower compared to nontreated swimmers, indicating that DOX not only produces ROS, but also decreases antioxidant enzyme capacity. Therefore it seems wise to prescribe exercise to patients for a time before chemotherapy, if possible, to augment antioxidant capacity before treatment. This also indicates that certain cancer treatment regimens may induce both acute (ROS production) and chronic (antioxidant defense disturbances) changes in redox balance in cancer patients.

Table 4

Enzyme activity following 9 weeks of exercise training

Group	Catalase	Glutathione Peroxidase	Superoxide Dismutase
Swim	122.2 ± 21.0*	10.9 ± 0.6*	247.1 ± 21.2*
Sedentary	74.9 ± 11.8	8.3 ± 0.6	164.2 ± 18.5

Note: Values are expressed as enzyme units/ml blood. * Significantly greater than sedentary. Adapted from Kanter et al. 1985.

A more recent article by Ascensao et al., (2005a) expanded on these findings. Using a similar swim-training protocol as Kanter, this group found that DOX treatment increased GSSG and GSSG% (indicating a strain on the glutathione antioxidant system) but this effect was countered by swim training. Cardiac damage was evaluated by cardiac troponin I (cTnI) presence in the plasma. Exercise trained mice treated with DOX had significantly less cTnI than non-exercise trained mice treated with DOX (0.65 vs. 1.40 ng/ml). Although swim training did not result in significant differences in total antioxidant status or CAT, SOD, GPx, or glutathione reductase activity between exercise and non-exercise DOX groups, there were clearly changes in antioxidant defense, as reduced glutathione levels increased substantially compared to exercised mice treated with a placebo or non-exercise mice treated with DOX. Additionally, both exercise groups (DOX and placebo) had significantly lower cardiac muscle protein carbonyls than the non-exercised DOX group.

Although there have been few human studies investigating the effect of exercise on antioxidant capacity in cancer patients, Bloomer et al. (2008) studied the effect of resistance training on blood oxidative stress in Parkinson disease, another disease implicated with oxidative stress. Thirteen subjects with Parkinson's disease completed this study, with six engaged in a resistance exercise program, and seven in a no-exercise

control group. Eight weeks of low volume resistance training (2d/wk) in Parkinson disease patients showed trends toward increased antioxidant capacity (SOD, CAT, GPx, Trolox-equivalent antioxidant capacity) and reduced hydrogen peroxide (H_2O_2) and malondialdehyde levels, but were not significant. Post hoc power analyses indicated that a total sample size of 16 and 22 subjects would be needed to observe a statistically detected interaction effect for MDA and H_2O_2 , respectively. It should be noted that there was no significant difference in baseline oxidative stress or antioxidant biomarkers between Parkinson's disease patients and a healthy control population.

Summary

Reactive oxygen species, once thought to be nothing more than biochemical byproducts of aerobic metabolism, are now recognized as crucial signaling molecules at low levels, but cytotoxic and potentially carcinogenic at high levels. This duality makes the study of ROS complicated, particularly in relation to chronic diseases, including cancer. Cancer patients often have high systemic oxidative stress, which is further aggravated by cancer treatments, particularly radiation and chemotherapy. Most cancer cells have marked oxidative stress due to increased ROS production and compromised antioxidant defense, but certain cancer cells, such as prostate and metastatic cells, are known to have increased antioxidant enzyme capacity. ROS are thought to play a role in cancer risk and etiology, but cancer itself is known to increase ROS production, resulting in further cellular mutation and proliferation. Ironically, ROS are known to induce apoptosis and necrosis in cancer cells, and ROS production is a mechanism shared by all non-surgical cancer treatments.

ROS in cancer patients are likely associated with many of the side effects of cancer and its treatments, including fatigue, cachexia and anorexia, muscle weakness, and reduced cardiorespiratory function. Therefore, it is reasonable to employ strategies which counteract both the oxidative stress and physiological decrements associated with cancer and its treatments. Both AOX therapy and exercise are potential strategies. Because antioxidants have been shown to protect cancer cells in some circumstances, this method is not suggested without further research, even following treatment, as micrometastases may still be present. Additionally, because the delicate redox balance is dose-responsive and synergistic by nature, exogenous administration of antioxidants may be unwise even as a preventative measure without a complete understanding of the interactions. Therefore, exercise is a recommended strategy to reduce ROS and cancer treatment side effects without artificially altering the redox status and potentially protecting cancer cells.

CHAPTER III

METHODOLOGY

Introduction

The primary purpose of this study is to determine the effects of a 10-week aerobic and resistance prescribed exercise intervention on plasma markers of oxidative stress and antioxidant enzyme capacity in cancer survivors versus a non-exercising control group. Markers of oxidative stress that were measured included 8-OHdG and reactive carbonyl derivatives. Trolox-equivalent antioxidant capacity (TEAC) was measured as a universal marker of antioxidant capacity in the blood. Additionally, selected physiological variables, including peak VO_2 , muscular strength, and fatigue were compared between groups. Baseline oxidative stress and antioxidant enzyme capacity in cancer patients were compared to healthy individuals. The relationship between oxidative stress and baseline physiological variables was determined. Similarly, the relationship between the change in oxidative stress and changes in physiological variables was determined. This chapter describes the research methodology for the study and includes discussion of the experimental design, data collection procedures, and data analysis.

Experimental Design

This study consisted of a 10-week prescribed exercise intervention in cancer patients following oxidative stress-inducing cancer treatments, including radiation and several chemotherapy agents (see Table 3 on page 65). Subjects had completed radiation or chemotherapy treatment within the previous 6 weeks. Currently there is no good characterization of the time-course of chronic oxidative stress status following the end of cancer chemotherapy or radiation treatment in the literature. Many papers evaluate oxidative stress while patients are undergoing treatment (Cetin et al., 2004; Conklin, 2000; Honda et al., 2000; Sabitha & Shyamaladevi, 1999; Weijl et al., 1998) or within hours and days after treatment (Sangeetha et al., 1990), but rarely evaluate markers of oxidative stress or antioxidants more than a week after the end of treatment. In mice, extracellular MDA levels remained elevated one week after chemotherapy, and oxidized glutathione was significantly higher than controls 6 weeks after treatment (Abushamaa et al., 2002). Unfortunately, it is difficult to extrapolate these data to humans due to varying lifespans. Jonas et al. (Jonas et al., 2000) found that GSH/GSSG ratio continually decreased over the two weeks following the end of treatment, indicating either that treatment directly impairs the antioxidant system, or that chronic oxidative stress is capable of depleting the glutathione pool and diminish reductive capacity.

Cancer patients were assigned to an exercise group or a standard care control group. Pre- and post- intervention fasting blood samples were drawn from cancer patients to determine markers of oxidative stress (8-OHdG, protein carbonyls) and antioxidant capacity (Trolox equivalent antioxidant status). An initial screening and physical assessment was conducted in each cancer survivor in the exercise and control

groups. These assessments determined fatigue, cardiorespiratory endurance, and muscular strength. Additionally a group of age-matched healthy individuals without cancer (NC) completed the Piper Fatigue questionnaire (Appendix A) and had fasting blood samples drawn to determine the relative degree of fatigue and oxidative stress in cancer patients at baseline. Reassessments were obtained after a 10-week individually prescribed exercise intervention. The same physiological and psychological parameters were assessed using identical protocols during the initial assessment. See Figure 5 for experimental design.

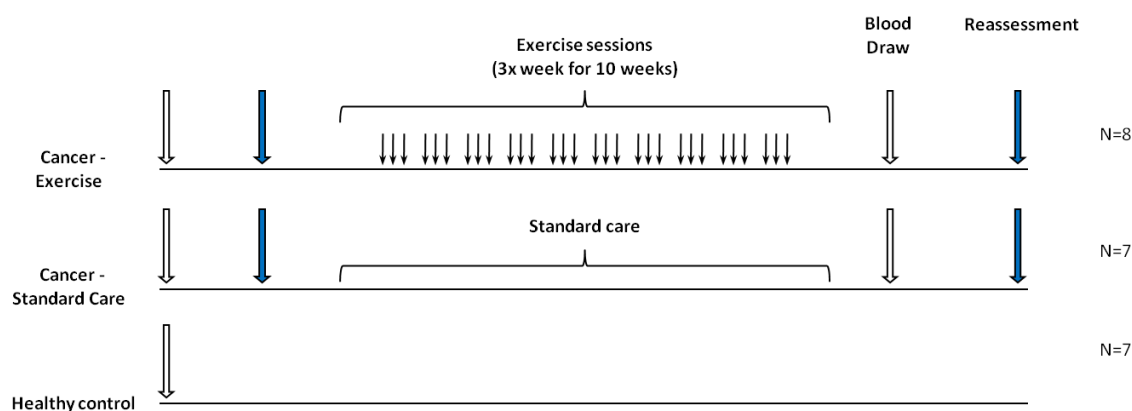


Figure 5. Experimental design

Subjects

Sixteen cancer survivors and eight healthy, age-matched subjects were recruited for participation in this study. A similar study on Parkinson's disease patients (Bloomer et al., 2008) determined that a sample size of 16 would be needed for adequate power, and this study was only twice per week, and only consisted of three sets of leg press, leg curl, and calf press for each session. Cancer patients were recruited from walk-in and

referred clients at RMCRI, as well as at McKee Hospital in Loveland, Poudre Valley Hospital in Fort Collins, and Northern Colorado Medical Center in Greeley.

Additionally, a newspaper article in the Greeley tribune was used to promote this study.

All cancer diagnoses were considered for this study, but subjects were required to have undergone radiation treatment, or one of the listed chemotherapy drugs (Table 3).

Cancer patient inclusion criteria consisted of: (1) completed radiation or approved cancer therapy regimen within 6 weeks of initial blood draw, (2) currently sedentary (less than two days/week or 20 minutes of aerobic or resistance exercise) (Brooks et al., 2005), (3) have not consistently supplemented with dietary antioxidants, including vitamins C and E, in the previous month (dietary half-life of ascorbate, for instance, is 8-40 days) (Otten, Hellwig, & Meyers, 2006), (4) non-smokers (for at least 6 months), and (5) were able to walk comfortably on a treadmill. Non-cancer, healthy controls were primarily recruited from among the faculty and staff at the University of Northern Colorado via campus-wide email and by word of mouth. This group was asked to fast for 12 hours, and avoid strenuous physical activity for 72 hours prior to their single, baseline blood draw.

Inclusion criteria for the non-cancer group included: (1) currently sedentary (less than two days/week or 20 minutes of aerobic or resistance exercise), (2) no history of cancer or other disease, (3) have not consistently supplemented with dietary antioxidants in the previous month, and (4) non-smokers. The University of Northern Colorado

Institutional Review Board (IRB) approved all procedures.

Data Collection Procedures

An initial phone interview with potential clients determined eligibility to participate, which included a complete cancer history discussion, and availability and willingness to complete the study. Subjects were informed that the study includes participation in 10 weeks of one-on-one exercise training at the Rocky Mountain Cancer Rehabilitation Institute, or standard care. Additionally, subjects were verbally given information regarding the nature of data collection, including the risk involved in a standard blood draw, and a maximal, graded treadmill test. After this brief discussion and clearance to exercise for high cardiac risk subjects by the RMCRI Medical Director, subjects met with the lead investigator and signed an informed consent.

Prior to arrival at RMCRI, subjects were mailed questionnaires to complete, including the Piper Fatigue Scale (Piper et al., 1998), lifetime physical activity, and medical history. For 72 hours prior to the blood draw, subjects were asked to avoid exhaustive exercise and antioxidant supplementation. The day before the cancer patient's initial exercise assessment, a 12-hour fasting blood sample was taken by the lead investigator, the RMCRI medical director, or an otherwise experienced phlebotomist. On the day of the initial assessment, the standard RMCRI assessment protocol (details to follow) was utilized. Height, weight, cardiorespiratory endurance, and muscular strength were evaluated, and results were used to develop individualized exercise interventions.

Piper Fatigue Inventory

The Piper Fatigue Inventory (Piper et al., 1998) was used to assess total cancer-related fatigue, as well as the subscales of behavioral, affective, sensory, and cognitive/mood fatigue. The behavioral fatigue subscale includes six questions and was

used to assess the impact of fatigue on school and/or work, interacting with friends, and overall interference with activities that are enjoyable. The affective fatigue subscale includes five questions and assessed the emotional meaning attributed to fatigue. The sensory fatigue subscale includes five questions and assessed the mental, physical, and emotional symptoms of fatigue. The cognitive and/or mood fatigue subscale includes six questions and assessed the impact of fatigue on concentration, memory, and the ability to think clearly. The average score on the 22 total questions from the subscales provides the total fatigue score. The range of possible scores on the subscales as well as total fatigue ranges from “0” to “10”. A score of a “0” indicates that the cancer survivor has no fatigue; scores ranging from “1” to “3” are indicative of mild fatigue; scores “4” to “6” suggest moderate fatigue; and scores of “7” or greater indicate severe fatigue.

Maximal Treadmill Testing

The RMCRI treadmill protocol (Table 5) was developed at the Rocky Mountain Cancer Rehabilitation Institute as a cancer-specific protocol, more suitable for the cancer population. Unlike the Bruce treadmill protocol, there are no abrupt increases in speed and grade. Smaller changes in effort and patient stride provide a more manageable progression through stages and results in reduced premature peripheral fatigue and more valid maximal treadmill tests (Bruce, Kusumi, & Hosmer, 1973). The RMCRI treadmill protocol begins at 1.0 mph and 0% grade during the first stage. For the next three stages, the speed increases 0.5 mph, but grade is maintained at 0%. Stage 5 increases the grade to 2% but maintains speed at 2.5 mph, and stage 6 increases to 3.0 mph and 2% grade. Stage 7 is 3.3 mph and 3% grade, and every subsequent stage increases 0.1 mph and 1%

grade. The subjects continue until volitional fatigue, at which point grade is reduced to 0% and speed is reduced to 1.5 mph for a three minute recovery period.

Table 5

RMCRIT Treadmill Protocol

Stage	Speed (mph)	Grade (%)	Time
1	1.0	0	1 min
2	1.5	0	1 min
3	2.0	0	1 min
4	2.5	0	1 min
5	2.5	2	1 min
6	3.0	2	1 min
7	3.3	3	1 min
8	3.4	4	1 min
9	3.5	5	1 min
10	3.6	6	1 min
11	3.7	7	1 min
12	3.8	8	1 min
13	3.9	9	1 min
14	4.0	10	1 min
15	4.1	11	1 min
16	4.2	12	1 min
17	4.3	13	1 min
18	4.4	14	1 min
19	4.5	15	1 min
20	4.6	16	1 min
21	4.7	17	1 min
Cool-Down	1.5	0	3 min

Prior to each incremental treadmill test, subjects were fitted with a heart rate monitor (Polar Inc., Lake Success, NY). Instructions regarding the specific changes in

speed and grade during each one-minute stage were given and the participant was encouraged to walk or run until exhaustion or volitional fatigue (to VO_2 peak). Blood pressure was assessed at rest, every three minutes during exercise, at immediate post exercise, and after three minutes of recovery (slow walking). Rating of perceived exertion was assessed every three minutes of exercise, according to Borg's 1-10 scale (Table 6). Heart rate and oxygen saturation was assessed at the end of every one-minute stage. Subjects were asked to only use handrails throughout the test if they required it for balance support. If they felt they would need handrails, they were asked to use them throughout the test.

Table 6

Borg Scale: Rating of Perceived Exertion

	Maximum Exertion
10	Very, very hard
9	
8	
7	Very hard
6	
5	Hard
4	Somewhat hard
3	Moderate
2	Light
1	Very light
0	No exertion

Adapted from Category-Ratio Perceived Exertion Scale: Relationship to Blood and Muscle Lactates and Heart Rate. *Med Sci Sports Exerc*, 15:523-528, 1983.

For individuals who were walking during the final stage, the ACSM metabolic equation (VO_2 (ml/kg/min) = $(0.1 \times S) + (1.8 \times S \times G) + 3.5$ ml/kg/min) was used, whereas the equation (VO_2 (ml/kg/min) = $(0.2 \times S) + (0.9 \times S \times G) + 3.5$ ml/kg/min) was used for individuals running during their final stage. For both equations "S" represents

treadmill speed, expressed as meters/minute, and “G” represents treadmill grade, expressed as a decimal, such that 0.08 would represent a grade of 8%, for example.

Muscular Strength Assessment

A true 1-repetition max (1-RM) assessment of strength can be the most accurate and reliable method of muscular strength assessment, but may be subject to variability due to unfamiliarity with weight lifting, lack of adequate facilities, and risk of injury, even in healthy individuals. In the cancer population, the variability and risk for injury are even greater. Therefore, the Brzycki equation,

$$1\text{-RM} = \text{weight lifted (lb)} / (1.0278 - (\text{repetitions to failure} \times 0.0278))$$

can be used to estimate 1-RM using the number of repetitions of a given submaximal load to fatigue, provided that the repetitions do not exceed 10 (Brzycki, 1993). Muscular strength was assessed using the lat pull-down, shoulder press, chest press, seated row, leg press, leg extension, and leg curl exercises, using Cybex® gym equipment (Cybex International, Medford MA). The goal was to choose a weight that will elicit muscle failure in 4-6 repetitions. Suggested values for the initial amount of weight lifted for the exercises were based upon previous muscular strength data from RMCRI (Table 7) (Schneider et al., 2003). Repetitions were performed without resting until muscle failure, or until the subject’s form deteriorates. If the client exceeded 10 repetitions for any exercise, they were stopped, and proceeded with the other muscular strength exercises. Following all exercises, those exercises which had been stopped were completed after increasing the weight to elicit muscle failure in less than 10 repetitions.

Table 7

Suggested initial resistance (% of Body Weight)

Exercise	Men	Women
Lat Pull-down	0.900	0.500
Shoulder Press	0.350	0.150
Chest Press	0.750	0.250
Seated Row	0.750	0.300
Leg Press	1.500	0.750
Leg Extension	0.750	0.400
Leg Curl	0.750	0.400

Lat Pull-Down

The participant sat on the bench seat with thighs positioned comfortably underneath the roller pads, which were adjusted if needed. The participant's hands were positioned on the bar slightly outside shoulder width, with palms facing inward (supinated) and torso remaining upright throughout the lift, without leaning forward or backward. A full repetition included a down motion, in which the training arm was pulled down until it is approximately even with the middle of the chest, and an up motion in which the training arm was allowed to rise until arms were extended but not locked.

Shoulder Press

The back of the seat was adjusted so that it was in the upright position, ensuring that the seat was locked. The participant sat on the seat with back and buttocks against the backrest. The participant's hands were placed on the lower handles at a comfortable width, depending on body size. Feet were placed flat on the floor approximately shoulder width apart. A full repetition included an up motion in which that training arm was raised

until arms were extended but not locked, and a down motion in which the training arm was lowered until the weight touched the top of the weight stack.

Chest Press

The participant sat on the seat with back and buttocks against the backrest and feet placed flat on the floor approximately shoulder-width apart. The seat was adjusted so the handles were at mid-chest height. A full repetition included an up motion in which the subject pressed outward until arms were at near full extension, and a down motion in which arms were lowered until elbows were at a 90° angle.

Seated Row

Seat height was adjusted to align the top of the participant's shoulders with the top of the handles. The chest pad was adjusted to allow both hands to reach the handles with arm fully extended. The participant sat upright, grasped the handles, and brought their elbows back, attempting to pinch shoulder blades together. The handles were returned to the starting position in a controlled manner, without slamming the weights back onto the stack.

Leg Press

The participant sat on the leg press seat with back flat against the backrest and buttocks tucked as far back as possible. The backrest was adjusted forward or backward until the upper leg was approximately perpendicular to the floor and there was a 90° bend in the participant's knees. The participant placed feet on the platform approximately shoulder width apart and in a position in which the quadriceps and hamstrings were exercised as evenly as possible. Prior to increasing the weight to the value indicated in Table 7, the participant performed several repetitions with no weight in order to

determine this position. A full repetition included an up motion in which the platform was pushed out until legs were fully extended, but knees not locked, and a down motion in which the platform was allowed to move back toward the participant until the weight touched the top of the weight stack.

Leg Extension

The back pad was adjusted to ensure that the participant's knees were aligned with axis of rotation. The participant's thighs were positioned parallel to each other and the shin pad was lifted to the appropriate position on the lower shin to ensure comfortable positioning. The participant's was instructed to relax ankles, grip handles firmly, and maintain proper posture by pressing back against the back pad. A full repetition including an up motion, in which the knees were straightened as far as possible without "kicking," and a controlled down motion in which the lower leg slowly returned to the starting position without slamming the weights back onto the stack.

Leg Curl

The back pad was adjusted to ensure that the participant's knees were aligned with axis of rotation. The leg starting position (0-12) was set so that knees were fully extended. The leg pad was adjusted for comfortable positioning slightly above ankles. The subject sat with lower legs resting upon pad, thighs parallel to each other and ankles relaxed. The thigh pad was lowered to fit securely across participant's thighs, while they held the grip handles on the thigh pad. A full repetition included a down motion in which the participant flexed their knees until they reached at least 90° without moving the pelvis or spine, and a controlled up motion in which the participant returned their legs to the starting position without slamming the weights back onto the stack.

Exercise Intervention

The lead investigator and Certified Cancer Exercise Specialists developed individualized exercise prescriptions and exercise interventions tailored to each subject in the exercise group based upon cancer history, health status, and results of the initial physical assessments. Subjects attended one-on-one supervised exercise sessions three days per week for the 10 week intervention. Prior to each training session, the lead investigator or Certified Cancer Exercise Specialists asked each participant a series of questions that elucidated whether it was necessary to alter that day's exercise intervention (Appendix E). Questions focused on how the participant felt after the last exercise session, if the participant had any soreness or specific problems that would affect training, and if changes in medication or treatment had been implemented since the last exercise session. The exercise sessions lasted one hour each, and consisted of 25 minutes of aerobic exercise, 25 minutes of resistance training, and 10-minutes of flexibility and/or balance training. Because the exercise intervention was personalized, there were some variations, but subjects spent a majority of the exercise session performing cardiovascular fitness, muscular strength and muscular endurance exercises.

The mode of aerobic exercise selected for each participant was based on the mode offering the greatest anticipated benefit. Options included treadmill walking, stationary cycling, and recumbent stepping. Initially, exercise intensity was based on the results from the treadmill assessment. Intensity was monitored via heart rate and RPE. The Karvonen, or percent heart rate reserve (HRR), method was used to determine exercise heart rate intensity utilizing the formula:

$$(220 - \text{age}) - \text{resting HR}] \times \text{percent of exercise intensity} + \text{resting HR}.$$

See Table 8 for recommended exercise intensity based on health and fitness status upon inclusion into the study. High intensity exercise was avoided, as it can result in excessive ROS production which may magnify, rather than reduce, chronic oxidative stress in cancer survivors due to inadequate antioxidant defenses associated with the disease and its treatments (Finaud et al., 2006; Fisher-Wellman & Bloomer, 2009; Poulsen et al., 1996).

Table 8

Recommended Exercise Intensity

Health and fitness status	Recommended intensity
Sedentary, poor health, low fitness	Start at 30-45% HRR; RPE of 1-3
Moderate health, average fitness	Start at 50-60% HRR; RPE of 4-5

Adapted from *Exercise and Cancer Recovery*, Schneider 2003, p. 87. Copyright 2003 Human Kinetics Publishers, Inc.

Resistance training followed the aerobic portion of the exercise session. Subjects progressively increased the resistance load over the 10-week intervention, and progressed through various modalities, including resistance tubing, Cybex® weight machines and free weights. Stability balls, balance pads, and Bosu™ balls were integrated into the resistance training exercises to increase proprioception and balance. Larger muscles were targeted first (chest, back, and thighs), followed by the smaller muscle groups (biceps and triceps). Patients performed 2-3 sets of each exercise, with 8-12 repetitions per set. Resistance was based upon the initial assessment, and was monitored by heart rate response to the exercise and RPE.

Cancer patients in the standard care control group were asked to maintain their current dietary and physical activity habits. Although standard care cannot be

“standardized” between hospitals and patients, it did not involve routine, systematic exercises (van Waart, Stuiver, van Harten, Sonke, & Aaronson, 2010). Following the 10-week study period, control subjects had the opportunity to exercise at RMCRI for three months at no cost. A stretching intervention was not utilized for the control group, as it may acutely increase oxidative stress, and chronically augment the antioxidant defense system. Although a control, stretching group was used by Campbell et al. (2010) without any change in F2-isoprostane levels in obese and overweight women, there was no significant difference between those engaged in aerobic exercise or stretching regimens. Moreover, passive stretch of isolated muscle fibers has been shown to increase cytosolic oxidant activity, (Chambers et al., 2009) and presumably could result in a hormetic response in antioxidant status over 12 weeks. Along the same lines, yoga is not a viable control intervention, as 40 days of yoga asanas (postures) reduced serum MDA from 5.9 ± 3.6 to 2.9 ± 2.3 mmol/ml in patients with non-insulin dependent diabetes mellitus (S. Singh et al., 2001).

Evaluation of Oxidative Stress

Blood was drawn from cancer patients on the day prior to both the initial assessment and the reassessment 10 weeks later. A minimum of 4mL of blood was withdrawn into sterile tubes containing potassium-EDTA and immediately put on ice. Samples were centrifuged at 3,000 rpm for 10 minutes, and the plasma was separated and stored in multiple aliquots at -80°C until analysis. All assays were performed in duplicate or triplicate on the first thaw. Protein carbonyls and 8-OHdG were measured in plasma using an enzyme-linked immunosorbent assay (ELISA) according to the procedures recommended by the manufacturer (Cell Biolabs, Inc., San Diego, CA).

Antioxidant capacity was measured in serum using the Trolox-equivalent antioxidant capacity (TEAC) assay using procedures outlined by the reagent provider (Sigma Chemical, St. Louis, MO).

Biochemical Assays

Reactive carbonyl derivatives were measured as a marker of oxidative protein damage. A pilot study at RMCRI indicated that an exercise intervention reduced plasma protein carbonyl concentration by 76% in an individual undergoing high dose chemotherapy (unpublished data). Oxidative DNA damage was determined by plasma 8-OHdG. This value is associated with the risk of cancer development and recurrence (Yamamoto et al., 1996). To assess plasma antioxidant capacity and concentrations of 8-OHdG and protein carbonyls, the following biochemical methodologies were used.

Protein Carbonyls

The OxiSelect™ Protein Carbonyl ELISA kit (Cell Biolabs, Inc., San Diego, CA) was used to assess byproducts of protein oxidation present in blood plasma. In this enzyme immunoassay, 100 µl of 10 µl/mL diluted protein samples or standards with known concentrations of oxidized bovine serum albumin (BSA) were adsorbed to each well in the provided 96-well Protein Binding Plate and incubated overnight at 4°C. Protein carbonyls present in the plasma sample or standard were derivatized to dinitrophenylhydrazine (DNPH), and probed with an anti-DNP antibody and a horseradish peroxidase (HRP) conjugated secondary antibody. After adding a stop solution, the results were read immediately at 405 nm using a plate reader.

8-OHdG

The OxiSelect™ Oxidative DNA Damage ELISA kit (8-OHdG Quantitation) (Cell Biolabs, Inc., San Diego, CA) was also utilized for analysis of DNA oxidation. 50 µl of plasma or 8-OHdG standard was added to each well of a 96-well 8-OHdG Conjugate coated plate provided by the manufacturer. Following a 10 minute incubation, 50 µl of anti-8-OHdG antibody was added to each well and incubated for an hour. Wells were washed and 100 µl of HRP secondary antibody was added and incubated for another hour. After washing well, 100 µl of room temperature substrate solution was added to each wells and incubated for approximately 30 minutes. 100 µl of stop solution was added to each well and the results were read immediately at 450 nm using a plate reader.

Trolox Equivalent Antioxidant Capacity

A commercially available assay kit (Sigma-Aldrich, Saint Louis, MO) was use to evaluate plasma antioxidant capacity, with 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) serving as a control antioxidant. The principle of this assay is the formation of a ferryl myoglobin radical from metmyoglobin (derived from horse heart) and hydrogen peroxide. This oxidizes with 2,2'azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) to produce ABTS^{•+}, a soluble chromogen radical that can be determined spectrophotometrically at 405nm. Antioxidants present in blood plasma samples or Trolox standards suppress the production of this radical in a concentration dependent manner, with a proportional decrease in color intensity. 10 µl or Trolox standard or test sample and 20 µl or Myoglobin Working Solution was added to each well in a 96 well plate. ABTS Substrate working solution was incubated in wells for 5

minutes, at which time a stop solution was added and the plate was read within an hour at 405 nm using a plate reader

Other studies have examined exercise-associated changes in antioxidant enzyme capacity in healthy (Bloomer, 2008; Ji, 2002; Powers et al., 1999), diseased (Bloomer et al., 2008; Fisher-Wellman, Bell, & Bloomer, 2009) and elderly (Carvalho et al., 2010; Hammeren et al., 1992) populations. Because the antioxidant defense system is complex and not fully elucidated, measurement of individual enzymes may not appropriately represent the dynamics of antioxidant status, as other mechanisms (non-enzymatic antioxidant status, transport mechanisms) may be involved in the adaptive response to exercise. For instance, despite an attenuation of doxorubicin-associated lipid peroxidation (as measured by MDA) and cardiotoxicity, exercise pre-training in rats failed to increase SOD activity (Chicco, Schneider, & Hayward, 2005). It should be noted that null findings in some studies were likely due to an insufficient variety of analyzed biomarkers (Fisher-Wellman et al., 2009). Therefore, TEAC may be a suitable marker for examination of changes in antioxidant status, as it is non-specific, and determines total antioxidant activity in a sample.

Statistical Analysis

Data were presented as means \pm SD. Subjects' baseline characteristics in the two cancer groups (exercise training versus control) and the non cancer group were compared using independent *t*-tests. A repeated measures 2 (group) \times 2 (pre- and post-intervention) analysis of variance (ANOVA) with Bonferroni post hoc was used to determine differences within (across time) and between the two groups related to markers of oxidative stress, antioxidant capacity, VO₂peak, muscular strength, and fatigue. Based on

a normal probability plot and the Kolmogorov-Smirnov test, the residuals from the model(s) show no evidence of a lack of normality. Therefore the standard repeated-measures ANOVA model appears appropriate for the data. Spearman correlation coefficients were calculated to determine associations between baseline markers of oxidative stress and baseline VO₂peak, muscular strength, and fatigue. Additionally, Spearman correlation coefficients were calculated to determine associations between post-intervention changes in markers of oxidative stress and post-intervention changes in VO₂peak, muscular strength, and fatigue. Although repeated measures ANOVA was used for analysis of change within the cancer population, the nonlinear Spearman's rank correlation was used due to the expectation of a nonlinear trend, as antioxidant and oxidative stress values may plateau at optimal levels in both healthy and cancer subjects, even as fitness and fatigue parameters continue to improve. A significance level of $\alpha = 0.05$ was used for all statistical analyses.

CHAPTER IV

CANCER RELATED FATIGUE AND MARKERS OF OXIDATIVE STRESS IN CANCER PATIENTS FOLLOWING AN EXERCISE INTERVENTION

Abstract

PURPOSE. The last two decades have produced an abundance of research indicating that exercise interventions in cancer patients are capable of reducing cancer-treatment related side effects, including fatigue. Cancer-related fatigue is a somewhat nebulous concept, as both physiological and psychological factors contribute to this phenomenon. Although the underlying mechanisms of cancer-related fatigue are not fully characterized, treatment-associated oxidative stress is thought to play a role. The purpose of this study was to determine the effect of an exercise intervention on blood markers of oxidative stress, antioxidant status, and cancer-related fatigue compared to a cancer control group. **METHODOLOGY.** An initial fasting blood draw and fatigue questionnaire were administered to fifteen cancer patients within six weeks of completing radiation or chemotherapy treatment, as well as seven age-matched individuals with no history of cancer (NC) to compare baseline values. Eight of the cancer patients participated in a 10-week exercise intervention (EX), while seven continued standard care (CON). Following the intervention, another fasting blood draw and fatigue questionnaire

were administered. Changes in plasma 8-OHdG and antioxidant status were compared between the EX and cancer CON groups. Baseline markers of oxidative stress were compared between NC subjects and cancer patients. A correlation analysis of fatigue and oxidative stress in all subjects was conducted. **RESULTS.** Mean total fatigue scores decreased significantly from 5.0 ± 2.2 to 2.6 ± 1.5 ($p < 0.05$) in EX, whereas changes in CON (4.7 ± 2.5 to 3.2 ± 2.4) were not significant. All fatigue subscales significantly decreased in EX, while only cognitive fatigue increased significantly in CON ($p < 0.05$). No group by time interaction effect between groups was found for fatigue scores. Total antioxidant capacity increased significantly over time in EX ($p < 0.01$), but not in CON. No significant changes over time were found in 8-OHdG, but a group by time interaction effect was detected ($p < 0.05$). Baseline antioxidant capacity significantly correlated ($p < 0.05$) with total ($r = -0.41$) affective ($r = -0.39$), sensory ($r = -0.40$), and cognitive fatigue ($r = -0.57$). Increases in antioxidant capacity were correlated with reductions in affective ($r = -0.49$), sensory ($r = -0.47$), and cognitive fatigue ($r = -0.58$). Although 8-OHdG was not correlated with any fatigue parameter at baseline, changes in total ($r = 0.46$) and affective ($r = 0.47$) fatigue exhibited significant correlations with changes in 8-OHdG over time. **CONCLUSION.** An exercise intervention reduced fatigue in cancer patients following cessation of treatment, whereas standard care resulted in non-significant reductions in fatigue. Oxidative stress may be implicated in cancer-related fatigue, while improved antioxidant capacity following an exercise intervention may play a role in mitigating fatigue.

Introduction

The National Cancer Institute estimates that 13.7 million Americans with a history of cancer were alive in 2012, and about 1,665,540 new cancer cases are expected

to be diagnosed in 2014 (American Cancer Society, 2014). Although survival rates continue to improve, these patients can expect to experience adverse side effects of surgery, radiation, chemotherapy, and other treatment modalities that may last for years. Typical side effects include muscle weakness (Christensen et al., 2014; Schneider et al., 2007a), reduced cardiorespiratory fitness (Burnett, Kluding, Porter, Fabian, & Klemp, 2013), and general fatigue (Campos, Hassan, Riechelmann, & Del Giglio, 2011; Puetz & Herring, 2012), resulting in an overall decrease in quality of life.

Acute and chronic cancer-related fatigue associated with radiation therapy, chemotherapeutic agents, and the tumor burden itself, have been well documented for decades (Piper et al., 1989; Questad et al., 1982; Rieger, 1988; Smets et al., 1993). Up to 90% of cancer patients report experiencing fatigue following cancer treatment, and it is commonly reported as the most problematic side effect for cancer survivors (Hofman, Ryan, Figueroa-Moseley, Jean-Pierre, & Morrow, 2007). Cancer-related fatigue is similar in nature to chronic fatigue syndrome in terms of symptoms and etiology (Rovigatti, 2012). Both conditions are more chronic and debilitating than standard fatigue, causing individuals to alter their behavior and demeanor, but all patients experience cancer-related fatigue differently (de Jong et al., 2002).

Because fatigue is both a physiological and psychological condition, it is difficult to identify a specific mechanism, but there are likely many. Some proposed factors contributing to fatigue are muscular wasting related to cachexia (Christensen et al., 2014; Gilliam & St Clair, 2011); sleep disturbances (Roscoe et al., 2007); psychological depression; (Piper et al., 1987); anemia (de Jong et al., 2002); and metabolic imbalance due to increased energy requirement (e.g., due to tumor growth), decreased metabolic

capacity (mitochondrial damage), or decreased substrate availability associated with anorexia, nausea, or vomiting (Glaspy, 2001). Additionally, many of the mechanisms responsible for exercise-associated fatigue have been postulated to contribute to cancer-related fatigue, including serotonin dysregulation, hypothalamic-pituitary-adrenal (HPA) axis dysfunction, vagal afferent nerve activation, and cytokine dysregulation (Ryan et al., 2007).

Although cancer-related fatigue is undoubtedly a product of a combination of these physiological and psychological factors, and may vary between individuals, oxidative stress is thought to play a pivotal role in many pathological processes associated with cancer and its treatments (Fang et al., 2009; Robbins & Zhao, 2004; Zhao & Robbins, 2009). Oxidative stress is a state in which reactive oxygen species (ROS) are produced at a rate that exceeds cellular adaptive and repair capacities, resulting in damage to local tissues, including lipid membranes, protein structures, and nucleic acids, (Halliwell & Gutteridge, 2007). Cancer cells often are characterized by elevated levels of oxidative stress. ROS production associated with breast cancer is common enough that a decreased GSH/GSSG ratio (reduced to oxidized glutathione ratio) has been identified as an important biochemical parameter for detecting breast malignancy (Yeh et al., 2006). Additionally, radiation and many chemotherapy regimens depend on ROS as an antineoplastic mechanism (Kovacic, 2007; Tandon et al., 2005). Although ROS exert a greater cytotoxic effect on cancer cells, cancer treatments are associated with collateral oxidative damage to healthy tissues (Chen, Jungsuwadee, Butterfield, & St Clair, 2007). Any disruption in antioxidant capacity of healthy cells can alter redox balance and health

status, potentially resulting in acute and chronic side effects, including cancer-related fatigue.

While cancer-related fatigue has been theoretically tied to oxidative stress (Gilliam & St Clair, 2011), and many of the proposed mechanisms previously mentioned can be driven by ROS and oxidative stress, there is a general lack of evidence explicitly relating oxidative stress to cancer-related fatigue. Gramignano et al. (2006), demonstrated that four weeks of L-carnitine administration simultaneously reduced fatigue and resulted in a non-significant decrease in oxidative stress in patients currently undergoing cancer treatment. Additionally, chronic fatigue syndrome has been associated with oxidative stress (Fulle et al., 2000; Jammes et al., 2005; Richards et al., 2000; Richards et al., 2007) and deficiency of the antioxidant glutathione (Bounous & Molson, 1999). Maes et al. (2009) found that 8-hydroxy-deoxyguanosine (8-OHdG), a marker of oxidative damage to DNA, was elevated in individuals suffering from both depression and chronic fatigue syndrome. DNA oxidation is linked to oncogenesis, as well as atherosclerosis and neurodegeneration, factors that are associated with psychological and cognitive dysfunction. Accordingly, oxidative stress may represent a potential mechanistic link between cancer and psychological dysregulation, including cancer-related fatigue.

Until recently, oncologists and primary care physicians treating cancer patients prescribed rest as a strategy to counteract cancer-related fatigue. Although it is somewhat counterintuitive, research has shown that physical activity is a more effective method of reducing acute and chronic fatigue in cancer survivors. Whereas exercise has been shown to have significant therapeutic benefit (Brown et al., 2011; F. C. Dimeo, 2001;

Schneider & Hayward, 2013), daytime inactivity may actually worsen fatigue symptoms (Berger & Farr, 1999). In particular, resistance and aerobic exercise have been found to be beneficial as a means to reduce fatigue, while improving quality of life (Ferrer, Huedo-Medina, Johnson, Ryan, & Pescatello, 2011), muscular strength and endurance (Focht et al., 2013; Schneider et al., 2007a), cardiorespiratory endurance (Courneya et al., 2007; L. W. Jones, Liang, et al., 2011; Lakoski, Eves, Douglas, & Jones, 2012; Schneider et al., 2007c), and even survival rates (Holmes, Chen, Feskanich, Kroenke, & Colditz, 2005; Irwin et al., 2011). Various methods of prescribed exercise, including resistance and aerobic exercise, both supervised and at home, have been shown to improve fatigue symptoms in cancer patients following treatment (Brown et al., 2011).

Although exercise interventions are known to improve antioxidant capacity in healthy individuals (Ji, 2008; Powers, Sollanek, Wiggs, Demirel, & Smuder, 2014; Radak, Chung, Koltai, Taylor, & Goto, 2008), minimal research on exercise-associated oxidative stress modifications in cancer patients is available. Allgayer et al (2008) demonstrated reductions in urinary 8-OHdG excretion, from 8.47 to 5.81 ng/mg creatinine ($p = 0.02$), after two weeks of moderate intensity exercise, while two weeks of high intensity exercise training resulted in a non-significant increase in 8-OHdG excretion. More recently, Jones et al (2011) demonstrated a nonsignificant gain (+ 32%, $p = 0.08$) in F2-isoprostanes following a high intensity 3-month exercise program. These disparate results indicate that, among other factors, exercise intensity likely plays a role in the redox balance response to exercise interventions. In animal models, exercise has been shown to attenuate oxidative damage and reduce treatment-associated side effects following treatment with doxorubicin (Ascensao et al., 2005a; Wonders et al., 2008).

Interestingly, individual antioxidant enzymes, such as superoxide dismutase, have been shown to increase with exercise training in rodents in some studies (Kanter et al., 1985) but not others (Chicco et al., 2005). This may be explained by the plastic and time-sensitive nature of redox signaling. For instance, although exercise training in rats resulted in increased activity of the antioxidant enzyme catalase in left ventricular tissue, upon cessation of exercise, catalase activity returned to pre-exercise values by day 9 (Lennon et al., 2004).

It is possible that the exercise-mediated reductions in oxidative stress are in part responsible for exercise-associated decreases in cancer-related fatigue, but to date, this relationship is largely uncharacterized. Therefore, the purpose of this study was to investigate changes in total antioxidant capacity, DNA oxidation, and fatigue parameters in cancer patients following a whole body exercise intervention versus a cancer control group. Furthermore, correlations between baseline fatigue and oxidative stress, as well as changes in oxidative stress and fatigue parameters were evaluated.

Methodology

This study consisted of a 10-week prescribed exercise intervention in cancer patients following oxidative stress-inducing cancer treatments, including radiation or chemotherapy. Subjects had completed radiation or chemotherapy treatment within the previous 6 weeks, with an average time out of treatment of 4 weeks. Fifteen cancer patients and seven individuals with no history of cancer or other chronic diseases (NC) participated in this study. Cancer patients were admitted to either an exercise intervention group (EX) or a standard care group (CON) based upon date of initial contact (pseudo-randomization), while NC subjects were only evaluated at baseline. Cancer patients were recruited from walk-in and oncologist referred patients at the Rocky Mountain Cancer

Rehabilitation Institute (RMCRI) at the University of Northern Colorado (UNC) in Greeley, Colorado. Additionally, oncology patient navigators and clinical oncology research specialists at Northern Colorado Medical Center in Greeley, Poudre Valley Hospital in Fort Collins, and McKee Hospital in Loveland, Colorado identified qualifying individuals as they completed radiation or chemotherapy treatments. Newspaper articles in the Greeley Tribune and UNC today, posters at the local American Cancer Society office and outpatient oncology waiting areas, and a recruitment page on the RMCRI webpage were used to promote this study.

Twenty six cancer patients made contact with the primary investigator, but exclusion criteria or attrition resulted in the loss of 11 potential subjects. Refer to Figure 6 for flow of the subjects through this study. All cancer diagnoses were considered for this study, but subjects were required to have undergone radiation or a chemotherapy treatment explicitly known to elicit oxidative stress, either as a side effect or as an antineoplastic mechanism. Chemotherapy agents included anti-neoplastic antibiotics, alkylating agents, platinum coordinating complexes, epipodophyllotoxins, fluorouracil and tamoxifen (Y. Chen et al., 2007; Conklin, 2004; Look & Musch, 1994). Cancer patient inclusion criteria consisted of: (1) completed radiation or approved cancer therapy regimen within six weeks of initial blood draw, (2) currently sedentary (less than two days/week or 20 minutes of aerobic or resistance exercise) (Brooks et al., 2005), (3) have not consistently supplemented with dietary antioxidants, including vitamins C and E, in the previous month, (4) non-smokers (for at least 2 months), and (5) were able to walk comfortably on a treadmill.

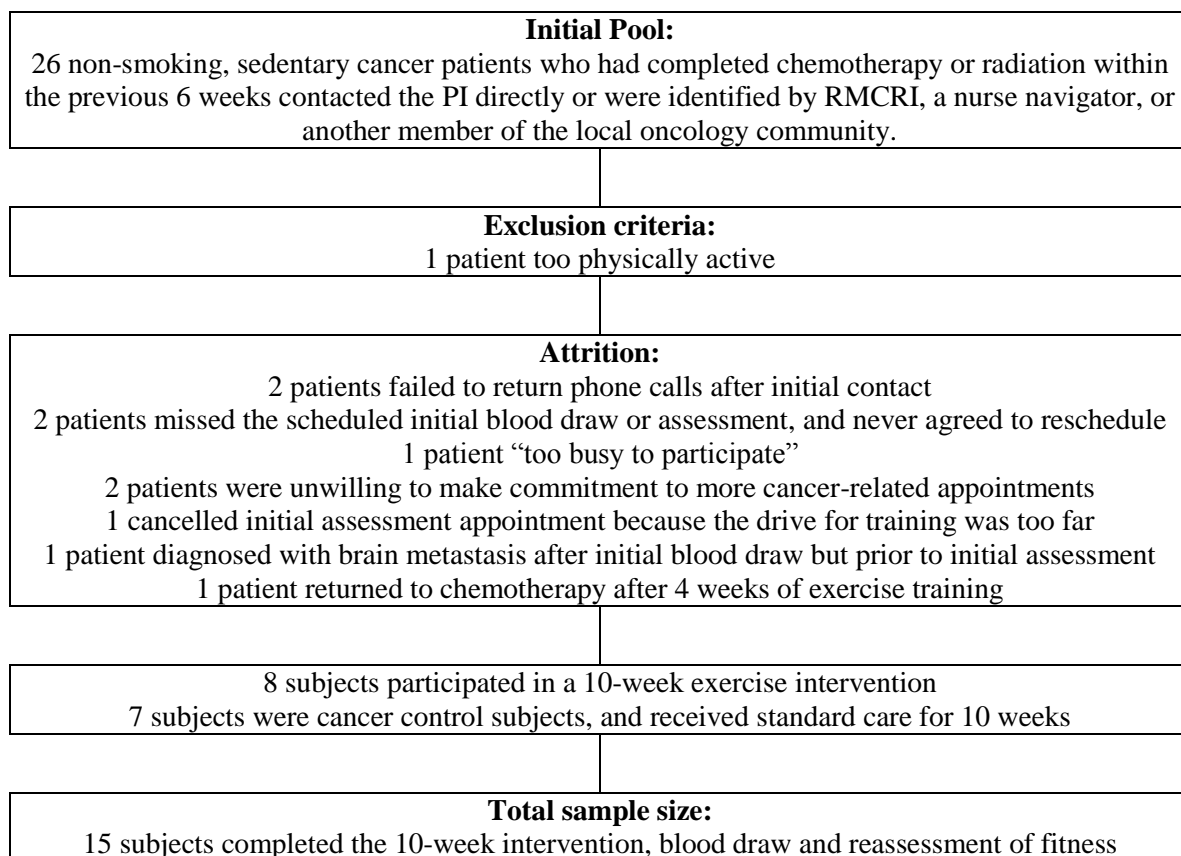


Figure 6. Flow of cancer patients through the study.

Non-cancer, healthy controls were primarily recruited from among the faculty and staff at the University of Northern Colorado via campus-wide email and by word of mouth. Inclusion criteria for the non-cancer group included: (1) currently sedentary (less than two days/week or 20 minutes of aerobic or resistance exercise), (2) no history of cancer or other major chronic disease, (3) have not consistently supplemented with dietary antioxidants in the previous month, and (4) non-smokers. The University of Northern Colorado Institutional Review Board (IRB) approved all procedures.

Prior to arrival at RMCRI, all cancer and non-cancer subjects were provided with the Piper Fatigue Scale (Piper et al., 1998) to complete within 24 hours of the initial blood draw. For 72 hours prior to the blood draw, subjects were asked to avoid

exhaustive exercise and antioxidant supplementation. The day before the cancer patient's initial exercise assessment, a 12-hour fasting blood sample was taken by the lead investigator or an otherwise experienced phlebotomist.

Piper Fatigue Inventory

The Piper Fatigue Inventory (Piper et al., 1998) was used to assess total cancer-related fatigue, as well as the subscales of behavioral, affective, sensory, and cognitive/mood fatigue. The behavioral fatigue subscale includes six questions and was used to assess the impact of fatigue on school and/or work, interacting with friends, and overall interference with activities that are enjoyable. The affective fatigue subscale includes five questions and assessed the emotional meaning attributed to fatigue. The sensory fatigue subscale includes five questions and assessed the mental, physical, and emotional symptoms of fatigue. The cognitive and/or mood fatigue subscale includes six questions and assessed the impact of fatigue on concentration, memory, and the ability to think clearly. The average score on the 22 total questions from the subscales provides the total fatigue score. The range of possible scores on the subscales as well as total fatigue ranges from "0" to "10". A score of a "0" indicates that the cancer survivor has no fatigue; scores ranging from "1" to "3" are indicative of mild fatigue; scores "4" to "6" suggest moderate fatigue; and scores of "7" or greater indicate severe fatigue.

Fitness Assessment

In order to appropriately prescribe an individualized exercise intervention, an initial assessment of cardiorespiratory endurance, muscular strength, flexibility, and balance was performed. An incremental treadmill test to volitional fatigue was used to assess initial cardiorespiratory fitness. A battery of seven muscular strength

measurements (predicted one-repetition maximum [1-RM]) was utilized to allow for appropriate prescription of resistance exercise, and included lat pull-down, shoulder press, chest press, seated row, leg press, leg extension, and leg curl exercises, using Cybex® gym equipment (Cybex International, Medford MA). Flexibility was assessed with a modified sit and reach test and the back scratch test, while the Bertec Balance Check™ Screener and Trainer Package (Bertec Corporation, Columbus OH) was used to assess postural stability and limits of stability. The RMCRI treadmill protocol, an internally validated graded exercise test to volitional fatigue, determined maximal aerobic capacity.

Exercise Intervention

Each exercise participant completed a 10-week exercise intervention that was individually supervised by a cancer exercise specialist 3 days per week. Each 1-hour session was in accordance with the subject's prescribed training modes, intensity, and volume, based upon their goals and assessment results. Exercise sessions varied depending on the subject's health and fitness status, but typically included a 5-minute warm-up, 20 minutes of aerobic exercise, 25 minutes of resistance training, and 10 minutes of flexibility and balance training. Blood pressure and HR were measured at the beginning and end of the exercise session, and HR, RPE, and SaO₂ were monitored throughout. Aerobic exercise intensity was based on the survivors' initial VO₂peak, and was generally low to moderate intensity, generally ranging from 30% to 60% of heart rate reserve (HRR). The Karvonen or percent heart rate reserve method was used to determine exercise heart rate intensity using the formula

$$[(220 - \text{age}) - \text{resting HR}] \times \text{percent of exercise intensity} + \text{resting HR}.$$

Subjects with poor overall health status health or low fitness were initially prescribed an intensity level of 30%-45% HRR or a RPE of 1-3, whereas subjects with a moderate health status or average fitness began their aerobic exercises at 50%-60% of HRR or an RPE of 4-5. The mode of aerobic exercise selected for each subject was based on the mode offering the greatest anticipated benefit. Options included outdoor or treadmill walking, stationary cycling, or recumbent stepping. Although rate of progression varied significantly by subject, cancer exercise specialists aimed to increase increases speed or resistance weekly, while keeping RPE values below 5 and HR values below 75% HRR.

At the beginning of the intervention, initial percent of 1-RM corresponded with the percent of HRR values for aerobic exercise, based upon initial health and fitness status (30%-45% 1-RM for low fitness, 50%-60% for average fitness). Cybex Eagle Selectorized Strength Machines[®] (Cybex International Inc, Medway, MA, USA), free weights, resistance bands and body weight exercises were all used for the exercise intervention, depending upon fitness, mobility, and comfort of the cancer patient. Muscular endurance was emphasized over hypertrophic training, as subjects performed 8-15 repetitions per set and typically ended each set at muscular fatigue rather than muscle failure. Resistance was increased when 8-15 repetitions no longer resulted in muscular fatigue.

All cancer patients were asked to avoid any substantial changes in their diet, assuming a physician or nutritionist did not prescribe a change in eating habits. Subjects in the standard care control group were asked to maintain their exercise habits during the 10-week study period. Although standard care cannot be “standardized” between hospitals and patients, it did not involve routine, systematic exercises (van Waart et al.,

2010). Neither yoga nor stretching programs were utilized for the control group because both interventions have the potential to alter markers of oxidative stress and antioxidant capacity (Chambers et al., 2009; S. Singh et al., 2001). Control subjects were asked to complete an exit survey inquiring about any changes in physical activity, dietary habits, medications, and medical procedures. Following the 10-week study period, control subjects had the opportunity to exercise at RMCRI for three months at no cost.

Blood Handling and Analysis

A minimum of 4mL of blood was withdrawn into sterile tubes containing potassium-EDTA and immediately put on ice. Samples were centrifuged at 3,000 rpm for 10 minutes, and the plasma was separated and stored in multiple aliquots at -80°C until analysis. All assays were performed in duplicate or triplicate on the first thaw. Oxidative DNA damage was determined by plasma 8-OHdG, which was measured using an enzyme-linked immunosorbent assay (ELISA) according to the procedures recommended by the manufacturer (Cell Biolabs, Inc., San Diego, CA). This value is associated with the risk of cancer development and recurrence (Yamamoto et al., 1996). Antioxidant capacity was measured in plasma using the Trolox-equivalent antioxidant capacity (TEAC) assay using procedures outlined by the reagent provider (Sigma Chemical, St. Louis, MO).

Statistical Analyses

Data are presented as means \pm SD. Subjects' baseline characteristics in the two cancer groups (EX versus CON) and the NC group were compared using independent *t*-tests. A repeated measures 2 (group) \times 2 (pre- and post-intervention) analysis of variance (ANOVA) with Bonferroni post hoc was used to determine differences within

(across time) and between the two groups related to markers of DNA oxidation, antioxidant capacity, and fatigue. A Kolmogorov-Smirnov was used to ensure that the normality assumption was met for this data set. Spearman correlation coefficients were calculated to determine associations between baseline markers of oxidative stress and baseline fatigue. Additionally, Spearman correlation coefficients were calculated to determine associations between post-intervention changes in markers of oxidative stress and post-intervention changes in fatigue. A significance level of $\alpha = 0.05$ was used for all statistical analyses.

Results

Baseline subject characteristics for the EX, CON, and NC groups are summarized in Table 9. The two cancer groups had statistically similar height, weight, body mass index, and time out of treatment, and no significant differences in age were found between the three groups. Although exit surveys in control subjects indicated no notable changes in dietary habits, qualitative physical activity in this group increased from baseline as, even in the absence of an exercise intervention, time out of treatment resulted in greater vigor for activities of daily living and leisure time activity. Increases in housework, light yard work, and walking were most commonly reported, but no control subjects reported participation in a structured exercise program.

Table 9

<i>Subject Characteristics</i>			
	Exercise	Control	Non-Cancer
N	8	7	7
Age (years)	64.0 ± 10.8	62.4 ± 9.7	55.1 ± 9.7
Females	7	4	5
Males	1	3	2
Height (in)	65.4 ± 2.5	66.8 ± 4.7	-
Weight (lbs)	174.0 ± 32.3	179.5 ± 40.8	-
BMI	28.4 ± 4.3	28.1 ± 3.8	-
Primary treatment (# of subjects)			
Radiation	4	4	-
Chemo	3	4	-
Days out of Treatment	29.9 ± 18.6	31.4 ± 21.7	-
Exercise adherence rate (%)	83.9 ± 12.8	-	-

Baseline fatigue parameters, including total fatigue as well as all four subcategories of the Piper Fatigue Scale, were significantly elevated in the cancer population compared to the age-matched, non-cancer population (Table 10). Mean total fatigue values were greater than 4 out of 10 in both cancer groups, resulting in a “moderate fatigue” classification. In the EX group, 3 subjects reported mild fatigue, 4 subjects reported moderate fatigue, and 1 subject was severely fatigued. The CON group displayed similar baseline fatigue distribution, as 2 subjects were mildly fatigued, 4 subjects moderately fatigued, and 1 subject severely fatigued. The NC group had a total fatigue score of 1.0, which corresponds with the cutoff for “no fatigue” and “mild fatigue.” Four subjects were classified as having “no fatigue” and 3 were classified with “mild fatigue.”

Table 10

Baseline Fatigue Scores

	Total	Behavioral	Affective	Sensory	Cognitive
Cancer (N = 15)	4.8 ± 2.3 ^a	4.4 ± 3.1 ^a	5.5 ± 2.4 ^a	5.5 ± 2.3 ^a	4.8 ± 2.1 ^a
NC (N=7)	1.0 ± 1.0	0.9 ± 1.3	0.7 ± 0.8	1.4 ± 1.3	1.2 ± 1.2

^a Significantly greater than NC

Following the 10 week study period, total fatigue and all four fatigue subcategories were significantly reduced in EX, while only cognitive fatigue significantly diminished in CON. Fatigue parameter means at all time points are displayed in Figures 7-11, and changes over time are tabulated in Table 11. No group by time interaction effects were found between EX and CON for any fatigue parameter. Mean total fatigue in the EX group fell from 5.0 ± 2.3 to 2.6 ± 1.9 ($p < 0.01$), indicating a change in group mean classification from “moderate fatigue” to “mild fatigue.” The only subject in this group failing to downgrade classification status was the individual with mild fatigue at baseline, and therefore little room for improvement. Mean total fatigue in the CON group dropped from 4.6 ± 2.4 at baseline to 3.2 ± 2.4 at follow-up, but this represented neither a statistically significant change, nor a change in group mean classification. Additional post-hoc analysis revealed that behavioral and affective fatigue in the exercise group no longer statistically differed from the NC group ($p > 0.05$) following the 10-week exercise intervention.

Table 11

Changes in Fatigue Scores

	Total	Behavioral	Affective	Sensory	Cognitive
EX (N=8)	-2.4 ± 0.9 ^a	-2.6 ± 2.4 ^b	-3.4 ± 1.9 ^a	-2.5 ± 1.2 ^a	-1.8 ± 1.1 ^a
CON (N=7)	-1.5 ± 2.1	-1.2 ± 2.1	-1.6 ± 2.9	-1.5 ± 2.2	-2.2 ± 1.7 ^a

^a Significant change over time ($p < 0.01$)

^b Significant change over time ($p < 0.05$)

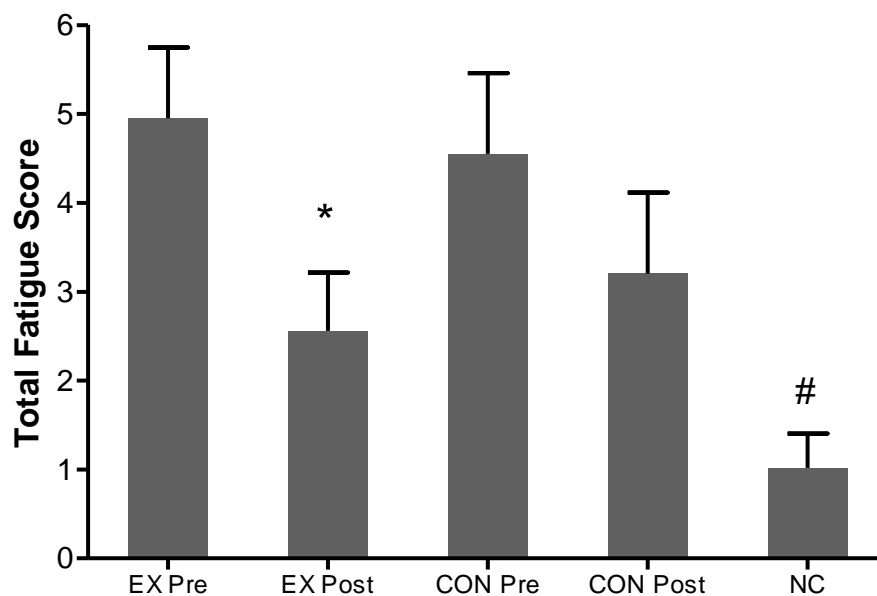


Figure 7. Total fatigue scores for all groups and time points.

*= significantly different than EX Pre ($p < 0.01$). # = Significantly different than EX Pre, CON Pre ($p < 0.01$), EX Post and CON Post ($p < 0.05$).

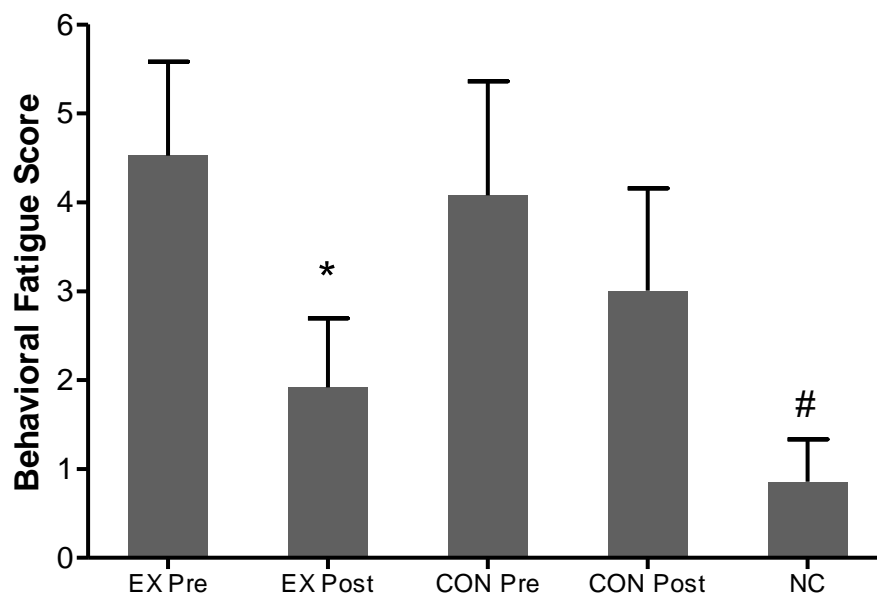


Figure 8. Behavioral fatigue scores for all groups and time points.

*= significantly different than EX Pre ($p < 0.05$). # = Significantly different than EX Pre, CON Pre ($p < 0.01$), and CON Post ($p < 0.05$).

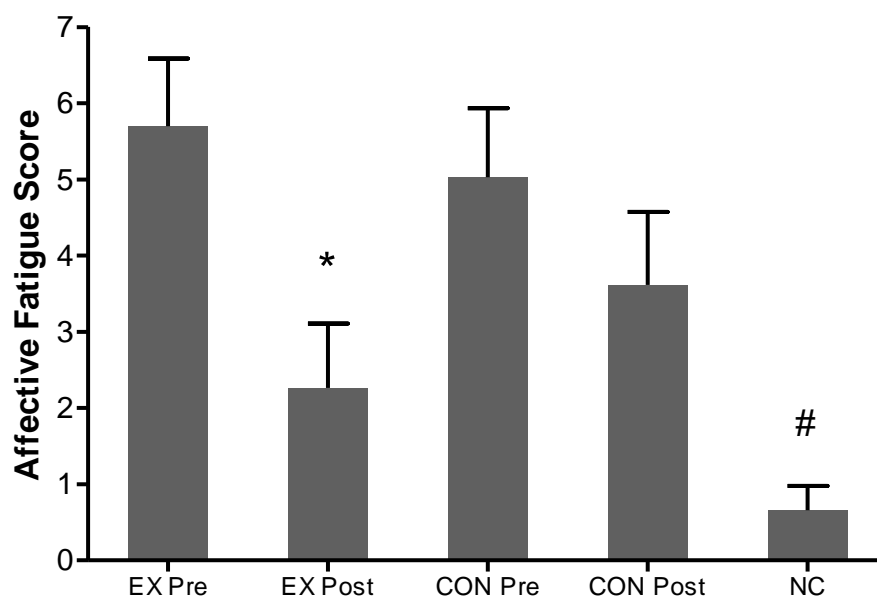


Figure 9. Affective fatigue scores for all groups and time points.
 *= significantly different than EX Pre ($p < 0.01$). # = Significantly different than EX Pre, CON Pre, and CON Post ($p < 0.01$).

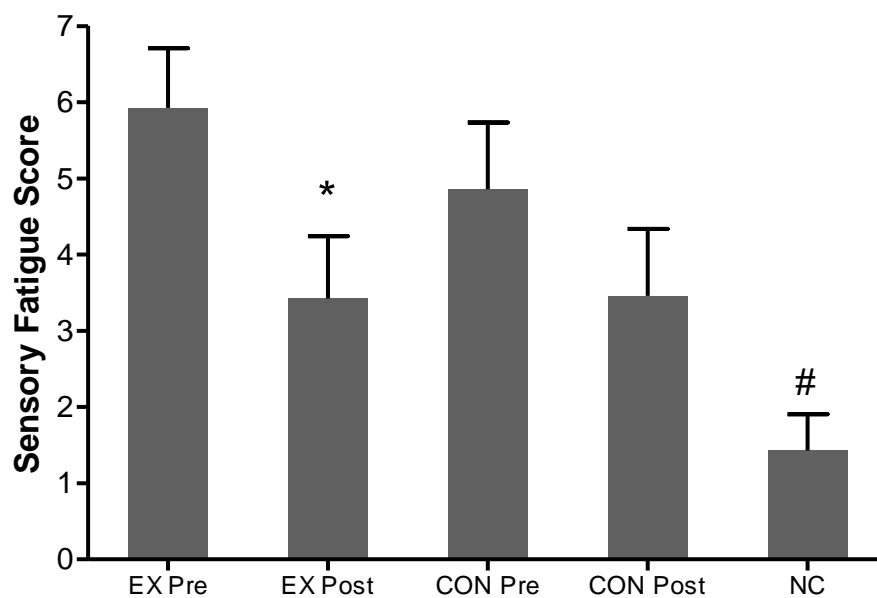


Figure 10. Sensory fatigue scores for all groups and time points.
 *= significantly different than EX Pre ($p < 0.01$). # = Significantly different than EX Pre, CON Pre ($p < 0.01$), EX Post and CON Post ($p < 0.05$).

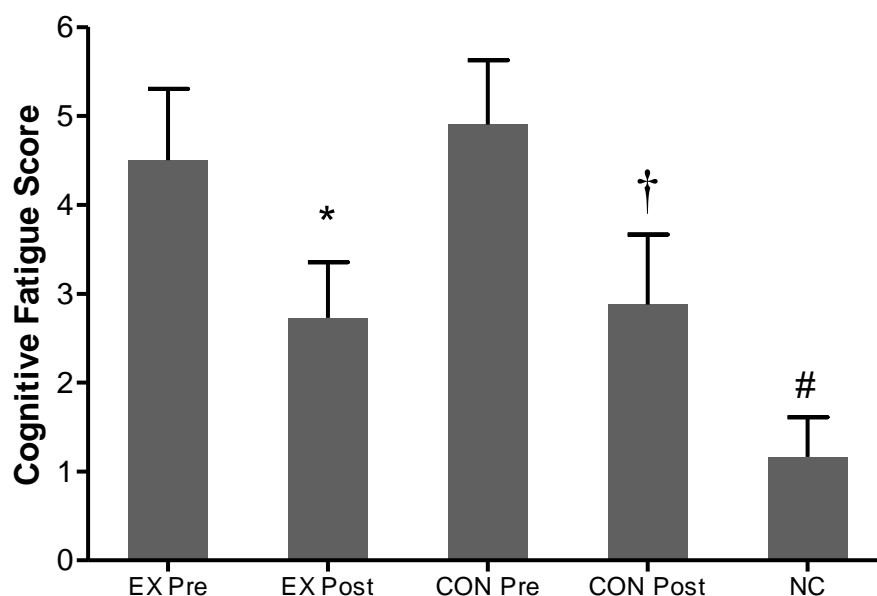


Figure 11. Cognitive fatigue scores for all groups and time points.
 * = significantly different than EX Pre ($p < 0.01$). † = significantly different than CON Pre ($p < 0.01$). # = significantly different than EX Pre, CON Pre ($p < 0.01$), EX Post and CON Post ($p < 0.05$).

Baseline markers of oxidative stress in cancer and NC patients are found in Table 12. Plasma antioxidant status, as determined by TEAC, did not differ between EX and CON, but was significantly greater in NC than in the cancer patients ($p < 0.01$). Plasma 8-OHdG, a marker of DNA oxidation, did not differ between any of the groups at baseline. ($p > 0.05$).

Table 12

Baseline Oxidative Stress Parameters

	TEAC (mM Trolox)	8-OHdG (ng/mL)
Cancer (N = 15)	0.27 ± 0.06 ^a	0.42 ± 0.26
NC (N=7)	0.37 ± 0.07	0.33 ± 0.18

^a Significantly lower than NC ($p < 0.01$)

Changes in antioxidant capacity and DNA oxidation for EX and CON are presented in Table 13. TEAC increased significantly in EX, from 0.28 ± 0.07 mM to

0.39 ± 0.05 mM Trolox equivalence ($p < 0.01$), while in CON, TEAC increased from 0.26 ± 0.05 to 0.32 ± 0.08 mM, but this did not represent a significant improvement ($p > 0.05$). Following the 10-week study period, antioxidant capacity in NC no longer differed significantly from either cancer group ($p > 0.05$). DNA oxidation did not change significantly over time in either group, but there was a significant interaction effect between EX and CON ($p < 0.05$). EX decreased plasma 8-OHdG concentration from 0.47 ± 0.33 to 0.29 ± 0.18 ng/mL, whereas CON increased from 0.35 ± 0.14 to 0.49 ± 0.22 ng/mL.

Table 13

Changes in Oxidative Stress Parameters

	TEAC (mM Trolox)	8-OHdG (ng/mL)
EX (N=8)	$+ 0.11 \pm 0.09^a$	$- 0.18 \pm 0.26^b$
CON (N=7)	$+ 0.06 \pm 0.09$	$+ 0.13 \pm 0.19$

^a Significant increase from baseline ($p < 0.01$)

^b Significant group by time interaction effect, compared to CON ($p < 0.05$)

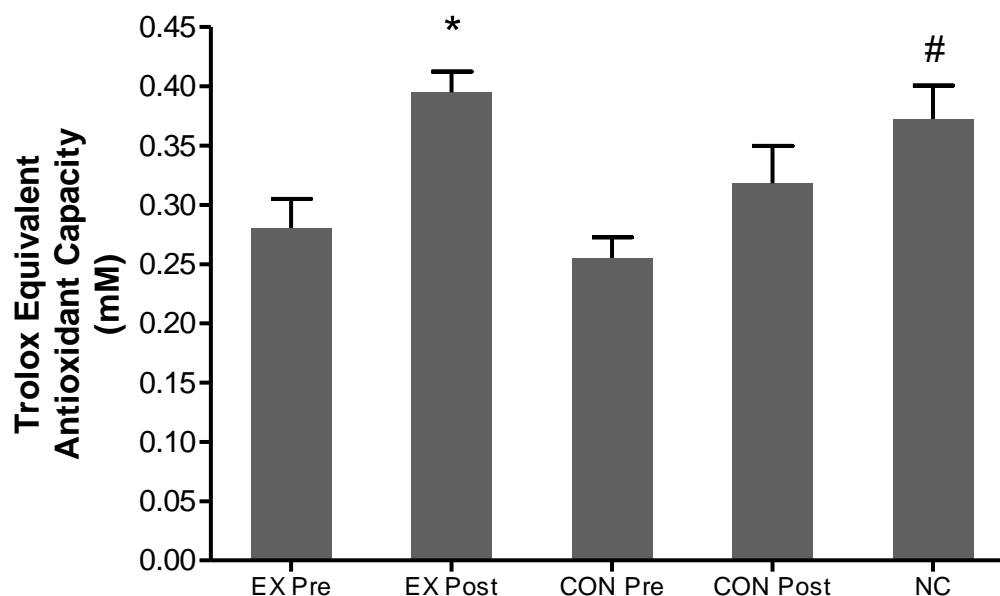


Figure 12. Trolox Equivalent Antioxidant Capacity for all groups and time points.

*= significantly different than EX Pre ($p < 0.01$). # = significantly different than EX Pre ($p < 0.05$) and CON Pre ($p < 0.01$).

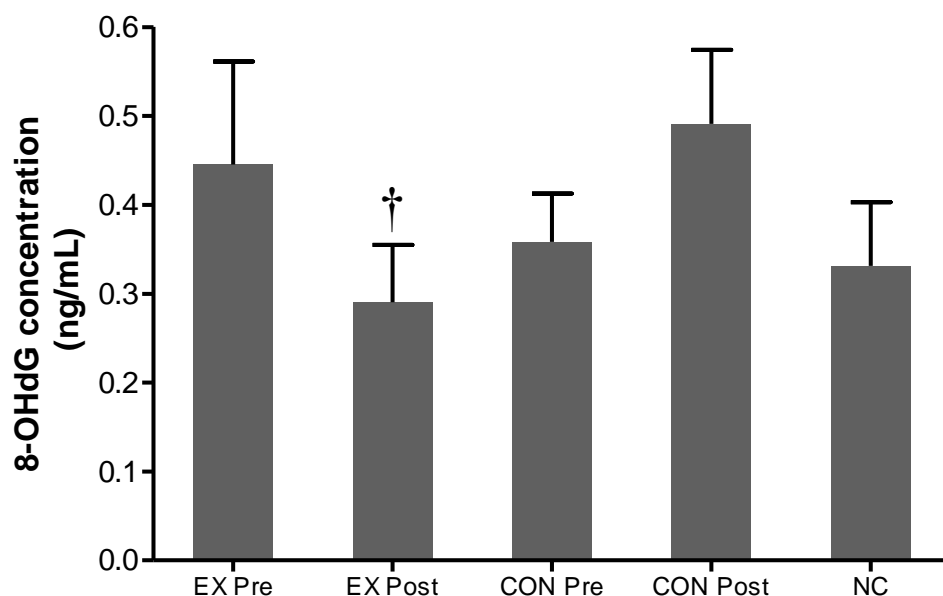


Figure 13. 8-OHdG plasma concentration for all groups and time points.
 † = significant group by time interaction effect, compared to CON ($p < 0.05$).

Spearman correlation analysis revealed negative correlations between baseline antioxidant capacity and total ($r = -0.41$, $p < 0.05$), affective ($r = -0.39$, $p < 0.05$), sensory ($r = -0.40$, $p < 0.05$), and cognitive fatigue ($r = -0.57$, $p < 0.01$). No correlations between baseline 8-OHdG and fatigue were detected. Significant negative relationships ($p < 0.05$) between changes in TEAC and changes in affective ($r = -0.49$), sensory ($r = -0.47$), and cognitive fatigue ($r = -0.58$) were revealed. Similarly, changes in 8-OHdG and changes in total ($r = 0.46$) and affective fatigue ($r = 0.47$) exhibited significant correlations ($p < 0.05$). There was no significant relationship between changes in TEAC and 8-OHdG.

Table 14

Correlations Between Oxidative Stress and Fatigue Parameters at Baseline

		Total Fatigue	Behavioral	Affective	Sensory	Cognitive
TEAC	Correlation	-0.41	-0.33	-0.39	-0.4	-0.57
	Significance	0.029 ^a	0.0659	0.036 ^a	0.033 ^a	0.003 ^a
8-OHdG	Correlation	0.28	0.30	0.27	0.26	0.19
	Significance	0.105	0.088	0.109	0.118	0.199

^a Significant correlation between blood parameter and fatigue score ($p < 0.05$)

Table 15

Correlations Between Changes in Oxidative Stress and Fatigue Parameters

		Total Fatigue	Behavioral	Affective	Sensory	Cognitive
TEAC	Correlation	-0.37	-0.097	-0.49	-0.47	-0.58
	Significance	0.0887	0.3661	0.0321 ^a	0.039 ^a	0.0124 ^a
8-OHdG	Correlation	0.49	0.62	0.34	0.26	-0.01
	Significance	0.033 ^a	0.007 ^a	0.106	0.174	0.485

^a Significant correlation between blood parameter and fatigue score ($p < 0.05$)

Adverse Events and Exercise Adherence

Several of the EX patients exhibited chronic musculoskeletal limitations at baseline, including osteoarthritis, rheumatoid arthritis, and complications associated with previous surgery (including knee surgery, hip replacement, double mastectomy, wrist surgery, lumbar fusion, and Whipple procedure for pancreatic cancer). Because of the individualized nature of the exercise intervention, modification of the exercise program was sufficient to allow for continued exercise participation and completion of the intervention in all EX subjects. Overall, exercise adherence was good, with subjects attending $84\% \pm 13\%$ of scheduled sessions. One patient missed three sessions during the intervention due to hip pain associated with a previously failed hip replacement, and another missed two sessions due to complications with a breast expander placed

following double mastectomy. Otherwise, scheduling conflicts and acute respiratory infections accounted for most missed training sessions.

Conclusions

Results from this study indicate that exercise training is an effective method of reducing fatigue in cancer patients who have completed treatment within the prior 6 weeks. It appears that time out of treatment is an important variable in that it clearly has a restorative effect on its own, as cognitive fatigue was significantly reduced in the CON group and there were no significant interaction effects between EX and CON for any fatigue variable. The attenuation of only cognitive fatigue in CON may indicate that physiological components of cancer-related fatigue are more persistent than the cognitive impairments in patients who are not actively counteracting the cancer treatment-induced physiological damage with physical activity. It should be noted though, that reported increases in physical activity and activities of daily living in several CON subjects represent control group contamination. This may partially explain the universal trend of reduced fatigue in control subjects, and subsequently no significant group by time interaction effects for fatigue parameters. Nonetheless, significant correlations between antioxidant capacity and both baseline and changes in fatigue indicate that improved antioxidant capacity may play a role in reduced fatigue, regardless of physical activity level.

Despite a lack of significant change in 8-OHdG over time, there was a significant group by time interaction effect between EX and CON. Matsumoto et al (2003) found that hepatic cancer patients with higher 8-OHdG levels in noncancerous liver regions were more likely to experience a cancer recurrence than those with lower 8-OHdG levels

($p < 0.01$). Therefore, it is possible that variable 8-OHdG response over time between the groups may be implicated in a reduced risk for cancer recurrence following the cessation of treatment in exercise subjects compared to standard care. Indeed, it has been reported that higher levels of physical activity are associated with an improved disease-free survival rate (Ibrahim & Al-Homaidh, 2011), and exercise following treatment may reduce the chance for cancer recurrence (Courneya, 2003; Loprinzi, Cardinal, Winters-Stone, Smit, & Loprinzi, 2012).

While antioxidant capacity was greater in NC than cancer patients at baseline, 8-OHdG did not differ between cancer and NC groups at baseline. This may be explained by the fact that all subjects were out of treatment, and presumably free of cancer burden. Gadjeva et al. (2008) found that plasma malondialdehyde (MDA) concentration dropped in melanoma patients following surgery due to the reduced cancer-induced oxidative stress. While chemotherapy subsequently increased oxidative stress compared to post-surgery levels, MDA levels were still significantly lower than pre-surgery values. Without pre-treatment data in our study, it is difficult to draw conclusions about this potential interaction.

There appeared to be a responder / non-responder effect in regards to 8-OHdG. Three EX subjects reduced 8-OHdG by more than 0.3 ng/mL, while the remainder changed less than 0.03 ng/mL. In contrast, while three CON subjects increased 0.2 ng/mL or more, the remainder changed approximately 0.1 ng/mL, with half exhibiting decreases. Kennedy et al (2005) found that in patients with chronic fatigue syndrome, oxidative stress, as measured by 8-iso-prostaglandin-F_{2α}-isoprostanes, were correlated with fatigue symptoms in normotensive, nonobese individuals, but was not in obese or

hypertensive individuals. This indicates that oxidative stress may significantly affect fatigue in cancer survivors, but due to the multi-causal nature of fatigue, this relationship may be uncoupled in certain clinical subsets of the cancer population where other physiological factors exist, including body composition, cardiac health, and medication status.

It is not entirely clear why there was a rise in 8-OHdG in the control group during the study period. Although the cancer treatments investigated in this study all are known to acutely introduce oxidative stress, the time frame of the persistence of markers of oxidative stress is still not wholly characterized, and may be affected by an undetected cancer burden. For instance, Erhola et al. (Erhola et al., 1997) found that the overall response to radiation and chemotherapy (“complete or partial remission”, versus “no change or progressive disease”) determined whether urinary 8-OHdG concentration increased or decreased with treatment. Furthermore, Jonas et al. (Jonas et al., 2000) found that GSH/GSSG ratio continually decreased over the two weeks following the end of chemotherapy, indicating that treatment either directly impairs the antioxidant system, or that chronic oxidative stress is capable of depleting the glutathione pool and diminish reductive capacity. Regardless, these data indicate that in certain cancer populations, oxidative stress may worsen for a period of time after the cessation of treatment, but the effects of variable prognostic criteria and differing cancer stages, types, and treatment regimens on the timeframe of chronic oxidative stress are unknown at this time.

As expected, baseline fatigue was greater, and antioxidant capacity lower, in cancer patients compared to non cancer patients. Previously, sparse data existed regarding the time course of antioxidant capacity following the cessation of treatment.

The current study indicates that additional time out of treatment (in this case, from one month to more than three months) helps restore antioxidant capacity, albeit nonsignificantly. Exercise, on the other hand resulted in a more robust, and statistically significant, increase in antioxidant capacity. Moreover, significant correlations between increased antioxidant capacity and reduced fatigue indicate that systemic oxidative stress may be a potential mechanism for cancer-related fatigue, much as it has been hypothesized in chronic fatigue syndrome (Kennedy et al., 2005). Results from our data presented here support the current physical activity guidelines for cancer survivors, which suggest moderate physical activity as a means to reduce treatment-associated side effects (Rock et al., 2012), an effect that may be in part due to changes in antioxidant capacity and oxidative stress.

Limitations

Pseudo-randomization resulted in different gender characteristics between the EX and CON groups, but as this was a repeated measures experiment and because changes in fatigue parameters over time were not expected to be gender-dependent, this is unlikely to represent sampling bias. NC fatigue values were unexpectedly classified as borderline “mild fatigue,” but this may have been influenced by the timing of the fatigue questionnaires, which were administered immediately prior to the early morning, fasted blood draws. Due to the self-selected nature of participation in this study, it is possible that psychological factors beyond the scope of this study may have independently affected fatigue values at baseline or fatigue changes over time. Although no substantial dietary alterations were explicitly reported by either the EX or CON group at follow-up, diets were not controlled and food logs were not utilized to evaluate subjects’

consumption of antioxidant-containing foods. It is possible that whole foods containing variable antioxidant compositions affected plasma antioxidant capacity or oxidative stress at either baseline or follow-up. Finally, although exercise interventions were similar, individualization of exercise regimens for each cancer patient based upon specific needs may have affected the biochemical and fatigue responses to exercise and could potentially limit the generalizability of this study.

CHAPTER V

**OXIDATIVE STRESS, MUSCULAR STRENGTH AND
CARDIOPULMONARY ALTERATIONS
FOLLOWING AN EXERCISE
INTERVENTION IN
CANCER SURVIVORS**

Abstract

PURPOSE: Exercise interventions in cancer patients have been shown to reduce cancer-treatment related side effects, including muscle wasting and diminished cardiorespiratory fitness (CRF). Despite this, the underlying mechanisms associated with the protective aspects of exercise are generally uncharacterized, but treatment-associated oxidative stress is thought to play a role. The purpose of this study was to determine the effect of an exercise intervention (EX) on muscular strength, CRF and blood markers of protein oxidation compared to a non-exercising, cancer control group (CON).

METHODOLOGY: Assessments of muscular strength and CRF and an initial fasting blood draw and were administered to fifteen cancer patients within six weeks of completing radiation or chemotherapy treatment. Eight of the cancer patients participated in a 10-week exercise intervention while seven continued standard care. Following the intervention, subjects completed a reassessment of strength and CRF and another fasted blood draw. Changes in plasma protein carbonyls and antioxidant status

were compared between the EX and CON groups using repeated measures ANOVA. Baseline markers of oxidative stress were compared between cancer patients and age-matched individuals with no cancer history (NC). A correlation analysis of changes in fitness parameters and oxidative stress in cancer patients was conducted. **RESULTS:** Baseline total antioxidant capacity was significantly lower, and plasma protein carbonyls significantly higher in cancer patients compared to NC ($p < 0.05$). An exercise intervention significantly increased antioxidant capacity and decreased protein carbonyls, while standard care did not ($p < 0.01$). Improvements in composite arm (41%) and leg strength (34%), isometric handgrip strength (11%), and VO_2 peak (16%) all significantly improved in the EX group ($p < 0.05$), while none of these parameters significantly changed in CON. No significant correlations between changes antioxidant capacity or protein oxidation and improvements in fitness parameters were revealed.

CONCLUSIONS: A whole-body exercise intervention is an effective method of increasing muscular strength, CRF and antioxidant capacity, while reducing markers of protein oxidation. Despite concurrent changes in oxidative stress, strength and CRF, Spearman correlations did not suggest a direct relationship between improvements in fitness parameters and reduction in oxidative stress.

Introduction

Between 1990 and 2010, cancer related mortality dropped from 215 to 172 deaths per 100,000 persons, demonstrating improved efficacy of cancer treatment methods (Howlader, 2012). Although this is an encouraging trend, the adverse side effects of surgery, radiation, chemotherapy, and other treatment modalities are associated with severe physiological side effects that may last for years. Typical side effects include muscle dysfunction (Christensen et al., 2014) and reduced CRF (Burnett et al., 2013).

While these decrements are multi-causal and multi-factorial, oxidative stress is thought to play a pivotal role in many pathological processes associated with cancer and its treatments (Fang et al., 2009; Robbins & Zhao, 2004; Zhao & Robbins, 2009).

Oxidative stress is a state in which reactive oxygen species (ROS) are produced at a rate that exceeds cellular adaptive and repair capacities (Halliwell & Gutteridge, 2007). The interrelationship between oxidative stress and cancer is complex. The development and progression of cancer are associated with high levels of oxidative stress-induced DNA modification (Valko et al., 2006), but cancer cells themselves can produce increased levels of ROS, perpetuating dysfunction and cancer growth (Hileman, Liu, Albitar, Keating, & Huang, 2004). In certain cancers, this allows for therapeutic selectivity, and many modes of cancer treatment depend on extremely high doses of ROS as a mechanism of cancer killing, including radiation and many chemotherapy regimens (Kovacic, 2007). Unfortunately, treatment-associated oxidative stress typically affects healthy tissue in addition to malignant tissue, resulting in both acute and chronic side effects (Ascensao et al., 2005b; Laviano et al., 2007). Doxorubicin (DOX), for instance, is a highly effective antineoplastic agent used for various cancers, yet causes a dose-dependent cardiotoxicity associated with oxidative stress (Chaiswing et al., 2004; Hayward et al., 2013; Kanter et al., 1985). More recently, DOX has been shown to elicit a similar degree of dysfunction in skeletal, smooth, and cardiac muscle tissues (Hayward et al., 2013). While the primary antineoplastic mechanisms of DOX are inhibition of topoisomerase II and intercalation in DNA (Chen, Jungsuwadee, Butterfield, & St Clair, 2007), resulting in inhibition of DNA replication and RNA transcription, the induction of ROS may contribute to antitumor activity (Taatjes et al., 1998). Other common

chemotherapy drugs, including tamoxifen, etoposide, 5-fluorouracil, alkylating agents and platinum coordinating complexes are known to induce significant oxidative stress, either as an antineoplastic mechanism or as a side effect (Y. Chen et al., 2007; Conklin, 2000). Radiation, by nature, is characterized by ROS production and exerts its physiological effects, both beneficial and harmful, via this mechanism (Ahn et al., 2006; Azzam, Jay-Gerin, & Pain, 2012).

Muscular dysfunction in cancer patients is likely caused by numerous factors, including physical inactivity, malnutrition, inflammatory cytokines, oxidative stress and advanced age (Christensen et al., 2014). Numerous studies link cancer and treatment-related ROS production with cachexia, among other potential mechanisms (Laviano et al., 2007; Mantovani et al., 2004). In healthy elderly women, oxidative protein damage has been shown to be associated with poor grip strength (Howard et al., 2007). The loss of muscle mass and cardiovascular fitness is compounded by significant decreases in physical activity following treatment. Studies have shown that muscular endurance declines after only two weeks of physical inactivity in healthy individuals (Haddad et al., 2003), while mitochondrial enzyme activity decreases following 3 months of inactivity (Coyle et al., 1984).

The selective reduction of oxidative stress in healthy tissues is a strategy which holds promise to reduce treatment-associated side effects while maintaining treatment efficacy. Although antioxidant supplementation may seem like a more direct method of increasing antioxidant capacity in cancer survivors as a strategy to reduce treatment associated side effects, this approach has been discouraged by several authors because of the protective effect it may have on cancer cells (D'Andrea, 2005; Lawenda et al., 2008),

although some groups disagree (Pathak et al., 2005). Post treatment, this concern is less pronounced, but the endogenous antioxidant system is complex and coordinately regulated, such that a supplementation of one antioxidant may result in the downregulation of others (McCord, 2008). Exogenous administration of any one of these alone could result in aberrant redox regulation (Kong & Lillehei, 1998), with the possibility of promoting oncogenesis and cancer recurrence (Acharya, Das, Chandhok, & Saha, 2010), particularly in the presence of undetected metastases. Furthermore, dietary antioxidant interventions have not proven particularly effective in the treatment treatment-associated side effects (Lawenda et al., 2008; Myers et al., 1983; Unverferth et al., 1983).

An increasingly popular rehabilitation method in cancer patients is prescriptive, whole-body exercise. Exercise has been shown to minimize many side effects of cancer treatment, including reduced CRF (Courneya et al., 2003; Courneya et al., 2007; Dimeo et al., 2004; Schneider et al., 2007c; Segal et al., 2009) and muscular strength (Lakoski et al., 2012; Schmidt et al., 2013; Schneider et al., 2007a). In a 2011 meta-analysis investigating the effect of exercise on VO_2 peak, pooled data of six studies (571 adult cancer patients; 344 exercise and 227 usual care control) indicated that exercise training significantly increased VO_2 peak ($+2.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), whereas usual care was associated with a decline in VO_2 peak ($-1.0 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) (L. W. Jones, Liang, et al., 2011). Similarly, a recent review of the impact of resistance exercise programs on muscular strength in over 1,000 cancer patients during or following treatment revealed large effect-size improvements (Cohen's $d = 0.86$) in muscular strength (Focht et al., 2013). A multi-center randomized, controlled study ($n=242$) demonstrated the magnitude of strength

gain, as an isolated resistance exercise training program increased muscular strength 25% to 35% in breast cancer patients receiving chemotherapy (Courneya et al., 2007).

Exercise may, in part, exert its rehabilitative effects by preferentially increasing antioxidant capacity in the tissues that are stressed by exercise. For instance, in healthy individuals, unilateral leg exercise resulted in increased antioxidant enzyme activity in the trained leg but not the untrained leg (Parise et al., 2005). It may be surmised that because of this localized change in antioxidant capacity, antioxidant capacity should increase in active tissue, but not in cancer cells. This also demonstrates the importance of total body exercise interventions in cancer survivors, as training effects and changes in antioxidant enzyme capacity may be local.

Minimal research regarding exercise-associated changes in oxidative stress in cancer patients has been published. The existing studies investigating this relationship in human cancer populations are not in agreement, as both increases and decreases in oxidative stress have been reported (Allgayer et al., 2008; L. W. Jones, Eves, et al., 2011). In non-cancer populations, exercise training can elicit acute and chronic augmentation of antioxidant defense and reductions in oxidative stress. Superoxide dismutase concentrations in skeletal muscle samples were significantly elevated for several days following a single non-damaging cycling bout, with a peak at 2 days, but values returned to baseline levels by day 6 (Khassaf et al., 2001). In obese individuals with no history of cancer, an exercise intervention resulted in significant increases in VO_2 peak and decreases in F_2 -isoprostane levels (a measure of lipid peroxidation), and these changes exhibited a negative linearly relationship (Campbell et al., 2010).

Similarly, F₂-isoprostane levels decreased 34% in premenopausal women following 15 weeks of aerobic exercise training (Schmitz et al., 2008).

Animal models provide us with more compelling evidence that exercise-induced alterations in oxidative stress can influence CRF and muscular strength improvements following cancer treatments. Kanter (1985) found that a swim-training protocol in rats improved antioxidant capacity and counteracted doxorubicin-mediated cardiotoxicity. Ascensao et al. (2005a) mirrored these results, and also found that cardiac damage, evaluated by cardiac troponin I (cTnI) presence in the plasma, and cardiac muscle protein carbonyls were significantly lower in exercise trained mice treated with DOX than non-exercise trained mice treated with DOX. Treadmill training in rats has been shown to reduce cardiotoxicity concurrently with increased glutathione peroxidase expression (Chicco, Hydock, et al., 2006) while an acute exercise bout had a cardioprotective effect accompanied by a reduction in myocardial lipid peroxidation (Wonders et al., 2008). More recently, Smuder et al. (2011) found that exercise training protects against doxorubicin-associated increases in both protein carbonyls and proteolysis in skeletal muscle tissue.

To address the lack of human research on the potential role that oxidative stress has in the restorative effects of exercise following cancer treatment, the purpose of this study was to investigate the effects of a 10-week exercise intervention on CRF, muscular strength, and plasma markers of antioxidant capacity and protein oxidation.

Methodology

Fifteen cancer patients and seven healthy age-matched individuals with no history of cancer or other chronic diseases participated in this study. This study consisted of a

10-week prescribed exercise intervention in an exercise group (EX), while a control group (CON) had standard care for the study period. Subjects were admitted to study groups based upon date of initial contact (pseudo-randomization). Cancer patients had completed oxidative stress-inducing cancer treatments, namely radiation or chemotherapy treatment, within the previous 6 weeks, with an average time out of treatment of four weeks. Cancer patients were recruited from walk-in and oncologist-referred patients at the Rocky Mountain Cancer Rehabilitation Institute (RMCRI) at the University of Northern Colorado (UNC) in Greeley, Colorado. Additionally, oncology patient navigators and clinical oncology research specialists at Northern Colorado Medical Center in Greeley, Poudre Valley Hospital in Fort Collins, and McKee Hospital in Loveland, Colorado identified qualifying individuals as they completed radiation or chemotherapy treatments. Newspaper articles in the Greeley Tribune and UNC today, posters at the local American Cancer Society office and outpatient oncology waiting areas, and a recruitment page on the RMCRI webpage were used to promote this study.

Twenty six cancer patients made contact with the primary investigator, but exclusion criteria or attrition resulted in the loss of 11 potential subjects. Refer to Figure 14 for flow of the subjects through this study. All cancer diagnoses were considered for this study, but subjects were required to have undergone radiation or a chemotherapy treatment explicitly known to elicit oxidative stress, either as a side effect or as an antineoplastic mechanism. Chemotherapy agents included anti-neoplastic antibiotics, alkylating agents, platinum coordinating complexes, epipodophyllotoxins, fluorouracil and tamoxifen (Y. Chen et al., 2007; Conklin, 2004; Look & Musch, 1994). Cancer patient inclusion criteria consisted of: (1) completed radiation or approved cancer therapy

regimen within six weeks of initial blood draw, (2) currently sedentary (less than two days/week or 20 minutes of aerobic or resistance exercise) (Brooks et al., 2005), (3) have not consistently supplemented with dietary antioxidants, including vitamins C and E, in the previous month, (4) non-smokers (for at least 2 months), and (5) were able to walk comfortably on a treadmill.

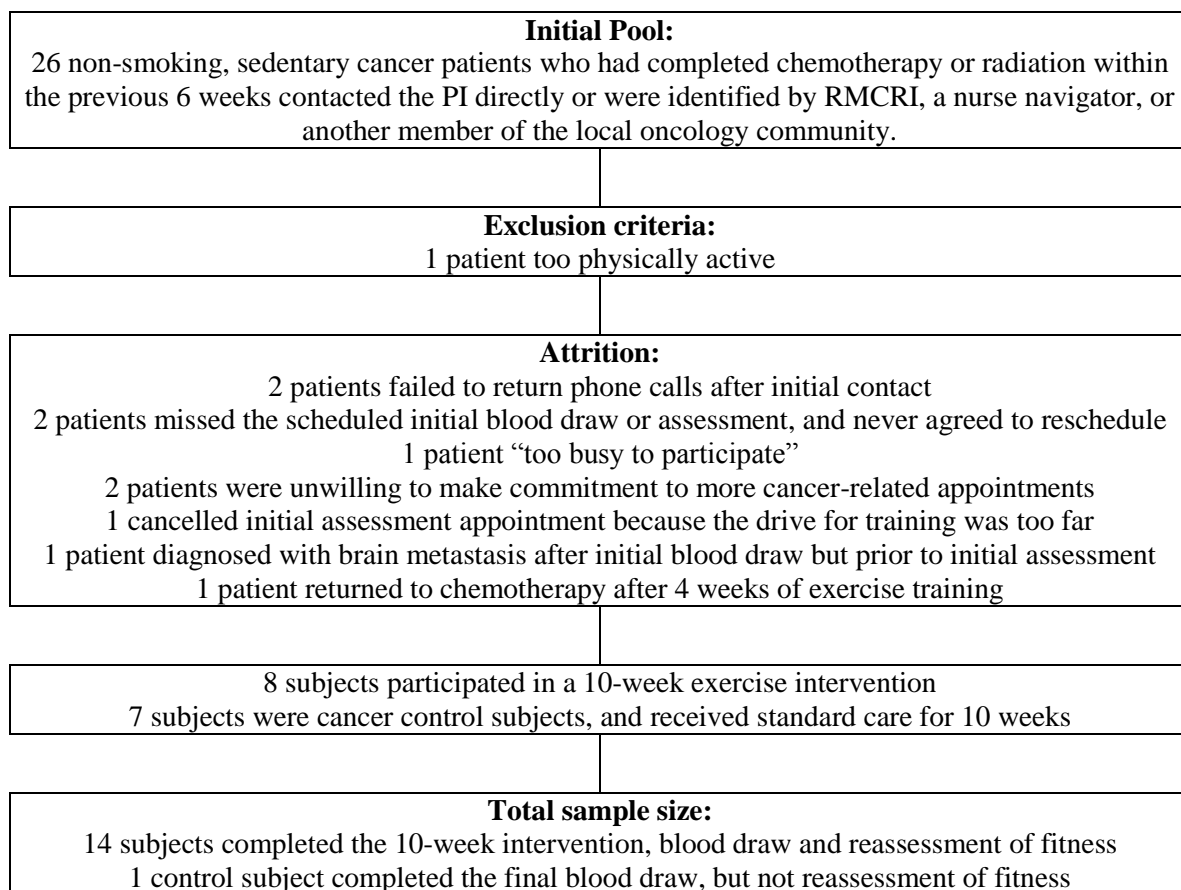


Figure 14. Flow of cancer patients through the study.

Non-cancer, healthy controls were primarily recruited from among the faculty and staff at the University of Northern Colorado via campus-wide email and by word of mouth. Inclusion criteria for the non-cancer group included: (1) currently sedentary (less than two days/week or 20 minutes of aerobic or resistance exercise), (2) no history of cancer or other major chronic disease, (3) have not consistently supplemented with

dietary antioxidants in the previous month, and (4) non-smokers. The University of Northern Colorado Institutional Review Board (IRB) approved all procedures.

For 72 hours prior to the blood draw, subjects were asked to avoid exhaustive exercise and antioxidant supplementation. The day before the cancer patient's initial exercise assessment, a 12-hour fasting blood sample was taken by the lead investigator or an otherwise experienced phlebotomist.

Maximal Treadmill Testing

The RMCRI treadmill protocol (Table 16) was developed at the Rocky Mountain Cancer Rehabilitation Institute as a cancer-specific protocol, more suitable for the cancer population. Unlike the Bruce treadmill protocol, there are no abrupt increases in speed and grade (Bruce et al., 1973). Smaller changes in effort and patient stride provide a more manageable progression through stages and results in reduced premature peripheral fatigue and more valid maximal treadmill tests.

Prior to each incremental treadmill test, subjects were fitted with a heart rate monitor (Polar Inc., Lake Success, NY). Instructions regarding the specific changes in speed and grade during each one-minute stage were given and the participant was encouraged to walk or run until exhaustion or volitional fatigue (to VO_{2peak}). Blood pressure was assessed at rest, every three minutes during exercise, immediately post exercise, and after three minutes of recovery (slow walking). Rating of perceived exertion was assessed every three minutes of exercise, according to Borg's 1-10 scale. Heart rate and oxygen saturation were assessed at the end of every 1-minute stage. Subjects were asked to use handrails only if it was required for balance support. If handrails were required for support, subjects were asked to use them throughout the test.

ACSM metabolic equations were utilized to quantify VO_{2peak} at time of volitional fatigue (Pescatello, Arena, Riebe, & Thompson, 2014).

Table 16

RMCRI Treadmill Protocol

Stage	Speed (mph)	Grade (%)	Time
1	1.0	0	1 min
2	1.5	0	1 min
3	2.0	0	1 min
4	2.5	0	1 min
5	2.5	2	1 min
6	3.0	2	1 min
7	3.3	3	1 min
8	3.4	4	1 min
9	3.5	5	1 min
10	3.6	6	1 min
11	3.7	7	1 min
12	3.8	8	1 min
13	3.9	9	1 min
14	4.0	10	1 min
15	4.1	11	1 min
16	4.2	12	1 min
17	4.3	13	1 min
Cool-Down	1.5	0	3 min

Muscular Strength Assessment

A true 1-repetition max (1-RM) assessment of strength can be the most accurate and reliable method of muscular strength assessment, but may be subject to variability due to unfamiliarity with weight lifting, lack of adequate facilities, and risk of injury, even in healthy individuals. In the cancer population, the variability and risk for injury are even greater. Therefore, the Brzycki equation,

$$1\text{-RM} = \text{weight lifted (lb)} / (1.0278 - (\text{repetitions to failure} \times 0.0278))$$

was used to estimate 1-RM using the number of repetitions of a given submaximal load to fatigue, provided that the repetitions do not exceed 10 (Brzycki, 1993). Muscular

strength was assessed using the lat pull-down, shoulder press, chest press, seated row, leg press, leg extension, and leg curl exercises, using Cybex® gym equipment (Cybex International, Medford MA). The goal was to choose a weight that elicited muscle failure in 4-6 repetitions. Suggested values for the initial amount of weight lifted for each exercise were based upon previous muscular strength data from RMCRI (Schneider et al., 2003) but varied greatly between individual (Table 17). Repetitions were performed without resting until muscle failure, or until the subject's form deteriorated. If the subject exceeded 10 repetitions for any exercise, he or she was stopped, and moved on the other muscular strength exercises. Following all exercises, those exercises which had been stopped were completed after increasing the weight to elicit muscle failure in less than 10 repetitions. For the purposes of data analysis, a composite score (sum of weight lifted) was used for upper body strength (lat pull-down, shoulder press, chest press, seated row) and lower body strength (leg press, leg extension, and leg curl).

Table 17

<i>Suggested initial resistance (% of Body Weight)</i>		
Exercise	Men	Women
Lat Pull-down	0.900	0.500
Shoulder Press	0.350	0.150
Chest Press	0.750	0.250
Seated Row	0.750	0.300
Leg Press	1.500	0.750
Leg Extension	0.750	0.400
Leg Curl	0.750	0.400

Following 1-RM testing, a Takei TKK 5101 handgrip dynamometer (Takei Scientific Instruments, Tokyo, Japan) was utilized to evaluate maximal isometric handgrip strength in both hands. Subjects held the dynamometer parallel to their side with the dial facing away from their body, and three maximal isometric contractions were

performed on each side. The average of the best trial for each hand was used for analysis. In order to appropriately prescribe an individualized exercise intervention, an evaluation of flexibility and balance was performed at the time of intake. Flexibility was assessed with a modified sit and reach test and the back scratch test, while the Bertec Balance Check™ Screener and Trainer Package (Bertec Corporation, Columbus, OH) was used to assess postural stability and limits of stability.

Exercise Intervention

Each exercise participant completed a 10-week exercise intervention that was individually supervised by a cancer exercise specialist 3 days per week. Each 1-hour session was in accordance with the subject's prescribed training modes, intensity, and volume, based upon their goals and assessment results. Exercise sessions varied depending on the subject's health and fitness status, but typically included a 5-minute warm-up, 20 minutes of aerobic exercise, 25 minutes of resistance training, and 10 minutes of flexibility and balance training. Blood pressure and HR were measured at the beginning and end of the exercise session, and HR, RPE, and SaO₂ were monitored throughout. Aerobic exercise intensity was based on the subjects' initial VO₂peak, and was generally low to moderate intensity, ranging from 30% to 60% of heart rate reserve (HRR). The Karvonen (percent heart rate reserve) method was used to determine target exercise heart rate intensity using the formula

$$(220 - \text{age}) - \text{resting HR}] \times \text{percent of exercise intensity} + \text{resting HR}.$$

Subjects with poor overall health status health or low fitness were initially prescribed an intensity level of 30%-45% HRR or a RPE of 1-3, whereas subjects with a moderate health status or average fitness began their aerobic exercises at 50%-60% of HRR or an

RPE of 4-5. The mode of aerobic exercise selected for each subject was based on the mode offering the greatest anticipated benefit. Options included outdoor or treadmill walking, stationary cycling, or recumbent stepping. Although rate of progression varied significantly by subject, cancer exercise specialists aimed to increase increases speed or resistance weekly, while keeping RPE values below 5 and HR values below 75% HRR.

At the beginning of the intervention, initial percent of 1-RM corresponded with the percent of HRR values for aerobic exercise, based upon initial health and fitness status (30%-45% 1-RM for low fitness, 50%-60% for average fitness). Cybex Eagle Selectorized Strength Machines[®] (Cybex International Inc, Medway, MA, USA), free weights, resistance bands and body weight exercises were all used for the exercise intervention, depending upon fitness, mobility, and comfort of the cancer patient.

Muscular endurance was emphasized over hypertrophic training, as subjects performed 8-15 repetitions per set and typically ended each set at muscular fatigue rather than muscle failure. Resistance was increased when 8-15 repetitions no longer resulted in muscular fatigue.

All cancer patients were asked to avoid any substantial changes in their diet, assuming a physician or nutritionist did not prescribe a change in eating habits. Subjects in the standard care control group were asked to maintain their exercise habits during the 10-week study period. Although standard care cannot be “standardized” between hospitals and patients, it did not involve routine, systematic exercises (van Waart et al., 2010). Neither yoga nor stretching programs were utilized for the control group because both interventions have the potential to alter markers of oxidative stress and antioxidant capacity (Chambers et al., 2009; S. Singh et al., 2001). Control subjects were asked to

complete an exit survey inquiring about any changes in physical activity, dietary habits, medications, and medical procedures. Following the 10-week study period, control subjects had the opportunity to exercise at RMCRI for three months at no cost.

Blood Handling and Analysis

A minimum of 4mL of blood was withdrawn into sterile tubes containing potassium-EDTA and immediately put on ice. Samples were centrifuged at 3,000 rpm for 10 minutes, and the plasma was separated and stored in multiple aliquots at -80°C until analysis. All assays were performed in duplicate on the first thaw. Reactive carbonyl derivatives were measured as a marker of oxidative protein damage. Protein carbonyls were measured in plasma using an enzyme-linked immunosorbent assay (ELISA) according to the procedures recommended by the manufacturer (Cell Biolabs, Inc., San Diego, CA). Antioxidant capacity was measured in plasma using the Trolox-equivalent antioxidant capacity (TEAC) assay using procedures outlined by the reagent provider (Sigma Chemical, St. Louis, MO).

Statistical Analyses

Data are presented as means \pm SD. Subjects' baseline characteristics in the two cancer groups (EX versus CON) and the NC group were compared using independent *t*-tests. A repeated measures 2 (group) \times 2 (pre- and post-intervention) analysis of variance (ANOVA) with Bonferroni post hoc was used to determine differences within (across time) and between the two groups related to markers of protein oxidation, antioxidant capacity, VO₂peak, and muscular strength. A Kolmogorov-Smirnov was used to ensure that the normality assumption was met for this data set. Spearman correlation coefficients were calculated to determine associations between baseline

markers of oxidative stress and baseline CRF and muscular strength. Additionally, Spearman correlation coefficients were calculated to determine associations between post-intervention changes in markers of oxidative stress and post-intervention changes CRF and muscular strength. A significance level of $\alpha = 0.05$ was used for all statistical analyses.

Results

Baseline subject characteristics for the EX, CON, and NC groups are summarized in Table 18. The two cancer groups had statistically similar height, weight, body mass index, and time out of treatment, and no significant differences in age were found between the three groups. Although exit surveys in control subjects indicated no notable changes in dietary habits, qualitative physical activity in this group increased from baseline as, even in the absence of an exercise intervention, time out of treatment resulted in greater vigor for activities of daily living and leisure time activity. Increases in housework, light yard work, and walking were most commonly reported, but no control subjects reported participation in a structured exercise program. One male control subject failed to complete a reassessment of fitness despite completing both blood draws.

Table 18

Subject Characteristics

	Exercise	Control	Non-Cancer
N	8	7	7
Age (years)	64.0 ± 10.8	62.4 ± 9.7	55.1 ± 9.7
Females	7	4	5
Males	1	2	2
Height (in)	65.4 ± 2.5	66.8 ± 4.7	-
Weight (lbs)	174.0 ± 32.3	179.5 ± 40.8	-
BMI	28.4 ± 4.3	28.1 ± 3.8	-
Primary treatment			
Radiation	4	4	-
Chemo	3	4	-
Days out of Treatment	29.9 ± 18.6	31.4 ± 21.7	-
Adherence rate (%)	83.9 ± 12.8	NA	-

Baseline oxidative stress parameters are found in Table 19. Plasma antioxidant status, as determined by Trolox equivalent antioxidant status (TEAC), did not differ between EX and CON, but was significantly reduced in cancer patients compared to NC ($p < 0.01$). Similarly, plasma protein carbonyls did not differ between cancer groups, but was significantly greater in cancer patients than NC ($p < 0.05$). Blood markers of oxidative protein oxidation and antioxidant capacity at all time points are illustrated in Figures 15 and 16, respectively.

Table 19

Baseline Oxidative Stress Parameters in Cancer and Non-Cancer Groups

	TEAC (mM Trolox)	Protein Carbonyls (nmol/mg)
Cancer (N = 15)	0.27 ± 0.06 ^a	1.24 ± 0.27 ^b
NC (N=7)	0.37 ± 0.07	0.89 ± 0.25

^a Significantly lower than NC ($p < 0.01$)

^b Significantly greater than NC ($p < 0.05$)

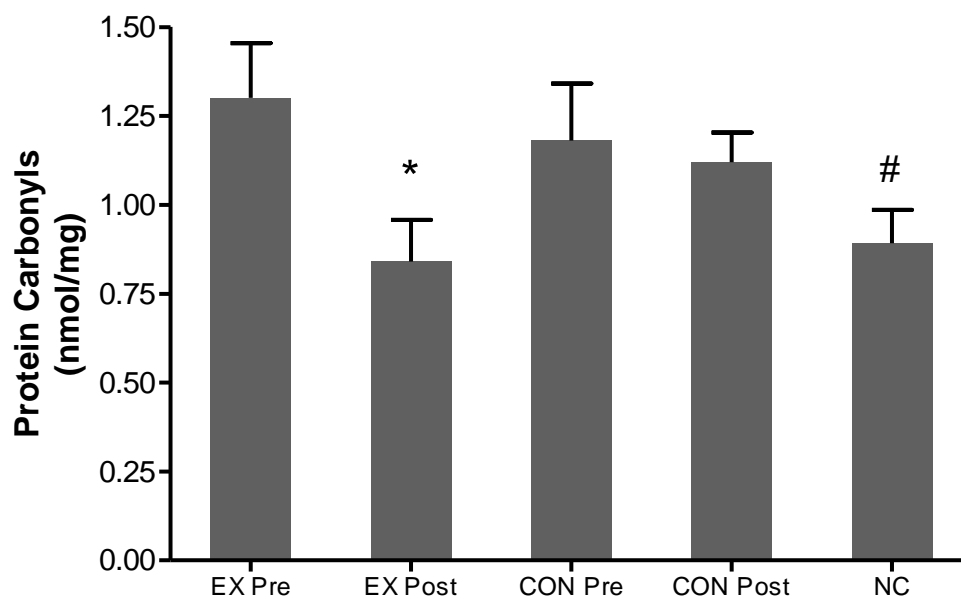


Figure 15. Plasma protein carbonyl concentration for all groups and time points. *= significantly different than EX Pre ($p < 0.01$). # = significantly different than EX Pre, CON Pre, and CON Post ($p < 0.05$).

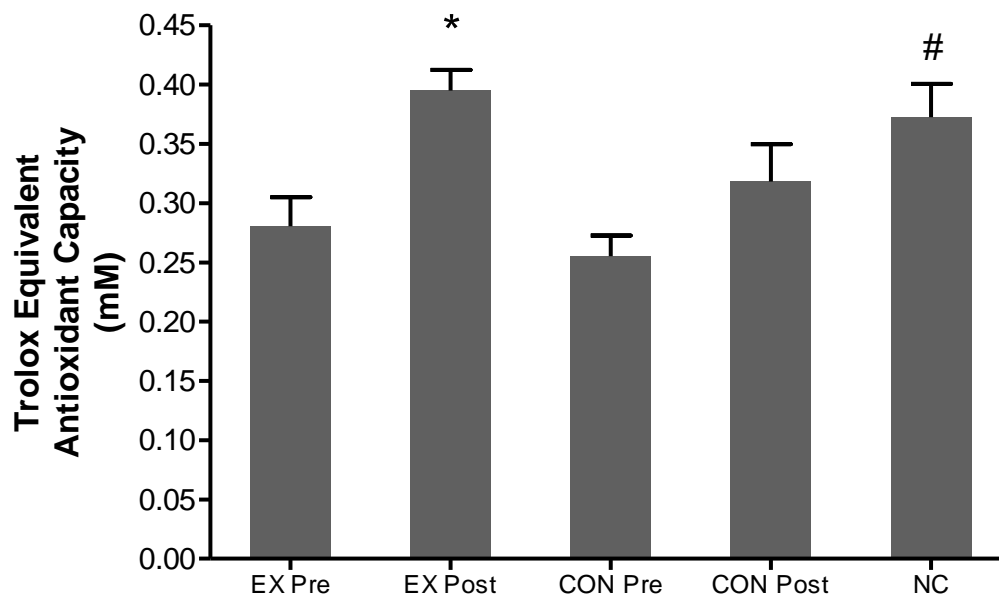


Figure 16. Trolox Equivalent Antioxidant Capacity for all groups and time points. *= significantly different than EX Pre ($p < 0.01$). # = significantly different than EX Pre ($p < 0.05$) and CON Pre, ($p < 0.01$).

Changes in antioxidant capacity and protein oxidation for EX and CON are presented in Table 20. Protein oxidation was significantly diminished at follow-up, from 1.30 ± 0.44 to 0.84 ± 0.33 nmol/mg in EX ($p < 0.05$), whereas no such response was apparent in CON (1.18 ± 0.42 to 1.12 ± 0.22 nmol/mg). Although no interaction effect was present, this trend approached significance ($p = 0.068$). Increases in antioxidant capacity were significant in EX (0.28 ± 0.07 mM to 0.39 ± 0.05 mM, $p < 0.01$), but not in CON (0.26 ± 0.05 to 0.32 ± 0.08 mM $p > 0.05$). Following the 10-week study period, EX no longer differed significantly from NC for protein oxidation, and neither cancer group differed in antioxidant capacity from NC ($p > 0.05$, Figures 15 and 16).

Table 20

Changes in Oxidative Stress Parameters in Exercise and Control groups

	TEAC (mM Trolox)	Protein Carbonyls (nmol/mg)
EX (N=8)	$+ 0.11 \pm 0.09^a$	$- 0.46 \pm 0.30^b$
CON (N=7)	$+ 0.06 \pm 0.09$	$- 0.06 \pm 0.47$

^a Significant increase from baseline ($p < 0.01$)

^b Significant decrease from baseline ($p < 0.05$)

Composite values for arm and leg strength were used for statistical analysis, and these results are found in Table 21, in addition to isometric handgrip strength and VO_2 peak. Both arm and leg strength improved significantly ($p < 0.01$) in EX, with 41% and 34% increases, respectively. There were essentially no changes in arm (+ 2.5%) or leg strength (-0.3%) strength in the control group. Accordingly, a significant time by group interaction effect was present for both arm and leg strength ($p < 0.01$). Handgrip strength gains in EX (+11.2%) were less compelling, but this change was significant nonetheless ($p < 0.05$). Although CON exhibited a 5.5% mean increase in handgrip strength, this result was not statistically significant. An analysis of individual strength exercises revealed significant increases in all exercises other than the leg curl in the

EX group, but no significant changes in the CON group. Furthermore, a significant interaction effect between CON and EX was apparent in all exercises other than leg curl and seated row. Detailed absolute and relative strength means and standard deviations for individual exercises are found in Table 22, while handgrip and composite arm and leg strength measurements at all time points are illustrated in Figures 17-19.

VO₂peak, as determined by a graded exercise test to volitional fatigue, increased 16% following the exercise intervention in EX, representing a significant increase over time ($p < 0.05$), whereas this parameter remained constant (+3.9%) in CON ($p > 0.05$). There was no significant time by group interaction between these two groups. Similarly, treadmill time improved significantly in the EX group (+2:05) but not in the CON group (+0:20). VO₂peak values and time to exhaustion on the treadmill at all time points are found in Figures 20 and 21, respectively.

Table 21

Muscular Strength and Cardiorespiratory Fitness Changes

	Pre	Post
EX (N=8)		
Arm Strength (lbs)	230.4 ± 91.4	324.4 ± 125.2 ^{a,c}
Leg Strength (lbs)	332.3 ± 130.9	445.5 ± 157.2 ^{a,c}
Handgrip Strength (lbs)	25.8 ± 4.8	28.6 ± 6.0 ^b
VO ₂ peak (ml·kg ⁻¹ ·min ⁻¹)	20.1 ± 9.7	23.4 ± 10.9 ^b
Treadmill Time (min)	8:44 ± 3:18	10:49 ± 3:15 ^a
CON (N=6)		
Arm Strength (lbs)	273.3 ± 148.1	280.1 ± 165.7
Leg Strength (lbs)	348.1 ± 166.3	346.9 ± 164.4
Handgrip Strength (lbs)	25.6 ± 9.7	26.8 ± 10.0
VO ₂ peak (ml·kg ⁻¹ ·min ⁻¹)	18.1 ± 4.4	18.8 ± 6.1
Treadmill Time (min)	8:59 ± 2:42	9:19 ± 3:31

^a Significantly lower than baseline ($p < 0.01$)

^b Significantly lower than baseline ($p < 0.05$)

^c Significant time by group interaction with control group ($p < 0.01$)

Table 22

Changes in Individual Muscular Strength Parameters

	EX (N=8)		CON (N=6)	
	Pre	Post	Pre	Post
Absolute Strength (lbs)				
Lat Pulldown	75.1 ± 13.4	102.6 ± 15.5 ^{a,c}	90.4 ± 39.4	89.9 ± 40.3
Shoulder Press	34.0 ± 14.7	53.7 ± 18.6 ^{a,c}	46.6 ± 29.2	48.3 ± 33.4
Chest Press	51.3 ± 24.6	77.8 ± 31.4 ^{a,c}	62.5 ± 46.8	68.6 ± 50.6
Seated Row	69.9 ± 8.5	90.2 ± 8.3 ^b	73.2 ± 36.5	73.6 ± 44.4
Leg Curl	74.1 ± 16.7	89.2 ± 10.1	82.3 ± 36.8	82.8 ± 40.4
Leg Extension	81.3 ± 26.5	113.8 ± 22.0 ^{b,c}	88.9 ± 48.1	73.3 ± 61.6
Leg Press	176.9 ± 39.2	242.6 ± 33.5 ^{a,c}	177.0 ± 84.9	172.5 ± 69.9
Relative strength (lbs / body weight)				
Lat Pulldown	0.44 ± 0.09	0.61 ± 0.12 ^{a,c}	0.50 ± 0.18	0.51 ± 0.21
Shoulder Press	0.21 ± 0.10	0.32 ± 0.13 ^{a,c}	0.26 ± 0.15	0.26 ± 0.16
Chest Press	0.30 ± 0.14	0.47 ± 0.21 ^{a,c}	0.34 ± 0.23	0.37 ± 0.25
Seated Row	0.41 ± 0.08	0.53 ± 0.11 ^b	0.41 ± 0.18	0.41 ± 0.23
Leg Curl	0.43 ± 0.08	0.53 ± 0.12	0.45 ± 0.15	0.46 ± 0.20
Leg Extension	0.48 ± 0.16	0.68 ± 0.18 ^{b,c}	0.49 ± 0.22	0.43 ± 0.34
Leg Press	1.04 ± 0.27	1.45 ± 0.43 ^{a,c}	0.98 ± 0.40	0.95 ± 0.28

^a Significantly lower than baseline ($p < 0.01$)

^b Significantly lower than baseline ($p < 0.05$)

^c Significant time by group interaction with control group ($p < 0.01$)

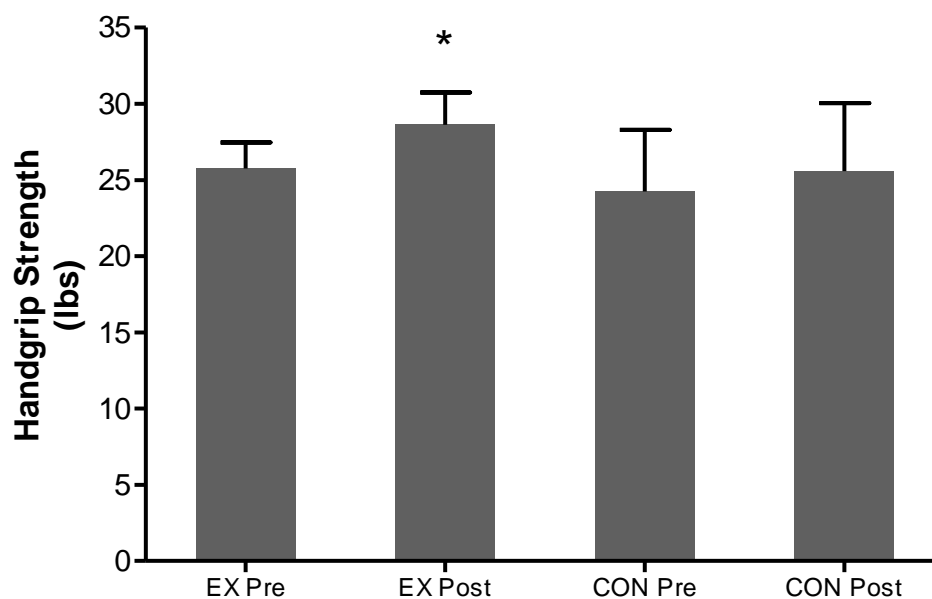


Figure 17. Handgrip strength (average, both hands) for all groups and time points.

*= significantly different than EX Pre ($p < 0.05$).

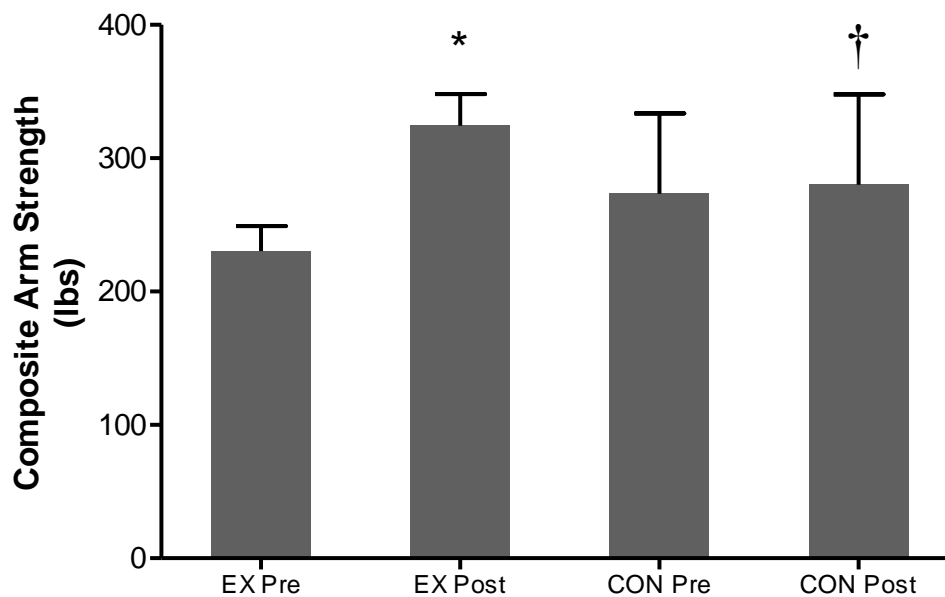


Figure 18. Composite arm strength for all groups and time points.
 *= significantly different than EX Pre ($p < 0.01$). † = significant group by time interaction effect, compared to EX ($p < 0.01$).

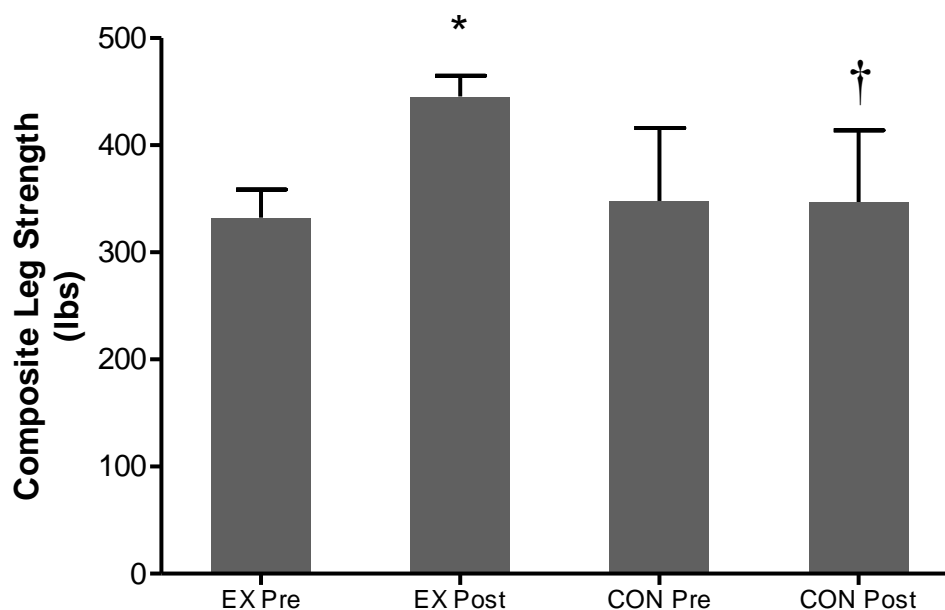


Figure 19. Composite leg strength for all groups and time points.
 *= significantly different than EX Pre ($p < 0.01$). † = significant group by time interaction effect compared to EX ($p < 0.01$).

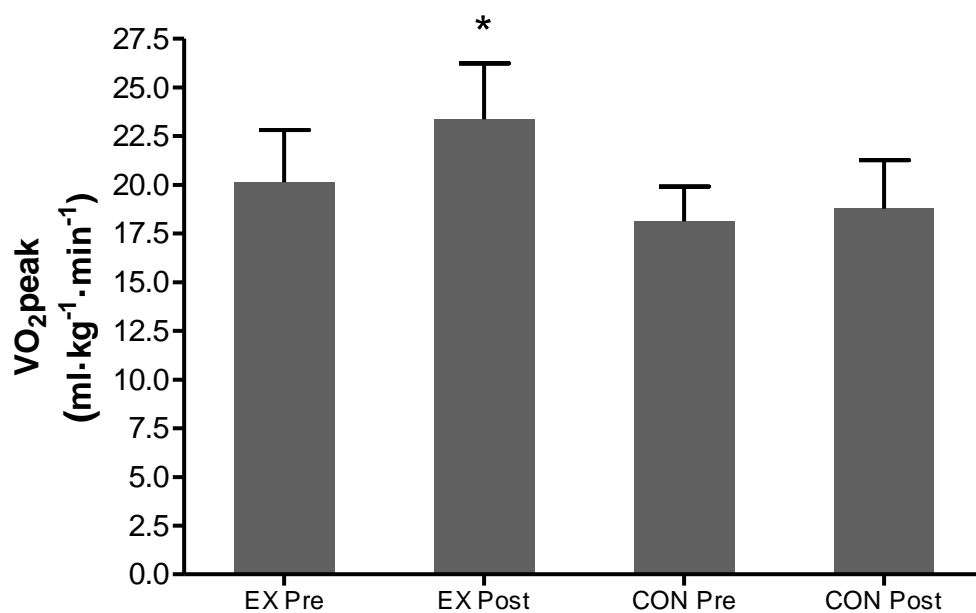


Figure 20. VO₂peak values for all groups and time points.
* = significantly different than EX Pre ($p < 0.05$).

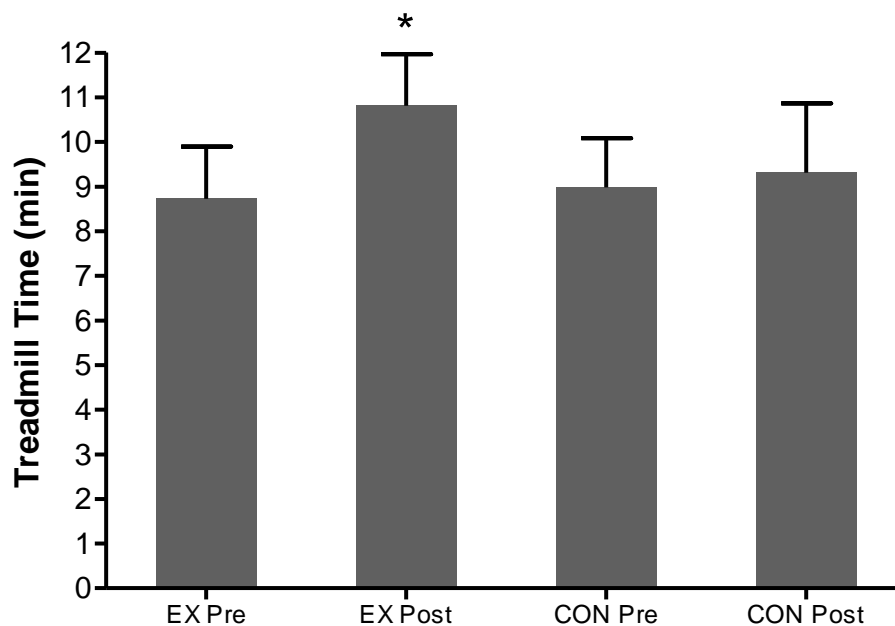


Figure 21. Time to exhaustion during treadmill test for all groups and time points.
* = significantly different than EX Pre ($p < 0.01$).

Initial VO₂peak exhibited a significant negative correlation ($r = -0.71$) with baseline antioxidant capacity ($p < 0.01$), but not with baseline protein carbonyls. Strength parameters did not significantly correlate with either protein oxidation or antioxidant capacity at baseline. Spearman correlation analysis showed no significant correlations between changes in VO₂peak or muscular strength and changes in protein carbonyls or antioxidant capacity. Correlations matrices are found in Tables 23 and 24.

Table 23

Correlations Between Oxidative Stress and Fitness Parameters at Baseline

		VO ₂ peak	Arm Strength	Leg Strength	Handgrip
TEAC	Correlation	-0.71	-0.23	0.1	-0.005
	Significance	0.001 ^a	0.206	0.361	0.492
Carbonyls	Correlation	-0.06	0.046	-0.06	-0.15
	Significance	0.422	0.435	0.415	0.301

^a Significant correlation between blood parameter and VO₂peak value ($p < 0.05$)

Table 24

Correlations Between Changes in Oxidative Stress and Fitness Parameters

		VO ₂ peak	Arm Strength	Leg Strength	HG
TEAC	Correlation	0.42	0.26	0.073	0.44
	Significance	0.070	0.189	0.403	0.059
Carbonyls	Correlation	-0.45	-0.4	-0.35	-0.21
	Significance	0.053	0.078	0.114	0.236

Adverse Events and Exercise Adherence

Several of the EX patients exhibited chronic musculoskeletal limitations at baseline, including osteoarthritis, rheumatoid arthritis, and complications associated with previous surgery (including knee surgery, hip replacement, double mastectomy, wrist surgery, lumbar fusion, and Whipple procedure for pancreatic cancer). Because of the individualized nature of the exercise intervention, modification of the exercise program

was sufficient to allow for continued exercise participation and completion of the intervention in all EX subjects. Overall, exercise adherence was high, with subjects attending $84 \pm 13\%$ of scheduled sessions. One patient missed three sessions during the intervention due to hip pain associated with a previously failed hip replacement, and another missed two sessions due to complications with a breast expander placed following double mastectomy. Otherwise, scheduling conflicts and acute respiratory infections accounted for most missed training sessions.

Conclusions

The present study demonstrated that an exercise intervention in cancer patients following treatment simultaneously increased muscular strength, CRF and antioxidant capacity, and reduced protein oxidation, whereas time out of treatment alone did not significantly alter these variables. Few prior studies have investigated oxidative stress changes in response to exercise in cancer patients, and results have varied. Our results are most consistent with Allgayer et al., (2008) who found that 2 weeks of moderate intensity exercise was capable of reducing oxidative stress, although a high intensity exercise protocol resulted in non-significant increases in oxidative stress. More recently, a 14-week exercise intervention resulted in increased urinary markers of oxidative stress in lung cancer patients, but this also was a high-intensity exercise intervention, which included 25-minute sessions at ventilatory threshold and 60-second interval bouts at peak workload (L. W. Jones, Eves, et al., 2011). The positive outcomes in our current study helps support the notion that an individualized, low to moderate intensity exercise intervention may be preferential to high intensity exercise in the rehabilitation of cancer patients.

Despite concurrent changes in protein oxidation, antioxidant capacity, muscular strength, and CRF, correlations between fitness and oxidative stress parameters were largely absent. Although baseline CRF unexpectedly exhibited a negative correlation with antioxidant capacity ($r = -0.71$), this relationship trended towards positive at follow-up ($r = 0.35$). Because muscular strength and CRF are strongly influenced by age, gender, body composition and lifetime physical activity, it is difficult to quantify cancer treatment-associated muscle damage and fitness decrements associated with cancer based solely on post-treatment fitness assessment. Therefore it was not surprising that oxidative stress was not correlated with baseline strength values within a population of cancer survivors of this size. Because the pre-treatment muscular strength was not known, we cannot exclude the possibility that protein carbonyls contributed to muscle wasting, as has been suggested in the literature (Barreiro et al., 2005; Mantovani et al., 2004; Moylan & Reid, 2007). Future studies may investigate decrements in muscular strength and CRF prior to and following cancer treatment and compare these changes to degree of oxidative damage.

Although it was anticipated that changes in oxidative stress and antioxidant capacity would correlate with changes in fitness parameters, no significant Spearman correlations were detected, but changes in VO_2 peak exhibited a strong trend towards correlation with changes in antioxidant capacity ($r = 0.42$, $p = 0.07$) and protein carbonyls ($r = 0.45$, $p = 0.053$). Due to musculoskeletal limitations in many of the patients, including arthritis and history or surgery, muscular strength may have been strongly limited by pain or limited range of motion, ultimately uncoupling a potential linear relationship between protein oxidation and decrements in strength.

Currently there is no good characterization of the time-course of chronic oxidative stress status following the end of cancer chemotherapy or radiation. In mice, extracellular malondialdehyde levels remained elevated one week after chemotherapy, and oxidized glutathione was significantly higher than controls 6 weeks after treatment (Abushamaa et al., 2002). Unfortunately, it is difficult to extrapolate these data to humans due to varying lifespans. Our data presented here suggest that cancer patients approximately one month out of treatment have persistent elevations in protein oxidation, and furthermore, CON continued to exhibit elevated plasma protein carbonyls 10 weeks later. It should be noted that, although the change in antioxidant capacity over was not significant in CON, this variable no longer significantly differed from NC at follow-up, suggesting some degree of redox normalization as time out of treatment progressed.

The $3.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ improvement in VO_2peak in the exercise group compared favorably with the $2.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ increase identified in a meta-analysis of 6 randomized controlled trials encompassing 344 cancer patients following various exercise interventions (L. W. Jones, Liang, et al., 2011). Despite significant improvements, CRF was poor in EX following the exercise intervention. Regardless, this approximately 1-MET ($3.5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) mean improvement is clinically relevant. Gulati et al. (2003) found that in apparently healthy individuals, for every 1-MET increase in aerobic capacity, there was an associated reduction in mortality rate of 12% in men and 17% in women. While such a relationship has yet to be elucidated in cancer populations, a clinical lower limit of $15.4 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ has been proposed for full independent living of cancer patients (L. W. Jones et al., 2012). In the same study, patients with an absolute VO_2peak greater than $1.09 \text{ L}\cdot\text{min}^{-1}$ had an adjusted hazard ratio

for death of 0.32 compared to patients with aerobic capacity values below $1.09 \text{ L}\cdot\text{min}^{-1}$. Two EX subjects in the current study were below this threshold at baseline, but both surpassed it during the course of the intervention, representing a meaningful clinical outcome.

One control subject demonstrated an impressive 30% increase in VO_2peak , representing an outlier in this group. When excluding this subject, VO_2peak in the control group actually decreased by 2.3%. Despite increased time out of treatment and improved familiarity with the treadmill protocol, CRF in 3 control subjects was diminished at follow-up, which may have been associated with prolonged sedentary activity or persistent oxidative stress, resulting in continued skeletal and cardiac muscle tissue degradation (Powers, Smuder, & Criswell, 2011; Smuder et al., 2011b). Nishayama et al. (1998) demonstrated that oxidative stress may be associated with exercise intolerance in clinical populations, as a significant negative correlation between exercise-induced malondialdehyde production and VO_2peak was present in cardiac patients, whereas no such relationship existed in healthy individuals. Our current investigation evaluated chronic redox status rather than acute, exercise-associated oxidative stress, but future studies investigating oxidative stress markers immediately following an exercise bout may further elucidate the complex dynamic between exercise and oxidative stress in cancer patients.

Summary

Cancer patients exhibited decrements in antioxidant capacity and increased protein oxidation compared to healthy, age-matched individuals. Fortunately, a prescribed, whole-body exercise intervention is capable of concurrently increasing

muscular strength, CRF, and antioxidant capacity, while reducing markers of protein oxidation. It therefore may be surmised that oxidative stress contributes to strength and cardiorespiratory decrements in cancer patients following cancer treatment. Similarly, improved antioxidant capacity may play a role in exercise-mediated cancer rehabilitation.

Limitations

Pseudo-randomization resulted in different gender characteristics between the EX and CON groups. Although baseline strength variables were substantially greater in males than females for this study, because this was a repeated measures experiment, changes in muscular strength over time were not expected to be gender-dependent, and this is unlikely to represent sampling bias. Because there were a variety of cancer types and treatments among the subjects in this study, it is possible that undetected prolonged physiological effects of treatment and cancer type may play a role in patient response to exercise or time out of treatment. Although no substantial dietary alterations were explicitly reported by either the EX or CON group at follow-up, diets were not controlled and food logs were not utilized to evaluate subjects' consumption of antioxidant-containing foods. It is possible that whole foods containing variable antioxidant compositions affected plasma antioxidant capacity or oxidative stress at either baseline or follow-up. Finally, although exercise interventions were similar, individualization of exercise regimens for each cancer patient based upon specific needs may have affected the biochemical and fitness responses to exercise and could potentially limit the generalizability of this study.

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APPENDIX A
PIPER FATIGUE INDEX

PIPER FATIGUE SCALE

Directions: Many individuals can experience a sense of unusual or excessive tiredness whenever they become ill, receive treatment, or recover from their illness/treatment. This unusual sense of tiredness is not usually relieved by either a good night's sleep or by rest. Some call this symptom "fatigue" to distinguish it from the usual sense of tiredness.

For each of the following questions, please fill in the space provided for that response that best describes the fatigue you are experiencing now or for today. Please make every effort to answer each question to the best of your ability. If you are not experiencing fatigue now or for today, fill in the circle indicating "0" for your response. Thank you very much!

1. How long have you been feeling fatigue? (Check one response only).

- 1. not feeling fatigue
- 2. minutes
- 3. hours
- 4. days
- 5. weeks
- 6. months
- 7. other (Please describe) _____

2. To what degree is the fatigue you are feeling now causing you distress?

No Distress											A Great Deal
1	2	3	4	5	6	7	8	9	10		

3. To what degree is the fatigue you are feeling now interfering with your ability to complete your work or school activities?

None											A Great Deal
1	2	3	4	5	6	7	8	9	10		

4. To what degree is the fatigue you are feeling now interfering with your ability to socialize with your friends?

None											A Great Deal
1	2	3	4	5	6	7	8	9	10		

5. To what degree is the fatigue you are feeling now interfering with your ability to engage in sexual activity?

None											A Great Deal
1	2	3	4	5	6	7	8	9	10		

6. Overall, how much is the fatigue which you are now experiencing interfering with your ability to engage in the kind of activities you enjoy doing?

None											A Great Deal
1	2	3	4	5	6	7	8	9	10		

7. How would you describe the degree of intensity or severity of the fatigue which you are experiencing now?

Mild
1 2 3 4 5 6 7 8 9 10 Severe

8. To what degree would you describe the fatigue which you are experiencing now as being?

Pleasant
1 2 3 4 5 6 7 8 9 10 Unpleasant

9. To what degree would you describe the fatigue which you are experiencing now as being?

Agreeable
1 2 3 4 5 6 7 8 9 10 Disagreeable

10. To what degree would you describe the fatigue which you are experiencing now as being?

Protective
1 2 3 4 5 6 7 8 9 10 Destructive

11. To what degree would you describe the fatigue which you are experiencing now as being?

Positive
1 2 3 4 5 6 7 8 9 10 Negative

12. To what degree would you describe the fatigue which you are experiencing now as being:

Normal
1 2 3 4 5 6 7 8 9 10 Abnormal

13. To what degree are you now feeling:

Strong
1 2 3 4 5 6 7 8 9 10 Weak

14. To what degree are you now feeling:

Awake
1 2 3 4 5 6 7 8 9 10 Sleepy

15. To what degree are you now feeling:

Lively
1 2 3 4 5 6 7 8 9 10 Listless

16. To what degree are you now feeling:

Refreshed
1 2 3 4 5 6 7 8 9 10 Tired

17. To what degree are you now feeling:

Energetic
1 2 3 4 5 6 7 8 9 10 Unenergetic

18. To what degree are you now feeling:

Patient
1 2 3 4 5 6 7 8 9 10 Impatient

19. To what degree are you now feeling:

Relaxed
1 2 3 4 5 6 7 8 9 10 A Great Deal

20. To what degree are you now feeling:

Exhilarated

1 2 3 4 5 6 7 8 9

Depressed

10

21. To what degree are you now feeling:

Able to Concentrate

1 2 3 4 5 6 7 8 9

Unable to Concentrate

10

22. To what degree are you now feeling:

Able to Remember

1 2 3 4 5 6 7 8 9

Unable to Remember

10

23. To what degree are you now feeling:

Able to Think Clearly

1 2 3 4 5 6 7 8 9

Unable to Think Clearly

10

24. Overall, what do you believe is *most* directly contributing to or causing your fatigue?

25. Overall, the *best* thing you have found to relieve your fatigue is: _____

26. Is there anything else you would like to add that would describe your fatigue better to us?

27. Are you experiencing any other symptoms right now? _____

APPENDIX B
INFORMED CONSENT

Informed Consent for Participation in Research
Project Title:
Oxidative Stress Alterations Following and Exercise
Intervention in Cancer Survivors

Rocky Mountain Cancer Rehabilitation Institute
 Chris Repka, M.S., Research Associate
 Phone Number: (908) 419-2767
 crepka@skidmore.edu
 Carole M. Schneider, Ph.D., Clinical Director
 Phone Number: (970) 351-2676

You are being asked to participate in a research study collecting information to assess the effect of an exercise program on oxidative stress following radiation and chemotherapy. The Rocky Mountain Cancer Rehabilitation Institute (RMCRI) supports the practice of protection of human subjects participating in research. The following information is provided for you to decide whether you choose to participate in the present study. You should be aware that even if you agree to participate, you are free to withdraw at any time without affecting your opportunities in other projects offered by the Institute.

This project involves the assessment of markers of oxidative stress, or free radical damage, which may have been induced by cancer chemotherapy and radiation therapy. Oxidative stress is known to play a role in the onset of numerous cancer treatment side effects, including fatigue, neurological and psychological detriments, and damage to cardiac and skeletal muscle. Blood samples will be obtained 2 times throughout the study at RMCRI and will be analyzed for these markers of oxidative stress. The blood samples will be obtained prior to initial assessment, and prior to reassessment, after 10 weeks of prescribed exercise.

In addition to blood draws, all subjects will be given the typical RMCRI fitness assessment. Measurement of cardiorespiratory capacity will be conducted on a motor-driven treadmill and will require you to run/walk at progressively increasing speed and grade until exhaustion. A pulmonary function test requires maximum exhalation into a sterile mouthpiece. Flexibility will be evaluated with a sit and reach test. A muscular strength assessment includes 2-10 repetitions of leg press, leg extension, leg curl, seated row, bench press, lat pulldown, and shoulder press. Heart rate, blood pressure, height, weight, and circumference measurements are also recorded. Forms to be completed include cancer history, medical history, cardiovascular risk profile, lifestyle/activity questionnaire, and fatigue and depression scales. Assessments will take place at the RMCRI and take approximately 2 hours to complete. Following the assessment of your cardiovascular endurance and pulmonary function, the results will be analyzed, and an exercise prescription written, by a cancer exercise specialist. During the course of the 10-week exercise intervention, a cancer exercise specialist will be your trainer and will write your daily, individualized exercise plan, which consists of 3, one-hour workouts per week at low to moderate intensity.

There is a possibility that you may be randomly selected for a control group. If this is the case, following the 10 week period of the intervention, you will have the opportunity to participate in the standard exercise intervention for the following 3 months at no cost.

This study will run under the supervision of the RMCRI director and lead investigator, but other persons will be associated with or assist in the data collection. The obtained data may be used in reports or publications but your identity will not be associated with such reports. A number will be used as your identification and your medical and exercise information kept in a locked file cabinet available only to the lead investigator. Confidentiality will only be broken if our assessment reveals that you are severely depressed or if you indicate you are a threat to yourself or to others, at which time you will be referred through our ancillary services for psychological counseling.

A great benefit for participating in this study is exercise training with trained cancer exercise specialists. Additionally, each participant will be provided a summary of his or her exercise data at the beginning and the end of the project period with a clear and concise exercise intensity recommendation based upon the exercise assessment results. There is no compensation for participating in this study.

Risks to you are minimal and may include the possibility of shortness of breath, mild muscular fatigue and soreness, and moderately uncomfortable test situations (such as during the pulmonary function test and the sit & reach test). Additionally, the VO₂peak fitness test used to assess your cardiorespiratory capacity can be uncomfortable. The duration of the discomfort is short. Before all testing, an explanation of the procedure will be given. Overall, the risks inherent in this study are no greater than during a regular physical exercise session a participant may conduct on his or her own time. All subjects will be cleared through the RMCRI Medical Director before the physiological testing commences. When collecting blood samples for research, there is no more risk than that which is encountered when one has blood drawn at a hospital or clinic. Proper containment and disposal of blood will be implemented. If you are injured as a result of this study, you will be treated in the usual manner and charges billed to your insurance/self. The study will not pay for health care costs.

Participation is voluntary. You may decide not to participate in this study and if you begin participation you may still decide to stop and withdraw at any time. Your decision will be respected and will not result in loss of benefits to which you are otherwise entitled, including continued exercise training at RMCRI. Having read the above and having had an opportunity to ask any questions, please sign below if you would like to participate in this research. A copy of this form will be given to you to retain for future reference. If you have any concerns about your selection or treatment as a research participant, please contact the Office of Sponsored Programs, Kepner Hall, University of Northern Colorado Greeley, CO 80639; 970-351-2161.

Participant Name

DATE

Signature of Researcher

DATE

Signature of Subject Agreeing to Participate.
By signing this consent you certify you are at least 18 years of age.

Signature of Medical Director **DATE**

APPENDIX C
INSTITUTIONAL REVIEW BOARD APPROVAL

Thursday, February 18, 2010 12:23 PM

Chris:

Thanks for your revised consent document and answers to my questions regarding your IRB application. **You now have UNC IRB approval.** At the end of your consent document, please make one minor change: the phone number for Sponsored Programs is 351-2161. I do not need to see that change.

I wish you the best with your work.

Sincerely,

Gary Heise

Co-chair, IRB

Gary D. Heise, Ph.D.

School of Sport and Exercise Science

University of Northern Colorado

2760 Gunter Hall

Greeley, CO 80639

voice: 970-351-1738 fax: 970-351-1762

Thursday, January 14, 2010 9:40 AM

Chris;

I completed my review of your IRB application and have some comments that I would like you to address. Comments on the informed consent document may be better addressed during a short meeting. I will be at RMCRI later today (Thursday), but could also meet tomorrow (Friday). Essentially, the consent document needs some details added (e.g., VO₂peak procedures - as it is written, the reader does not know that he/she will be wearing a mouthpiece, nose-clip and walking until he/she can no longer continue). The discussion of risks and benefits need to be re-worded - your narrative reads more clearly. I have a few other comments, but again can be quickly addressed with a meeting.

The control group assignment worries me. People will be coming to RMCRI because they want to exercise, so telling them that they are in a control group and cannot exercise is an issue that must be addressed (delicately). In contrast to your comment to Dr. Weiler, I have heard some granting agencies that do not allow non-exercising control groups for certain studies because it would be unethical to withhold a treatment that is known to do good (can this be said for cancer patients?). One other comment - "already approved by UNC IRB" should read "previously approved by UNC IRB" IRB approval are for specific protocols. Dr. Schneider and I talked about a more "blanket" IRB approval for RMCRI, but such approval is not currently in place.

Please let me know when you are available.

Thanks,

Dr. Heise

APPENDIX D
SUBJECT UPDATE QUESTIONS

Subject Update Questions

1. Did you have any problems as a result of your last training session? (soreness, pain, etc.)
Yes No If yes, identify the specific problem on the Client Update Response Sheet
2. Are you having any specific problems today that would affect training? (feeling ill, headache, pain, nausea, dizziness, extreme fatigue, etc.)
Yes No If yes, identify the specific problem on the Client Update Response Sheet
3. Are you taking any new medications? Has a medication been discontinued or the prescribed amount changed? Has your health status changed?
Yes No If yes, identify the change on the Client Update Response Sheet and notify the Clinical Coordinator.
4. Since your last exercise session, have you been diagnosed with cancer recurrence?
Yes No If yes, write an explanation and notify the Clinical Coordinator.
5. Since your last exercise session, have you begun chemotherapy treatment?
Yes No If yes, immediately report the date and specific chemotherapy drugs to the Clinical Coordinator.
6. Since your last exercise session, have you completed your chemotherapy treatment?
Yes No If yes, immediately report the date completed to the Clinical Coordinator.
7. Since your last exercise session, have you begun your radiation treatment?
Yes No If yes, immediately report the date of initiation and type of radiation to the Clinical Coordinator.
8. Since your last exercise session, have you completed your radiation treatment?
Yes No If yes, immediately report the date completed to the Clinical Coordinator.
9. Since your last exercise session, did you perform any additional exercise?
Yes No If yes, describe the type, duration, and intensity. If lengthy, include on separate sheet in training log.

APPENDIX E
EXIT SURVEY FOR CONTROL SUBJECTS

Exit Survey for control subjects, Chris Repka's Oxidative Stress Study

Name: _____

Date: _____

1. Since your initial assessment, how many times per week did you engage in physical activity?
2. Have you changed your dietary habits
3. Have you made any other lifestyle changes since we last met? (yoga, meditation, support groups, changes in medication).
4. Have you had any further medical procedures?
5. How many days a week would you like to exercise at RMCRI?

APPENDIX F

STANDARD CURVES FOR BIOCHEMICAL ANALYSES

