THE EFFECT OF A SINGLE 30-MINUTE BOUT OF MODERATE INTENSITY INTERMITTENT EXERCISE ON THE INFLAMMATORY RESPONSE IN BREAST CANCER SURVIVORS

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ABSTRACT

Robert Coleman Mills III: The Effect of a Single 30-Minute Bout of Moderate Intensity
Intermittent Exercise on the Inflammatory Response in Breast Cancer Survivors
(Under the direction of Claudio L. Battaglini)

This retrospective study examined the effect of an acute 30-minute bout of moderate intensity intermittent exercise on inflammatory markers (TNF-α, CRP, and IL-10) in breast cancer survivors and healthy controls. Relationships between inflammatory markers and NK cell count were also explored. Eighteen subjects (9 breast cancer survivors, 9 healthy controls) completed the study. Markers of inflammation and NK cell count were analyzed from blood samples taken at pre-exercise, immediately post-exercise, 2 hours post-exercise, and 24 hours post-exercise. There were no significant changes in inflammatory markers across time for both groups ($p > 0.05$). There were no significant relationships between changes in inflammatory markers and NK cell across time ($p > 0.05$). In conclusion, both groups responded similarly to the acute bout of exercise relating to markers of inflammation, and from this sample changes in NK cell did not seem to be mediated by the inflammatory response.

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CHAPTER I

INTRODUCTION

Breast cancer is the second leading cause of cancer death in women after lung

cancer, and is currently the most common form of cancer in women other than skin

cancer in the United States (ACS, 2013). In the United States in 2013, a total of 232,340

new breast cancer cases and 39,620 new breast cancer deaths are projected to occur in

women (Siegel et al., 2013). Surgery, chemotherapy, radiation, and hormone

replacement therapies are the most commonly used treatments for those with cancer

(ACS, 2013). Treatment improvements and early diagnosis have increased survival

rates, and more women are surviving longer than ever before. There have also been

other factors contributing to the increased survival rate of cancer patients such as a

higher awareness of the importance of self-examination, greater education, and

advancements in modern technology (ACS, 2013).

Breast cancer treatment results in many negative side effects. These include

fatigue, nausea, pain, muscle weakness, and loss of functional capacities among others.

In addition, cancer treatment has been shown to result in inflammation along with

decreased immune system function (Battaglini et al., 2006; Grivennikov et al., 2010).

Exercise has been shown to be an effective method for alleviating some of these side

effects of cancer treatment (Schmitz et al., 2010). Improvements in cardiorespiratory

and muscular fitness, and overall quality of life along with reductions in fatigue, nausea,

and pain have been well documented in the literature (Dimeo et al., 1997; Herrero et al., 2005; Kim et al., 2006; Battaglini et al., 2006; De Backer et al., 2008; Hayes et al., 2012; Cormie et al., 2013).

Most recently, one of the areas that has sparked interest among researchers in exercise oncology is the effect of exercise on markers of inflammation (Cytokines) and immune response. One of the main reasons for the increased interest in this area of research is due to the important roles cytokines have in immune response. These roles include: coordination of inflammatory responses which permits communication between immune cells, immune regulation through interactions with specific membrane receptors, and activation of intracellular signaling transduction pathways to induce gene transcription and synthesis of new cellular proteins (Chaplin, 2010; Zhou, 2010). Cytokines are also mediators of innate and adaptive immunity, and are often involved in inflammation through their effects on cell recruitment, cell activation, and antigen presentation (Zhou, 2010). Cytokines have been shown to mediate the proliferative and cytolytic activities of different cell types that target malignant cells such as NK cells, cytotoxic T cells, and monocytes (Abbas, 2005).

There is preliminary evidence that populations with moderate to high levels of inflammatory markers may reduce circulating levels of pro-inflammatory cytokines with regular aerobic exercise (Petersen and Pedersen, 2006). However, studies that have investigated the exercise-induced inflammatory and immune response in breast cancer survivors are scarce and have presented conflicting results, with some showing positive exercise effects while others showing no response (Pederson, 2000; Löf et al., 2012). Possible explanations for the variable results in the inflammatory and immune

response presented in the current literature may be due to different study populations, different types of anti-cancer treatment, and the type, intensity, and duration of the exercise. In addition, the sensitivity of the assays used in different studies can affect the cytokine profile (Pederson, 2000).

An interesting observation when examining the current literature regarding the effects of exercise on cytokines and immune marker responses in cancer patients is that all previous studies have only examined the immune and inflammatory marker responses in relation to chronic exercise; in other words, these studies had cancer patients exercise train for a period of time, and cytokines and inflammatory markers were assessed at baseline and follow-up (prior to training and at the end of the exercise training period) (Fairey et al., 2005a; Hutnick et al., 2005; Gomez et al., 2011; Janelsins et al., 2011; Jones et al., 2012). The focus of these studies was to examine the effects of exercise training interventions on potential up-regulation of immune function through mediation of positive changes in inflammatory markers (↑ anti-inflammation while ↓ pro-inflammatory markers) (Fairey et al., 2005a; Peterson and Pedersen, 2005).

In cancer survivors, the potential positive mediation of cytokine response to exercise training on the up-regulation of immune function may translate into better immune surveillance and therefore a potential risk reduction of a possible recurrence and ultimately longer survival. It is also important to note that repeated bouts of acute high intensity/long duration exercise without sufficient recovery has been suggested to further suppress the immune system and lead to chronic impairments in immune function (Smith, 2003). For cancer survivors, an immune suppressed state could promote an undesirable long-term prognosis.

Cancer treatments may often have considerable effects on the immune system of cancer patients and survivors, particularly by decreasing cell number and function of immune cells including components that are associated with anti-cancer defense (NK Cells, neutrophils, macrophages, and T lymphocytes) (Fairey et al., 2002). In order to better understand how these treatments impact immune function and perhaps how exercise may alter the immune system in cancer patients, it is paramount that the examination of an acute bout of exercise on the immune response in cancer patients is investigated. Examining the acute effects of an exercise bout on markers of inflammation and immune function in this population will allow for a better understanding of the effect of exercise on the immune response of cancer patients and the role of inflammation in the regulation of immune function. This will also help identify the immune system recovery throughout the exercise timeline so more precise exercise prescriptions in regards to intensity, volume, and frequency of exercise training can be devised for cancer patients. Studying these effects can help provide certainty for promoting positive immune adaptations while avoiding the potential of exercise to create an immune suppression response in this population.

The majority of breast cancer survivors have been shown to have higher levels of inflammation compared to healthy populations (Seruga et al., 2008). Exercise prescriptions for breast cancer survivors are designed after those for healthy populations, but it is currently unknown whether breast cancer survivors have similar exercise-induced inflammatory responses as healthy individuals (Schmitz et al., 2010). Therefore, it is necessary to examine the inflammatory response to an acute bout of exercise in order to compare the inflammatory/immune response during and post-

completion of an exercise bout (recovery from exercise) so comprehensible exercise prescription guidelines for breast cancer survivors can be designed to be safer and promote optimal benefits while minimizing the potential issue of promoting immune suppression.

In a previous dissertation, Dr. Elizabeth Evans evaluated the immune response to an acute bout of exercise in breast cancer survivors and healthy controls. The current study is retrospective in nature, and will use stored blood samples from Dr. Evans' dissertation to examine the inflammatory response between breast cancer survivors and healthy controls during one acute bout of moderate intensity intermittent exercise. With the goal of researching the pro and anti-inflammatory response, Tumor Necrosis Factor (TNF-α), C-Reactive Protein (CRP), and Interleukin 10 (IL-10) were the inflammatory markers selected for the current study. These markers were also selected because they are commonly seen in research examining this population. NK cell count has already been measured in the previous dissertation of Dr. Evans. NK cells are highly associated with tumor progression, and they were selected for correlational purposes in order to investigate if inflammatory markers can potentially modulate immune function due to exercise in breast cancer survivors.

Statement of the purpose

The purpose of this study was to investigate the effect of one acute bout of moderate intensity intermittent exercise on selected markers of inflammation in post-treatment breast cancer survivors.

A secondary purpose was to examine the relationship between selected markers of inflammation and natural killer cell count to explore if the potential mediation of the immune response was also influenced by the inflammatory response due to exercise in breast cancer survivors.

Research questions

R1. Does one bout of acute aerobic exercise alter the inflammatory response in breast cancer survivors and healthy controls immediately post-exercise?

R2. How long does the inflammatory response to an acute bout of exercise last in both the breast cancer and healthy control groups?

R3. Does the inflammatory response due to exercise in breast cancer survivors differ from healthy controls?

R4. Is there a relationship between the changes in inflammatory markers and the change in NK cell count after an acute bout of aerobic exercise in breast cancer survivors?

Hypotheses

H_1: From pre-exercise to immediately post-exercise both TNF-α and CRP will be significantly reduced in both groups.

$\qquad H_{1a}$: From pre-exercise to immediately post-exercise IL-10 will be significantly increased in both groups.

H_2: From pre-exercise to 2 hours post-exercise both TNF-α and CRP will be significantly reduced in both groups.

H_{2a}: From pre-exercise to 2 hours post-exercise IL-10 will be significantly increased in both groups.

H_3: From pre-exercise to 24 hours post-exercise both TNF-α and CRP will be significantly reduced in both groups.

H_{3a}: From pre-exercise to 24 hours post-exercise IL-10 will be significantly increased in both groups.

H_4: There will be a significant inverse correlation between changes in NK cell count and changes in both TNF-α and CRP from pre-exercise to immediately post-exercise in both groups.

H_{4a}: There will be a significant inverse correlation between changes in NK cell count and changes in both TNF-α and CRP from pre-exercise to 2 hours post-exercise in both groups.

H_{4b}: There will be a significant inverse correlation between changes in NK cell count and changes in both TNF-α and CRP from pre-exercise to 24 hours post-exercise in both groups.

H_5: There will be a significant positive correlation between changes in NK cell count and changes in IL-10 from pre-exercise to immediately post-exercise in both groups.

H_{5a}: There will be a significant positive correlation between changes in NK cell count and changes in IL-10 from pre-exercise to 2 hours post-exercise in both groups.

H_{5b}: There will be a significant positive correlation between changes in NK cell count and changes in IL-10 from pre-exercise to 24 hours post-exercise in both groups.

Operational definitions

- *Major Cancer Treatment*: Surgery, chemotherapy, and radiation therapy.

- *Breast Cancer Survivors*: Women between the ages of 40-70 years who had confirmed diagnosis of Stages I-III invasive breast cancer and had completed all planned surgery, radiation therapy, and chemotherapy 3-6 months prior to participation in the study.

- *Healthy Controls*: Study group which included women 40-70 years of age who were healthy, sedentary, and had never received treatment for cancer of any type. Healthy controls were matched to breast cancer survivors based on age.

- *Sedentary*: Not participating in regular physical activity for at least 1 year prior to enrollment in the study. Regular physical activity would be considered as 30-minutes of moderate-vigorous activity, 3 days per week.

- *VO2peak*: A subject's peak aerobic capacity, measured during a maximal cardiopulmonary exercise test (CPET).

- *Acute Bout of Intermittent Exercise*: The 30-minute exercise bout was comprised of ten 3-minute exercise intervals performed at 60% of VO_{2peak} alternating with 1.5 minutes of rest. A discontinuous exercise protocol was used to ensure that all subjects would be capable of completing the exercise session.

- *Baseline*: The study time point that will occur immediately before commencing the acute bout of moderate intensity exercise. Baseline is synonymous with pre-exercise.

- *Immediately post-exercise*: The study time point that occurred immediately upon completion of the acute bout of moderate intensity exercise.

- *2 hours post-exercise*: The study time point that occurred 2 hours after completion of the acute bout of moderate intensity exercise.

- *24 hours post-exercise*: The study time point that occurred 24 hours after completion of the acute bout of moderate intensity exercise.

- *Innate immune system*: The initial host response due to the broad number of recognition molecules that act rapidly after an invading pathogen or toxin is encountered (Chaplin, 2010; Seruga et al., 2008).

- *Inflammation*: A complex reaction of the innate immune system in tissues involving the accumulation and activation of plasma proteins and leukocytes at a site of cell injury, toxin exposure, or infection (Abbas, 2005).

- *NK cells*: Major cellular components of the innate immune response that attack virally infected cells as well as tumor cells (Abbas, 2005).

- *Cytokines*: Proteins produced by macrophages and many other types of innate immune system cells in response to microbes and other antigens that mediate inflammatory and immune reactions (Abbas, 2005). They help control various aspects of cellular growth and differentiation (Corwin, 2000)

- *C-reactive protein*: An acute phase protein produced in the liver that has pro-inflammatory characteristics and is a highly significant marker of inflammatory disease (Abbas, 2005).

- *TNF-α*: A pro-inflammatory cytokine that is responsible for many of the systemic complications of severe infections. TNF-α is also an important mediator of the acute inflammatory response to infectious microbes (Abbas, 2005).

- *IL-10*: Produced by T cells and is an inhibitor of host immune responses, especially

those involving macrophages (Corwin, 2000). IL-10 has anti-inflammatory properties and is considered the most profound immunosuppressive cytokine (Smith, 2003).

<u>Delimitations</u>

- All subjects were women between the ages of 40 and 70 years old.

- All subjects in the breast cancer survivor group had completed their major cancer treatments and surgeries 3-6 months prior to the time of participation in the study.

- All subjects in the control group had been sedentary for at least 1 year prior to enrollment; i.e., they had not participated in regular organized physical activity within the past year.

- Subjects in the breast cancer survivor group were recruited from the Medical Oncology clinic and the Radiation Oncology clinic at the NC Cancer Hospital on the campus of the University of North Carolina at Chapel Hill (UNC-Chapel Hill) as well as from the waitlist of the Get REAL and HEEL Breast Cancer Program in the Department of Exercise and Sport Science at UNC-Chapel Hill.

- Subjects in the healthy control group were recruited from the faculty, staff, and student populations at UNC-Chapel Hill, as well as from across the Triangle region of North Carolina.

- Baseline study time points occurred between 7:00-10:00am in order to control for daily variations in study variables.

- All the subjects were cleared by their medical oncologist or primary physician prior to enrolling in the study, and had no major health issues that precluded their ability to engage in low to moderate exercise training.

- Before the VO_{2peak} assessment and the acute bout of exercise, subjects were asked to refrain from eating 2 hours prior to testing and not to exercise for at least 12 hours prior to testing. Also, subjects were asked to refrain from caffeine use at least 12 hours prior to testing and not consume alcohol at least 48 hours prior to testing.

Assumptions

- All breast cancer survivors strictly adhered to all of the pre-assessment guidelines given to them prior to reporting to all laboratory-testing sessions.

- The impact of different cancer treatments and drugs would result in similar side effects experienced by the subjects in the breast cancer survivor group.

- All subjects accurately reported medical history, and abstained from using any medications that may affect the response of the study variables.

- Breast cancer survivors did not alter their dietary practices during the study.

Limitations

- This is a retrospective study and all data collection procedures could not be altered for this study.

- The results of this study may only apply to sedentary women between the ages of 40-70 years, as well as breast cancer survivors for whom it has been 3-6 months since receiving any major cancer treatment. The results of this study may not be

able to be generalized to women of all ages, men, all cancer survivors, cancer

patients currently undergoing major cancer treatment, or individuals who are

exercise-trained.

- Cancer survivors' exercise training prior to treatment could alter the inflammatory

  and immune response.

- Changes in cytokines were measured only in the blood.

- The different treatment regimens that breast cancer survivors underwent prior to

  the study could have influenced their exercise-induced response.

- The intermittent nature of the exercise bout may differently influence the immune

  and inflammatory response compared to continuous exercise.

Significance of study

This study helped to clarify if inflammation is acutely altered after a bout of

moderate intensity exercise in breast cancer survivors. Few studies have attempted to

examine potential mediators of the exercise-induced immune response in breast cancer

patients, such as inflammatory markers. Most studies in this area have only looked at

the inflammatory response to long-term exercise training interventions in this

population. However, it is important to understand the acute inflammatory response to

exercise in order to monitor the safety and efficacy of implemented exercise programs

for this population. Since inflammation is a mechanism of innate immunity, the

immune system can potentially be helped if an acute bout of moderate intensity

exercise can positively alter inflammatory markers (Bruunsgaard, 2005). Thus,

exercise may help mitigate some of the chronic inflammation and immunosuppression

commonly seen in breast cancer survivors. In order to assess whether exercise may reduce the risk of illness in cancer patients, clinicians and researchers need to understand how exercise affects the inflammatory response in these patients. Consequently, more specific and relevant exercise prescriptive guidelines can be developed to allow these patients to achieve maximum health benefits without risking further illness (Jones et al., 2012). More precise aerobic exercise prescriptions for breast cancer survivors can be designed as a result of knowledge gained from this study, especially with regard to full recovery time needed from one bout of moderate intensity exercise to a subsequent one.

CHAPTER II

REVIEW OF LITERATURE

For the purpose of organization, Chapter II will be organized into the following sections: I. Breast cancer facts, treatment-related side effects, and general exercise benefits; II. Inflammation and immunology overview; III. Effects of exercise on markers of inflammation in healthy populations; IV. Cancer and markers of inflammation; V. Effects of exercise on markers of inflammation in breast cancer survivors; VI. Summary. Measures from the following reviewed articles have been delimited in relation to the measurements pertinent to the current study.

Breast cancer facts, treatment-related side effects, and general exercise benefits

Breast cancer facts

Breast cancer is defined as a malignant tumor starting in the cells of the breast that may metastasize to distant areas of the body or invade surrounding tissues. Except for skin cancers, breast cancer is the most common form of cancer in women in America, and approximately 12% of American women will develop invasive breast cancer throughout their lifetime (ACS, 2013). In 2013, it is estimated that approximately 232,340 new cases will be diagnosed along with 39,620 deaths among women from breast cancer in the United States (ACS, 2013; Siegel et al., 2013).

Breast cancer treatment primarily consists of surgery, chemotherapy, radiation therapy, and hormone therapy. The method of administration depends on the type and stage of the breast cancer, and many of these treatments are combined according to the needs of the patient (NCI, 2013). Common surgical treatments are used to remove cancer from the breast and may include lumpectomy, partial mastectomy, or total mastectomy. Chemotherapy is a treatment that destroys cancer cells by killing them or stopping their division and is administered both orally and intravenously. Radiation is another breast cancer treatment that uses high-energy x-rays to destroy cancer cells or prevent them from growing larger. Hormone therapy treatment removes hormones by preventing their release from endocrine glands and blocking their action in order to help stop cancer growth (NCI, 2013). Targeted therapies are being developed that are tumor specific. These types of therapies are growing in number and include trastuzumab also known as Herceptin, which is a monoclonal antibody given to breast cancer survivors who overexpress the protein called human epidermal growth factor receptor 2 (HER2/neu receptor) that is responsible for promoting the growth of cancer cells (ACSM's Guide to Exercise and Cancer Survivorship, 2012).

Breast cancer treatment-related side effects

Breast cancer survival rates have improved due to earlier detection through increased awareness and screening, advancements in modern technology, increased self-examination, and improvements in treatment (Battaglini et al., 2008). Although survival rates have increased in the last several years, many negative adverse side effects can result from breast cancer treatment. Treatment-related side effects may be acute, lasting over a period of days or weeks, or they may be persistent, lasting years

after the completion of treatment (ACSM's Guide to Exercise and Cancer Survivorship, 2012). Pain, infection, tenderness, bleeding, and temporary swelling are among the side effects of surgical treatment for breast cancer (ACS, 2013). Chemotherapy side effects may include weight changes, nausea, hair loss, fatigue, vomiting, and an increased chance of infections (ACSM's Guide to Exercise and Cancer Survivorship, 2012). Radiation treatments may cause patients to encounter soreness, fatigue, skin changes, and swelling (ACSM's Guide to Exercise and Cancer Survivorship, 2012). Side effects of hormone therapy may involve hot flashes, fatigue, vaginal discomfort, and mood swings (ACSM's Guide to Exercise and Cancer Survivorship, 2012).

Overall, usual side effects observed in cancer patients that have undergone treatment are depression, worry, pain, cachexia, dyspnea, nausea, and fatigue (Battaglini et al., 2006). Studies have reported that 70 percent of patients undergoing chemotherapy and radiation have fatigue (Battaglini et al., 2007). Both radiation and chemotherapy have also been shown to cause necrotic death of cancer cells and surrounding tissues, which can result in increased inflammation in breast cancer patients (Grivennikov et al., 2010). In addition, cancer patients may experience depressed immune system parameters. Patients receiving chemotherapy have been shown to have suppressed NK cell counts compared to healthy populations (Shore et al., 1999).

Exercise benefits on treatment-related side effects

There is now strong evidence found in epidemiologic studies that exercise may affect breast cancer risk reduction. In a systematic review conducted by C. M. Friedenreich, 73 epidemiologic studies were reviewed that provide evidence that

physical activity reduces breast cancer risk by about 25% (Friedenreich, 2010).

Exercise may also improve overall general health, and studies have shown that exercise

can be a helpful tool in attenuating the physiological effects associated with breast

cancer treatment. Improvements in cardiorespiratory fitness, body composition,

physical functioning, quality of life, and fatigue have been shown by systematic review

evidence in cancer survivors who exercise (DeBacker et al., 2008).

Patients receiving cancer treatments in previous years were advised to rest and

avoid activity known to further decrease energy levels. Exercise has been shown by

scientific research to help alleviate the routine symptoms of cancer treatments such as

pain, nausea, and fatigue. Possible benefits of exercise used to alter normal cancer

treatment side effects include improved cardiovascular efficiency, increased

mobilization, muscle regeneration, energy production enhancement, and stimulation of

erythrocyte, leukocyte, and thrombocyte cell production (Battaglini et al., 2006).

Numerous studies have generally demonstrated that exercise does indeed reduce

insulin resistance, endogenous estrogen levels, adiposity levels, and inflammation

(Friedenreich, 2010).

Inflammation and immunology overview

Inflammation

Inflammation can be described as a complex reaction of the innate immune

system in tissues involving the accumulation and activation of plasma proteins and

leukocytes at a site of cell injury, toxin exposure, or infection (Abbas, 2005).

Inflammation involves a progression of synchronized immune responses to tissue

damage caused by physical agents such as trauma, surgery, or radiation. Other causes

of inflammation may be due to infections by pathogens, either viral or bacterial (Zhou et

al., 2010). The inflammatory process is considered to be homeostatic. In this process

the host begins a complex series of humoral and cellular responses in an effort to limit

tissue damage, isolate and destroy the invading organism, and activate the course of

repair (Smith, 2003). Inflammation can serve as a protective mechanism in promoting

tissue repair and controlling infections, but it can also cause tissue damage and disease

(Abbas, 2005).

Cytokines

Cytokines are proteins produced by many different types of cells in response to

microbes and other antigens that mediate inflammatory and immune reactions (Abbas,

2005). The production of cytokines can be from macrophages and lymphocytes as well

as other cells such as fibroblasts and endothelial cells (Smith, 2003). Production of

these proteins is increased in response to various stressors including injury, infection

by microorganisms, normal growth demands, psychological distress, and inflammation

(Corwin, 2000). At low concentrations, many cytokines have beneficial effects, whereas

tissue damage and multiple organ failure are associated with higher concentrations

(Smith, 2003). Cytokines are often involved in inflammation through their effects on

cell recruitment, bone marrow differentiation, antigen presentation, cell activation, and

cell adhesion molecule expression (Zhou et al., 2010). There are three broad classes of

cytokines including lymphokines and monokines, growth factors, and colony-

stimulating factors (Corwin, 2000).

Depending on the microenvironment, cytokines can be functionally separated into two categories: those that are inherently pro-inflammatory and anti-inflammatory. Pro-inflammatory cytokines are important in the inception and advancement of inflammation. On the other hand, anti-inflammatory cytokines inhibit certain conditions of adaptive immunity (Abbas, 2005). Pro-inflammatory cytokines are especially of interest to clinicians since they guide the immune response by the stimulation of white blood cell proliferation and cytotoxicity. These cytokines can be controlled by anti-inflammatory cytokines through negative feedback (Corwin, 2000).

Cytokines are regarded as communication molecules that can act at any range (autocrine, paracrine, endocrine), and are important in activating local inflammatory/immune cells and regulating invasion of white blood cells into traumatized tissue (Smith, 2003). Similar to classic hormones, cytokines act by binding to a receptor on a target cell to alter the function of the target cell (Corwin, 2000). They act as immune regulators through interactions with specific membrane receptors (Zhou et al., 2010). Cytokines elicit cellular responses by activating signal transduction pathways within the cell. This is accomplished with the binding of cytokines to transmembrane cell surface receptors and ultimately leads to the introduction of new gene transcription and the synthesis of new cellular proteins (Chaplin, 2010).

The actions of cytokines are often pleiotropic, meaning one cytokine has the ability to act and induce a variety of responses on different cell types. This pleiotropic nature allows cytokines to mediate diverse biological effects whether it be regulating innate immunity, adaptive immunity, or stimulating hematopoiesis (Abbas, 2005). Cytokines also exert redundant effects with multiple cytokines having the same

functional effects (Abbas, 2005). Virtually every system in the body is affected by cytokines. They also have an effect on all aspects of growth and development as well as every host response to injury, infection, and inflammation (Corwin, 2000).

Specific markers of inflammation

Tumor necrosis factor (TNF-α) is released from activated macrophages in response to increased concentrations of IL-1 and IL-2 and bacterial endotoxin (Corwin, 2000). This pro-inflammatory cytokine is responsible for many of the systemic complications of severe infections. TNF-α is also an important mediator of the acute inflammatory response to infectious microbes (Abbas, 2005). This cytokine acts to induce expression of IL-1 and IL-2 and stimulates the production of IL-6, leading to increased signs of infection and inflammation as well as the production of acute phase proteins (Corwin, 2000). One of the main physiological functions of TNF-α is to stimulate the recruitment of monocytes and neutrophils to infection sites and activate these cells to eliminate microbes (Abbas, 2005).

C-reactive protein (CRP) is an acute phase protein produced in the liver that has pro-inflammatory characteristics and is a highly significant marker of inflammatory disease. CRP is an acute phase reactant due to the increase in its plasma levels during the acute stages of many infections (Abbas, 2005). CRP is involved in the innate immune response to bacterial infections and is considered to be a member of the pentraxin family of plasma proteins. This protein is known to bind to the capsule of pneumococcal bacteria (Abbas, 2005). Sedimentation rate is a blood test that can identify the presence of inflammation by measuring how quickly red blood cells settle in a test tube in one hour. The elevation in CRP as well as other acute phase proteins

contributes to sedimentation rate increases that frequently accompany infective and inflammatory disease. In some circumstances the measurement of CRP may be considered to be a key indicator of morbidity relating to infective and inflammatory diseases in general (Corwin, 2000).

Interleukin-10 (IL-10) is involved in controlling innate immunity reactions and cell-mediated immunity through the suppression of activated macrophages and dendritic cells. IL-10 is produced by T cells and is an inhibitor of host immune responses, especially those involving macrophages (Abbas, 2005; Corwin, 2000). On the basis of cell-mediated immunity and macrophage function, IL-10 is considered the most profound immunosuppressive cytokine (Smith, 2003). IL-10 has an anti-inflammatory property known to down-regulate inflammatory cytokine production including TNF-α, IL-1, and IL-6 (Zhou et al., 2010). IL-10 may suppress cytokine synthesis by inhibiting the transcription of the genes of cells and by post-transcriptional mechanisms (Petersen and Pedersen, 2005).

Cytokines and immunity

Cytokines are considered the principal mediators of communication between cells of the immune system, functioning as key contributors to immunity and inflammation (Abbas, 2005). These soluble proteins allow communication between immune cells with coordination of inflammatory responses (Zhou et al., 2010). Since cytokines are released from immune cells in a signaling fashion, they have strong roles in the up-regulation of particular immune parameters (Pedersen, 2000). The immune system employs many mechanisms working together to combat infection by microbes.

This fully integrated immune response draws elements from multiple effector systems in order to modify a specific response to the invading pathogen (Chaplin, 2010).

Immune function

The immune system is commonly divided into two main sectors including innate immunity and adaptive immunity. Both innate and adaptive mechanisms are used to detect and eliminate pathogenic microbes (Chaplin, 2010). NK cells as well as monocytes/macrophages, T cytotoxic cells, and polymorphonuclear cells are involved in innate immunity. These cells help to form the "first line of defense," and generally respond to any invading pathogen by rapidly recognizing common molecular patterns and indiscriminately attacking anything appearing to be foreign (Abbas, 2005). The innate system is the initial host response due to the broad number of recognition molecules that act rapidly after an invading pathogen or toxin is encountered (Chaplin, 2010; Seruga et al., 2008). NK cells are an important component of cell-mediated immunity, and they are the most sensitive cells to typical stress-induced immunosuppression (Smith, 2003). NK cells are morphologically defined as large granular lymphocytes that recognize their targets using an elaborate accumulation of activating and inhibitory cell surface receptors (Chaplin, 2010). These cells develop in bone marrow and have no antigen-specific receptors. They can destroy target cells through antibody dependent cell-mediated cytotoxicity. In addition, NK cells have prominent anti-tumor effects and are potent killers of virally infected cells (Chaplin, 2010).

Effects of exercise on markers of inflammation in healthy populations

Exercise and cytokine response

        Exercise modulates the immune system in healthy individuals and can work as a model of temporary immunosuppression (Pedersen and Hoffman-Goetz, 2000). The inflammatory regulation of exercise has been linked to the production of cytokines (Petersen and Pedersen, 2005). Cytokines are released at the site of inflammation as a result of exercise, infection, or tissue injury. The exercise-induced local inflammatory response is accompanied by a systemic response known as the acute phase response (Pedersen and Hoffman-Goetz, 2000; Ostrowski et al., 1999). This response includes the production of a large number of hepatocyte-derived acute phase proteins, such as CRP (Petersen and Pedersen, 2005).

        Although many different cell types produce plasma cytokines, muscle cells are a major source during exercise (Zhou et al., 2010). Muscle contractions can induce a myokine response, which is characterized by the release of cytokines, such as IL-6, from working muscles. Regular muscle contraction can mediate signals with myokines that may suppress pro-inflammatory activities at both distant sites as well as within the skeletal muscle itself (Bruunsgaard, 2005).

        The cytokine response in relation to exercise may be initiated due to the mechanical disruption of myofibers. The production of cytokines resulting from exercise resembles that observed in relation to trauma (Pedersen and Hoffman-Goetz, 2000). However, many of the effects that come with the fully developed, systemic pro-inflammatory response occurring with trauma do not happen with exercise. Although the initiation of the acute-phase response develops, the lack of a fully developed

systemic response with exercise may be due to exercise only resulting in a transient

release of cytokines (Pedersen and Hoffman-Goetz, 2000). Increases in pro-

inflammatory cytokines such as TNF-α are minute due to exercise without muscle

damage, and this indicates that in non-traumatic exercise, the cytokine cascade differs

importantly from the classical acute-phase response in infectious systems

(Bruunsgaard, 2005). The fact that pro-inflammatory cytokines such as TNF-α may not

increase with exercise provides evidence for the difference in the cytokine response to

exercise compared to that of severe infections (Petersen and Pedersen, 2005). Also,

anti-inflammatory cytokines, soluble receptors, and receptor antagonists can restrict

the inflammatory response to exercise, potentially having an effect on exercise not

producing a full systemic response (Ostrowski et al., 1999).

   Numerous studies have shown that several cytokines can be found in plasma

both during and after exercise. While acute exercise protocols result in short term

changes in particular cytokines, chronic exercise training may result in decreased levels

of many circulating cytokines (Zhou et al., 2010). Exercise may also induce an increase

in the systemic levels of a number of cytokines with anti-inflammatory properties, and

thereby can protect against chronic medical disorders associated with low-grade

systemic inflammation (Petersen and Pedersen, 2005).

Exercise in relation to the inflammatory process

   Epidemiological evidence supports the notion that a lifestyle with a high degree

of physical activity is associated with attenuated circulating levels of TNF-α, IL-6, and

CRP independently of age, gender, BMI, or blood glucose. Furthermore, a high level of

physical activity is associated with reduced levels of peripheral inflammatory mediators

in the range of 20-60% compared with a sedentary lifestyle (Bruunsgaard, 2005). Even

physical inactivity has been shown to be associated with low-grade systemic

inflammation in healthy subjects according to several cross-sectional studies (Petersen

and Pedersen, 2005). In healthy individuals, elevated levels of CRP may be associated

with increased body fat and sedentary lifestyles (Pierce et al., 2009b). Overall, multiple

studies have found a strong inverse relationship between physical fitness and markers

of inflammation (Markovitch et al., 2008).

More frequent physical activity may help reduce the levels of systemic

inflammation in the general population. Several cross-sectional reports using general

population samples have indicated that increased levels of exercise are associated with

decreased levels of markers of inflammation such as C-reactive protein (CRP)

(Abramson and Vaccarino, 2002). Abramson and Vaccarino (2002) found that a higher

frequency of physical activity was independently associated with lower odds of having

elevated inflammation levels, such as CRP and white blood cell count, among healthy

middle-aged and older US adults. This association was found even after adjustments

were made for potential confounding factors including measures of general obesity

(body mass index) and central obesity (waist-to-hip ratio), which have been shown to

influence levels of inflammation (Abramson and Vaccarino, 2002). Also, physical

activity has been shown to be inversely associated with CRP concentrations in a

representative sample of healthy adults in the US, suggesting that physical activity may

mitigate inflammation (Ford, 2002).

Multiple studies indicate that the association between exercise and inflammation

is not entirely mediated by reductions in obesity, and other mechanisms such as the

antioxidant effects of exercise may be involved in the relationship between exercise and decreasing levels of inflammation (Abramson and Vaccarino, 2002). It is possible that exercise training in general may reduce markers of inflammation, such as CRP, both directly and indirectly. Exercise may reduce CRP directly by reducing cytokine production in muscle, fat, and mononuclear cells and indirectly by reducing body weight, increasing insulin sensitivity, and improving endothelial function (Kasapis and Thompson, 2005). Multiple studies have found an association between exercise and inflammation, but the mechanisms through which physical activity influences the inflammatory process are not all currently known (Ford, 2002).

Even light to moderate physical activity has been shown to be associated with lower blood concentrations of inflammatory markers, especially in cross-sectional designs. Specifically, Pitsavos et al. (2003) found that leisure time physical activity was associated with 33% lower concentrations of CRP and 10% lower concentrations of white blood cell counts independent of age (Pitsavos et al., 2003). Also, only 30 minutes of moderate exercise on a regular basis may have the ability to facilitate an anti-inflammatory environment represented by enhanced levels of cytokines such as IL-10 (Bruunsgaard, 2005). Very few studies have examined the inflammatory response due to acute moderate-intensity exercise, and the findings are controversial. While some studies have found increases in inflammatory markers due to acute moderate-intensity exercise, others have found no changes in these markers (Nieman et al., 2005; Markovitch et al., 2008). It may be important to assess the inflammatory response to exercise by studying the long-term versus acute effects of exercise, as well as the duration and intensity of the exercise (Markovitch et al., 2008).

<u>Exercise-induced anti-inflammatory response</u>

Several studies have shown that physical activity mediates strong anti-inflammatory effects in skeletal muscle and fat tissue. Exercise has the ability to produce acute increases in various anti-inflammatory mediators (Kasapis and Thompson, 2005). The anti-inflammatory effects of exercise have been attributed to mechanisms that involve the cytokine IL-6 (Bruunsgaard, 2005; Steensberg et al., 2003). IL-6 can increase as much as 100-fold after strenuous exercise, and this increase is the earliest as well as the most prominent cytokine response to exercise (Kasapis and Thompson, 2005). There is controversy over whether IL-6 has pro- or anti-inflammatory properties, but some studies suggest IL-6 should be classified as an anti-inflammatory cytokine due to the fact that IL-6 may stimulate the production of IL-10 and IL-1ra while inhibiting the production of TNF-α (Starkie et al., 2003; Steensberg et al., 2003). IL-6 has also been found to enhance the levels of both IL-10 and IL-1ra, independently of TNF-α (Steensberg et al., 2003).

Starkie et al. (2003) found that physical activity may mediate anti-inflammatory activity, and exercise-induced IL-6 production may help to mediate the effect of exercise on TNF-α production. Eight healthy subjects received an endotoxin bolus to induce low-grade inflammation after 2.5 hours into a 3-hour bout of exercise on a cycle ergometer. Increases in TNF-α from the endotoxin bolus were totally attenuated due to the exercise, whereas the endotoxin induced a two- to threefold increase in circulating levels of TNF-α during rest in the same subjects (Starkie et al., 2003). There are potentially other mediators, such as epinephrine, that may contribute to the anti-inflammatory effects of exercise as well. Epinephrine is highly induced by exercise and

has the ability to blunt the appearance of TNF-α production (Starkie et al., 2003). The

finding that exercise suppresses TNF-α production has also been supported in animal

models in which exercise normalized the overexpression of TNF-α in TNF-R knockout

mice (Keller et al., 2004).

In a 2005 review article by Petersen and Pedersen, circulating levels of anti-

inflammatory cytokines were commonly found in relation to exercise. As a result of

exercise, increases primarily in IL-6 followed by increases in IL-1ra and IL-10 have been

thoroughly reported (Petersen and Pedersen, 2005). The production of IL-10 that has

been shown with exercise can lead to inhibition of the synthesis of a large range of pro-

inflammatory cytokines by different cells, notably of the monocytic line of descent.

Therefore, IL-10 may be able to inhibit the production of TNF-α by attenuating the

surface expression of TNF-α receptors (Petersen and Pedersen, 2005; Steensberg et al.,

2003). IL-10 may also be able to inhibit the production of IL-1α and IL-1β as well as the

production of some chemokines. The anti-inflammatory effects of an acute bout of

exercise may protect against chronic systemic low-grade inflammation, but a similar

link between the acute effects of exercise and long-term benefits has yet to be

determined (Petersen and Pedersen, 2005).

Simultaneous increases in both pro- and anti-inflammatory markers due to exercise

Exercise may induce an increase in pro-inflammatory cytokines, such as TNF-α.

This release of pro-inflammatory cytokines may also be balanced by the release of

cytokine inhibitors and anti-inflammatory cytokines, such as IL-10. These findings

suggest that anti-inflammatory cytokines as well as cytokine inhibitors may restrict

both the duration of the exercise and the exercise-induced inflammatory response

(Pedersen and Hoffman-Goetz, 2000; Ostrowski et al., 1999). Therefore, there is a parallel anti-inflammatory counter-regulation that is also part of the acute phase response to exercise (Kasapis and Thompson, 2005; Pedersen, 2000).

Ostrowski et al. (1999) found a significant increase in TNF-α after a strenuous bout of exercise (marathon) in healthy adult males. Significant increases in the inflammation responsive cytokine IL-6 were also found following the exercise with only a modest increase in plasma CRP. Both anti-inflammatory cytokines (IL-10) and cytokine inhibitors were significantly increased due to the exercise, serving to balance the exercise-induced release of pro-inflammatory markers (Ostrowski et al., 1999). Contraction-induced IL-6 expression may be followed by a systemic anti-inflammatory response, providing a common underlying pathway by which pro-inflammatory markers (TNF-α activity) are attenuated after a single bout of exercise (Bruunsgaard, 2005). Although strenuous exercise has been shown to result in positive changes in inflammatory markers, severe exercise or exercise in an immunosuppressed state may induce a negative inflammatory state as well as immunosuppression (Nieman et al., 1999; Smith, 2000). This phenomenon called the "open window theory," describes a period of time after exercise in which an individual may be subject to increased risk of infection due to a depression in immune function. "Over-trained" states may result in the depression of key immune parameters, which have been linked to imbalances in cytokines (Nieman et al., 1999).

Even though strenuous exercise has been shown to induce an initial pro- and anti-inflammatory response, there may be a different effect as a result of moderate exercise in healthy individuals (Markovitch et al., 2008). Markovitch et al. (2008) found

that acute moderate intensity exercise had no effect on pro- or anti-inflammatory responses. Twelve sedentary men underwent 30 minutes of walking at 50% VO_{2max} in which no significant changes were found in CRP, IL-6, or IL-10 concentrations over the 7 days following the single bout of exercise. The results from this study may suggest that the long-term anti-inflammatory effects that were previously reported with exercise of moderate-intensity must be explained by something other than a net anti-inflammatory response to each exercise bout (Markovitch et al., 2008).

<u>Variability found in the levels of inflammatory markers across studies</u>

There have been many differences found between markers of inflammation due to exercise. Inconsistent findings have been reported for TNF-α and CRP in response to exercise (Ostrowski et al., 1999). There are several possible explanations for these variable results on cytokines in relation to exercise including: 1) the intensity and duration of the exercise as well as the type of physical activity; 2) the specificity and the sensitivity of the assays used in particular studies; 3) heterogeneity of subjects from sample to sample (Pedersen and Hoffman-Goetz, 2000; Pedersen, 2000). Increased cytokine levels have mainly been described due to eccentric training, but concentric exercise can induce cytokine production as well. Also, the magnitude of the increases found in markers of inflammation is closely related to the duration of the exercise (Pedersen and Hoffman-Goetz, 2000; Pedersen, 2000).

Cancer and markers of inflammation

Cancer and inflammation

Increased circulating levels of cytokines are known to be associated with cancer, and epidemiological findings have shown a strong association between chronic inflammation and some types of cancer (Gomez et al., 2011; Seruga et al., 2008). Inflammatory cells may have powerful effects on tumor development, and inflammation has been shown to act as a tumor promoter. Inflammation can affect tumor development and progression in addition to the response to therapy (Balkwill and Mantovani, 2001; Grivennikov et al., 2010). Cytokines are mediators that govern a vast range of processes involved in the development of cancer, and markers of inflammation form a major part of the tumor microenvironment (Candido and Hagemann, 2013). Inflammatory cells as well as cytokines regulate the entire tumor organ, managing the migration, growth, and differentiation of all types of cells in the tumor microenvironment (Coussens and Werb, 2002). These cytokines can also influence immunosuppression and tissue remodeling in the inflammatory microenvironment, which is a critical component of all tumors (Grivennikov et al., 2010; Seruga et al., 2008).

An irregular balance between pro- and anti-inflammatory mechanisms often occurs in the cancer population, leading to chronic inflammation as well as chronic immune activation (Seruga et al., 2008). Cytokines such as TNF-α may play a role in tumor progression by producing an optimal environment for tumor growth, promoting angiogenesis, and assisting genomic instability (Coussens and Werb, 2002). Tumor growth initially depends on increased cell proliferation and reduced cell death, both of

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which are stimulated by inflammation-driven mechanisms. Tumor promotion that is induced by inflammation may occur early or late in tumor development (Grivennikov et al., 2010). A common view about the cause of cancer is that inflammation may promote cancer, but recent evidence has shown that cancer may cause inflammation. Cancer-induced inflammation may be due to the activation of intrinsic inflammation pathways by genetic events that cause neoplasia (Candido and Hagemann, 2013).

Recruitment of inflammatory markers may also interfere with tumor development and mediate the suppression of tumor growth. Although inflammatory responses may also be anti-tumor, cancer patients often have abnormal inflammatory responses (Standish et al., 2008). These abnormal responses may arise from two different mechanisms: failure to up-regulate anti-inflammatory cytokines or disruption of the host response from the desensitization of receptors. The latter of the two mechanisms may lead to increased cytokine concentrations, attenuating systemic responses (Coussens and Werb, 2002). The development of malignancy in some cancers may be preceded by inflammatory conditions, while other cancers may have oncogenic changes driving a tumor-promoting inflammatory environment. Regardless of the origin, this inflammation may aid in the growth and survival of malignant cells, angiogenesis, and metastasis. In addition, responses to hormones and chemotherapy drugs may be potentially altered (Candido and Hagemann, 2013).

Pre-treatment markers of inflammation in breast cancer patients

Before surgery, breast cancer patients have been shown to exhibit elevated levels of CRP, with higher stages of breast cancer resulting in even larger concentrations of CRP. This may insinuate that CRP is related to tumor progression in

these patients (Pierce et al., 2009a). Also, high levels of the cytokine IL-6 has been shown to potentially be a predisposing genetic factor contributing to worse breast cancer prognosis (Germano et al., 2008; Hutnick et al., 2005). It has been found that cancer survivors with chronic inflammation may have an overall higher risk of recurrence due to the effects of the inflammatory processes on cell growth (Pierce et al., 2009a).

Altered inflammatory responses occur frequently in women with breast cancer, and studies have related breast cancer to an inflammation etiology (Standish et al., 2008). It has been reported that CRP, TNF-α, and IL-6 are elevated in breast cancer patients (Seruga et al., 2008). As previously mentioned, IL-6 has been found to correlate with both cancer stage and degree of metastasis as well as breast cancer recurrence (Hutnick et al., 2005). Also, increased CRP levels may be associated with mortality in women diagnosed with breast caner (Jones et al., 2012).

Breast cancer treatments and inflammation

Surgery, chemotherapy, and radiation can all induce local or systemic increases in inflammation (Jones et al., 2009). Surgery may result in tissue injury through the activation of stress-sensing pathways. Chemotherapy and radiation may result in cancer cell death through necrosis, which is a pro-inflammatory form of cell death (Grivennikov et al., 2010). Different cancer treatments can activate the immune system to generate pro-inflammatory cytokines that have been associated with toxic effects of treatment, such as bone loss, flu-like effects, and fatigue. Increases in this category of cytokines have also been related to cachexia, pain, and resistance to treatment in cancer patients (Seruga et al., 2008). Cancer and some of its treatments can encourage an up-

regulation of pro-inflammatory cytokines in which many of these cytokines may promote a metabolic state or other condition that results in tissue losses (Battaglini et al., 2012). There is also growing evidence that links inflammation and fatigue both during and after cancer treatment. Chemotherapy has been associated with acute increases in markers of inflammation as well as fatigue, which may last long into survivorship (Bower et al., 2011).

On the other hand, chemotherapy and radiation may be used to eliminate the tumor-promoting inflammatory microenvironment by causing the death of tumor-promoting immune/inflammatory cells (Grivennikov et al., 2010). Activation of the immune system by cancer treatments might also have a role in producing anti-cancer effects by differentially altering the secretion of cytokines (Seruga et al., 2008). Although cancer treatments attempt to reduce markers of inflammation, the majority of breast cancer survivors have been shown to display increased levels of inflammation compared to healthy women (Seruga et al., 2008).

Breast cancer survivors and higher levels of inflammation

Survivors of breast cancer have been shown to have higher levels of circulating cytokines and receptors than their healthy counterparts (Gomez et al., 2011; Seruga et al., 2008). Systemic inflammation has been shown to potentially be an important long-term prognostic factor for breast cancer. A significant association has been found between reduced overall survival and increased concentrations of inflammatory markers, such as CRP (Pierce et al., 2009a). According to Seruga et al. (2008), preliminary data indicates that circulating levels of different pro- and anti-inflammatory cytokines are significantly higher in breast cancer survivors up to 5 years

after diagnosis than in healthy controls (Seruga et al., 2008). Measurement of percent body fat has been found to be the most important predictor of inflammatory markers in breast cancer survivors. Increased body fat may be associated with increases in inflammation, and considering that many breast cancer survivors are overweight or obese, increased body fat could cause additional problems in the inflammatory microenvironment of these patients (Pierce et al., 2009b). It has been reported that more than 50% of breast cancer survivors are overweight or obese, and the breast cancer survivors who gain weight may have increased levels of inflammation (Jones et al., 2012). With weight gain, fat cells in the breast expand and are more likely to die. The death of these cells may trigger the release of saturated fatty acids, which activate macrophages. Fatty acids that bind to the receptors of macrophages may start the cascade of inflammation by triggering the production of IL-1, IL-6, and TNF-α (Patlak and Nass, 2012).

Breast cancer survivors with higher stages of breast cancer and metastases may have higher levels of pro-inflammatory cytokines than those with lower stages and non-metastatic cancer (Gomez et al., 2011). Higher levels of pro-inflammatory cytokines may be a primary cause of fatigue in these patients, since persistent fatigue has been associated with increased levels of these inflammatory markers in breast cancer survivors (Seruga et al., 2008). Chemotherapy has been associated with increases in inflammation and fatigue, and findings have suggested that TNF-α may play a significant role in chemotherapy-induced fatigue. Also, animal models have shown chemotherapy-induced rises in TNF-α have led to increased oxidative stress and inflammation in the brain (Bower et al., 2011). Considering fatigue is one of the most

common side effects experienced among breast cancer survivors, the reduction of pro-inflammatory cytokines may be essential in improving the quality of life in breast cancer survivors (Seruga et al., 2008)

Potential reasons for elevated inflammation in breast cancer survivors

It has been noted that future cancer therapies should focus on normalizing the inflammatory network in order to re-establish an overall normal host response (Coussens and Werb, 2002). The occurrence of therapy-induced inflammation is possible, and the various forms of therapy that can induce the death of malignant cells may activate cytokine-producing inflammatory cells (Grivennikov et al., 2010). Current cancer treatments may not be able to completely diminish the high levels of tumor-promoting mediators, such as pro-inflammatory cytokines while increasing tumor-suppressing factors, such as anti-inflammatory cytokines (Candido and Hagemann, 2013). The destruction of tumor cells has been the concentration of cancer treatments for many years. Proposed strategies to moderate the host microenvironment offer supplemental approaches to cancer treatment, and cancer-associated inflammation may be an interesting target of anti-tumor therapies in the future (Germano et al., 2008; Mantovani et al., 2008).

Inhibiting antibodies that have been shown to have a significant therapeutic effect in other inflammatory diseases may be applicable to cancer therapies (Coussens and Werb, 2002). Even though cancer therapies attempt to reduce inflammation, this is not always successful and may be one of the reasons why many cancer survivors have elevated levels of inflammation. In some instances, inflammation has been shown to

diminish the beneficial effects of cancer therapy (Balkwill and Mantovani, 2001). Also, cancer therapy, such as chemotherapy and radiation, may cause a strong tumor-associated inflammatory response due to the necrotic death of cancer cells and surrounding tissues that can occur with these types of treatments (Grivennikov et al., 2010).

Discussions about the breast cancer population and inflammation in different studies allude to things, but mechanisms as to why breast cancer survivors have increased inflammation are speculative as of now. Although it is evident that inflammatory processes may have a role in the progression of tumors, not much is known why these survivors still have increased levels of inflammation after cancer treatment (Grivennikov et al., 2010). The following are possible mechanisms for why this is occurring: 1) Treatment-related increases in inflammation with muscle and tissue damage due to cancer treatments; 2) Treatments potentially not being able to alleviate inflammatory markers that could have led to metastasis in the first place (Chaplin, 2010; Mantovani et al., 2008). How far the cancer progression was in each patient before treatment could potentially be a big determinant in how well the treatment can decrease and return levels of inflammation to normal in the breast cancer population (Gomez et al., 2011).

Effects of exercise on markers of inflammation in breast cancer survivors

Exercise and inflammation in breast cancer survivors

It has been suggested by several studies that physical activity is associated with a modest decrease in mortality for breast cancer patients (Pierce et al., 2009). In

observational studies, better lifestyle choices including moderate intensity exercise have resulted in improved survival after treatment of patients with breast cancer (Janelsins et al., 2011; Seruga et al., 2008). It is evident that appropriate amounts of physical activity may promote an up-regulation of anti-inflammatory cytokines while down regulating the expression of some pro-inflammatory cytokines (Battaglini et al., 2012). Preliminary findings support this shift in a healthier inflammatory cytokine profile with physical activity in cancer patients, which may result in an improved muscle tissue status as well as an acceleration of protein synthesis within the skeletal muscle (Battaglini et al., 2012). Several exercise trials with breast cancer survivors have found reductions in both pro-inflammatory markers and adiposity, and it has been postulated that physical activity's effects on cytokine levels may be mediated through fat loss (Jones et al., 2012).

Exercise has been shown to reduce inflammatory markers, such as CRP, independent of changes in body fat due to the modification of cytokine production at non-adipose sites such as mononuclear cells and skeletal muscle. Reductions in inflammatory markers with exercise may occur indirectly through improved endothelial function, reduced body weight, or increased insulin sensitivity (Pierce et al., 2009b). The potential anti-inflammatory effect of physical activity could explain the better outcome of cancer found in observational studies. Cumulative anti-inflammatory effects of repeated exercise bouts could be one of the mechanisms responsible for the health benefits of regular exercise (Gomez et al., 2011). A physically active lifestyle has been shown to decrease production of pro-inflammatory cytokines in non-cancer populations and has been reported to improve blood immune function in breast cancer

survivors. Increasing evidence suggests that exercise may be an important part of the lifestyle of breast cancer survivors in improving treatment-related fatigue as well as preventing cancer recurrence and death (Seruga et al., 2008).

Regular exercise has been shown to reduce pro-inflammatory cytokines in people with chronic conditions, such as cardiovascular disease, but the beneficial effects of exercise in cancer survivors remains to be determined (Gomez et al., 2011). To date, very few studies in post-treatment breast cancer patients have examined the effects of exercise on inflammatory markers, especially the inflammatory response to an acute bout of exercise. Most studies have only reported the inflammatory response due to long-term exercise interventions in this population. Also, the majority of these studies are generally small, and the evidence is not consistent (Löf et al., 2012).

Specific studies examining exercise and inflammatory markers in breast cancer survivors

Fairey et al. (2005) examined the effect of an exercise intervention on markers of inflammation in breast cancer survivors. Fifty-three postmenopausal breast cancer survivors were randomized into either a control or exercise group in which the subjects in the exercise group trained on cycle ergometers three times per week for 15 weeks. The researchers found no significant changes between the groups in cytokine production by blood mononuclear cells. Although the study did not find changes in cytokine production, the authors did find significant improvement in blood immune function due to the exercise training with an increase in NK cell cytotoxic activity (Fairey et al., 2005a).

Gomez et al. (2011) found no significant changes in TNF-α, IL-10, or IL-6 among other cytokines in breast cancer survivors after a combined aerobic and resistance

exercise training program. The breast cancer survivors underwent three sessions per week for a total of 8 weeks. The training program was not able to decrease circulating levels of pro-inflammatory cytokines or increase levels of major anti-inflammatory cytokines. However, the authors did observe a decrease in the IL-10/TNF-α ratio in these subjects due to the exercise that approached significance. The authors noted that the decrease found in the IL-10/TNF-α ratio might indicate that the exercise intervention did have an anti-inflammatory effect on the breast cancer survivors. The research team then went on to suggest that perhaps a more intense or longer exercise intervention may be necessary in order to induce a significant anti-inflammatory benefit (Gomez et al., 2011).

Hutnick et al. (2005) were interested in the effect of a 6-month exercise intervention on both inflammatory and immune function markers in post-treatment breast cancer patients. The study consisted of two groups, an exercise group (n=28) and a non-exercise control group (n=21). Subjects in the exercise group underwent combined aerobic and resistance training sessions three times per week, but twelve of these subjects participated in the exercise training for only three months instead of the full six months. The exercise group had higher but non-significant levels of IL-6 throughout the exercise program compared to the control group. Although there were no significant changes found in cytokines throughout the exercise intervention, there were greater levels of lymphocytes, such as CD4^{+} cell activation, found in the exercise group. These findings suggest that a moderate intensity exercise program may result in an increased immune system function in breast cancer survivors (Hutnick et al., 2005).

While some studies have found no changes in inflammatory markers due to an exercise intervention, other studies have. Fairey et al. (2005) found exercise-induced changes in CRP in postmenopausal breast cancer survivors. Fifty-three breast cancer survivors were randomly assigned to an exercise group (n=25) or control group (n=28). The exercise group trained 3 times per week for 15 weeks on cycle ergometers. At the end of the exercise intervention, CRP decreased by 1.39 mg/L in the exercise group whereas it increased 0.10 mg/L in the control group. Although the mean between group change in CRP was -1.49 mg/L, the exercise training was found to have a change in CRP that only approached significance (p = .066). According to the authors, proposed mechanisms for the change found in CRP included alterations in: cytokine production by blood mononuclear cells, NK cell cytotoxic activity, oxidized low-density lipoprotein cholesterol, intra-abdominal fat, insulin resistance, nitric oxide, antioxidants, and leukocyte adhesion molecules (Fairey et al., 2005b).

Janelsins et al. (2011) assessed the effects of a 12-week moderately intense Tai Chi Chuan (traditional Chinese martial art) intervention on breast cancer survivors. Post-treatment breast cancer patients were randomized into the Tai Chi Chuan exercise group or a psychosocial support therapy control group. Subjects in the Tai Chi Chuan group met for 60 minutes 3 times per week. Breast cancer survivors who participated in the Tai Chi Chuan were found to have non-significant decreases in IL-6 along with non-significant increases in IL-2. The researchers found that changes in fat-free mass were positively correlated with changes in IL-6 and negatively correlated with changes in IL-2. As fat-free mass increased, IL-6 also increased but IL-2 decreased. This lead the authors to believe that the elevated IL-6 levels were positive markers of the reduction

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in fat mass that resulted from the moderate-intensity form of exercise (Janelsins et al., 2011).

Sixty-eight breast cancer survivors were randomized into either a 6-month aerobic exercise intervention (n = 36) or usual care (n = 32) in a study conducted by Jones et al. (2012). At baseline, the study found that both IL-6 and CRP were positively correlated with percent body fat, body weight, and BMI, and inversely correlated with pedometer steps per day. These results suggest that IL-6 and CRP were associated with higher adiposity as well as lower levels of physical activity at baseline. After 6 months, plasma concentrations of IL-6, CRP, and TNF-α did not differ between the exercise and usual care groups. However, secondary analyses showed a significant decrease in IL-6 among exercisers who exercised at least 120 minutes per week compared with those who exercised less than 120 minutes per week. The authors also reported a borderline significant correlation between change in percent body fat and change in CRP in women randomized to exercise (p = 0.069). Poor adherence to the exercise intervention may have impacted the results since women who met at least 80% of the exercise goal showed significant decreases in IL-6. The authors concluded by stating potential mechanisms through which physical activity may reduce inflammation. These mechanisms include: anti-inflammatory cytokine release during exercise, effects of muscle-derived IL-6, reductions in adipose tissue, and inhibition of TNF-α production by epinephrine (Jones et al., 2012).

Summary

Overview of the gaps in the current literature and exercise prescription guidelines for cancer survivors

Given that exercise modulates the immune system in healthy individuals, research considering clinical populations for which the immune system has a role, such as breast cancer survivors, is of importance. Although there are specific risks related to cancer treatments that need to be considered when survivors exercise, there seems to be consistent evidence that exercise is safe and well tolerated without adverse effects in breast cancer survivors (Schmitz et al., 2010). Few studies have examined markers of inflammation in breast cancer survivors, and even fewer studies have investigated the inflammatory response to exercise in this population (Seruga et al., 2008). The majority of studies that have explored the relationship between exercise and markers of inflammation in breast cancer survivors have only focused on the inflammatory response due to long-term exercise interventions in these patients. Most exercise oncology studies have only observed pre- and post-levels of inflammatory markers in response to an exercise intervention, and not the effect of an acute bout of exercise on these markers in breast cancer survivors.

It is necessary to observe the magnitude and temporal response of cytokines in order to understand the inflammatory response to exercise. Exercise prescriptions for breast cancer survivors are modeled after those for healthy populations (Schmitz et al., 2010). It is currently unknown whether breast cancer survivors have similar exercise-induced inflammatory responses as healthy individuals. The acute effects of exercise need to be explored in order to examine the more direct relationship between exercise and the role of inflammation in breast cancer survivors. Understanding the more acute

effects of exercise will allow for the enhancement of exercise prescription guidelines, as

well as the time course needed before prescribing the next exercise session. This

understanding will be helpful in order for these patients to fully recover and receive the

maximal health benefits of exercise without increasing the risk of further illness.

CHAPTER III

METHODOLOGY

Subjects

Data from 18 subjects were analyzed in this retrospective study. The recruitment process for the study was completely voluntary as subjects were made aware of the study through contact by a member of the research team as well as through fliers. Subjects were recruited into a breast cancer survivor and a healthy control group: each consisting of 9 subjects. Attempts were made to pair subjects in the control group with subjects in the breast cancer survivor group based on age and physical activity level. Recruitment sites for subjects in the breast cancer survivor group included the Medical Oncology clinic and the Radiation Oncology clinic at the North Carolina Cancer Hospital as well as from the waitlist of the Get REAL and HEEL Breast Cancer Program in the Department of Exercise and Sport Science at the University of North Carolina at Chapel Hill. The control group consisted of subjects recruited from the staff, faculty, and student populations at UNC-Chapel Hill, as well as from across the Triangle region of North Carolina (Raleigh, Durham, Chapel Hill, and surrounding areas). Approval from the Institutional Review Boards in both the Department of Exercise and Sport Science and School of Medicine at UNC-Chapel Hill, along with approval from the Protocol Review Committee in the Lineberger

Comprehensive Cancer Center at UNC-CH, was acquired before progressing with the recruitment of subjects.

All subjects in both groups were between the ages of 40 to 70 years old, were either post-menopausal or had not experienced a menstrual period for approximately 1 year before enrollment, and were not normal users of anti-inflammatory medications. Additional inclusion criteria for participation in the breast cancer survivor group were as follows: 1) Confirmed diagnosis of Stage I, II, or III invasive breast cancer; 2) Had received chemotherapy; 3) Must had completed all major cancer treatments 3-6 months prior to enrollment. Additional inclusion criteria for participation in the control group were as follows: 1) No history of cancer diagnosis or treatment; 2) Were free from musculoskeletal and cardiovascular disease that would make participation in aerobic exercise unsafe; 3) No participation in organized physical activity for at least 1 year prior to enrollment. Subjects were screened for exclusion based on the criteria put forth by the American College of Sports Medicine (ACSM) as contraindications to exercise testing (Whaley et al., 2006- ACSM's Guidelines for Exercise Testing and Prescription).

Instrumentation

Height was measured to the nearest 0.01 cm using a Portable stadiometer (Perspective Enterprises, Portage, MI USA), and weight was measured to the nearest 0.1 kg using a Mechanical scale (Detecto, Webb City, MO USA). A Discovery Dual Energy X-Ray Absorption (DEXA) scanner (Hologic, Inc., Bedford, MA USA) was used in order to assess percent body fat. A medical history questionnaire (Department of Exercise and

Sport Science Medical History form) was also used to record information about each subject's medical history, race, age, physical activity level over the past year, menopausal status, and cancer treatment type (for subjects in the breast cancer survivor group only). Also, the medical history questionnaire was used by a research team member authorized to do physical evaluations for exercise testing prior to subjects participating in the study. This was used in concert with the resting EKG and the physical exam to determine if the subjects were cleared to participate in the study.

Heart rate was assessed via Pacer Polar heart rate monitor (Polar Electro Inc., Lake Success, NY USA). Blood pressure was assessed via Diagnostix 700 aneroid sphygmomanometer (American Diagnostics Corporation, Hauppauge, NY USA) and Litmann stethoscope (3m, St. Paul, MN USA) during rest and exercise. A resting electrocardiogram (EKG) was performed prior to testing and EKG monitoring was continued during exercise using a GE CASE Cardiosoft V. 6.6 ECG diagnostic system (General Electric, Palatine, IL USA). A Parvo Medics TrueMax 2400 Metabolic System (Parvo Medics, Salt Lake City, UT USA) was used for the assessment of Cardiopulmonary function (VO_{2peak}). Both the VO_{2peak} test and the acute aerobic exercise bout consisted of cycling using a Lode electronically braked cycle ergometer (Lode, Gronigen, The Netherlands). The Borg's 6-20 Rate of Perceived Exertion (RPE) scale was used to measure RPE.

The following instruments were used for the assessment of blood sampling procedures as well as markers of inflammation and immune system function. Whole blood samples were collected in K_3EDTA Vaccutainer tubes. An IEC Centra-8R refrigerated centrifuge (International Equipment Company, Needham Heights, MA USA)

were used in order to isolate the plasma component of the whole blood samples. For

the determination of plasma volume shifts, hematocrit and hemoglobin were measured

from complete blood count data using a COULTER Ac•T diff Hematology Analyzer

(Beckman Coulter, Inc., Brea, CA USA). NK cell count was analyzed using a CyAn 3

laser/9 color flow cytometer (Beckman Coulter, Inc., Brea, CA USA). Flow cytometry

data was viewed and analyzed using Summit 4.3 software (Dako North America, Inc.,

Carpinteria, CA USA). Enzyme-linked immunosorbant assay (ELISA) kits (Rocky

Mountain Diagnostics, Inc, Colorado Springs, CO USA; R&D Systems, Minneapolis, MN

USA) were used to measure markers of inflammation (TNF- α, CRP, and IL-10). In order

to read the ELISA assays, a Finstruments 347 microplate reader and Spectra software

analysis (MTX Lab Systems Inc., Vienna, VA USA) was used.

Procedures

Due to the nature if this retrospective study, data were already collected as part

of a previous dissertation. All procedures about the exercise protocol, blood sampling,

and NK cell analysis using flow cytometry can be found in the dissertation of Dr.

Elizabeth Evans, 2012 (UNC-CH). This study analyzed the blood samples from Dr.

Evans dissertation study in order to evaluate the inflammatory response of breast

cancer survivors due to an acute bout of moderate intensity intermittent exercise.

All subjects visited the laboratory on three different occasions. Prior to each

visit to the laboratory, subjects were asked to follow a set of pre-assessment guidelines.

These included the maintenance of adequate hydration, no eating for at least two hours

prior to testing, no exercise or caffeine intake for at least 12 hours prior to testing, and

no alcohol consumption for at least 48 hours prior to testing. All subjects included in this study were required to have a physical screening by either a physician or other certified professional, complete a medical history questionnaire, and undergo a 12-lead resting electrocardiogram. All visits to the laboratory took place in either the Integrative Exercise Oncology Research Laboratory (IEORL) and/or in the Applied Physiology Laboratory (APL) in the Department of Exercise and Sport Science at UNC-CH. In order to reduce the potential for diurnal variation in the study variables, the acute exercise session (i.e., the one where the blood samples were taken) began between 7:00-10:00 am. Keeping similar times of day when subjects reported to the lab allowed for more controlled measurements of inflammatory markers.

The following is an overview of each visit to the laboratory. The three laboratory visits will be further broken down in the proceeding text. Visit 1 consisted of medical and physical screening, the assessment of peak aerobic capacity on the cycle ergometer, and an orientation to the study. Visit 2 included each subject performing a moderate aerobic bout of exercise for 30 minutes on the cycle ergometer at 60% of their VO_{2peak}. During this laboratory visit, blood samples were collected pre-exercise (baseline), immediately post-exercise, and 2 hours post-exercise. Visit 3 only included the collection of one blood sample from each subject 24 hours post-exercise.

Fig 1. Blood sampling timeline
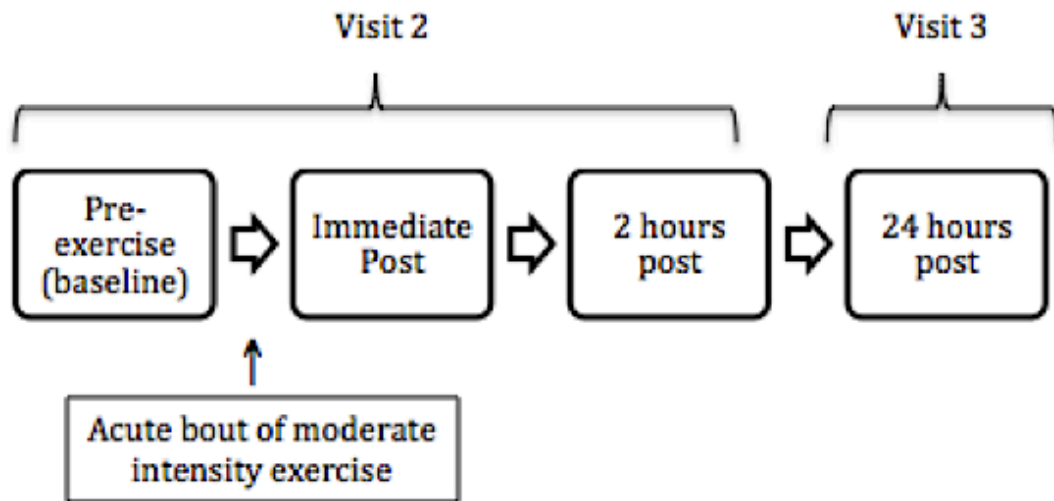
Visit 1: Medical/Physical Screening and VO_{2peak} Test

      The first visit to the laboratory consisted of all subjects completing a comprehensive medical history form and undergoing both a physical exam and 12-lead resting electrocardiogram. Subjects received information regarding potential risks of participation in the study, a description of the study protocol, and were asked to sign informed consent documentation. Age, height, body mass, and race were recorded for all subjects, and height and body mass were used to calculate body mass index (BMI). Percent body fat was also measured using a Dual Energy X-ray Absorption (DEXA) scanner. Cancer treatment type was documented for subjects in the breast cancer survivor group.

      VO_{2peak} was also measured during the first visit to the laboratory. The VO_{2peak} test was performed on an electronically braked cycle ergometer using the Astrand Cycle Ergometer Maximal Test Protocol. VO_{2peak} was used to assess peak aerobic capacity in order to estimate the submaximal workload that all subjects would perform during visit

2. The VO_{2peak} test began with the collection of resting metabolic data as subjects sat

quietly for 3 minutes on the cycle ergometer. The first stage of the test started with

subjects cycling at 50 Watts for 3 minutes. Each stage was 3 minutes long, and at the

end of each stage workload was increased by 25 Watts until volitional fatigue. Each

subject's VO_{2peak} was the highest VO_2 measured by the metabolic system during the last

stage obtained by the subject. The corresponding workload on the cycle ergometer at

each subject's VO_{2peak} was recorded as the subject's peak workload.   The subject

pedaled at a low workload (<20 Watts) once the VO_{2peak} test was complete, with

continued blood pressure and EKG monitoring until subjects returned to near-baseline

levels. Heart rate, expired gases, rating of perceived exertion (RPE) (6-20 Borg scale),

and 12-lead ECG monitoring were performed throughout the test. Both heart rate and

RPE were recorded at the end of every minute during the VO_{2peak} test. Workloads at

60% VO_{2peak} for all subjects were estimated by regressing VO_2 values for each stage

against corresponding workloads for that stage (See Figure 2 below for an example).

Fig 2. Example regression for determining workload corresponding to 60% of VO_{2peak}
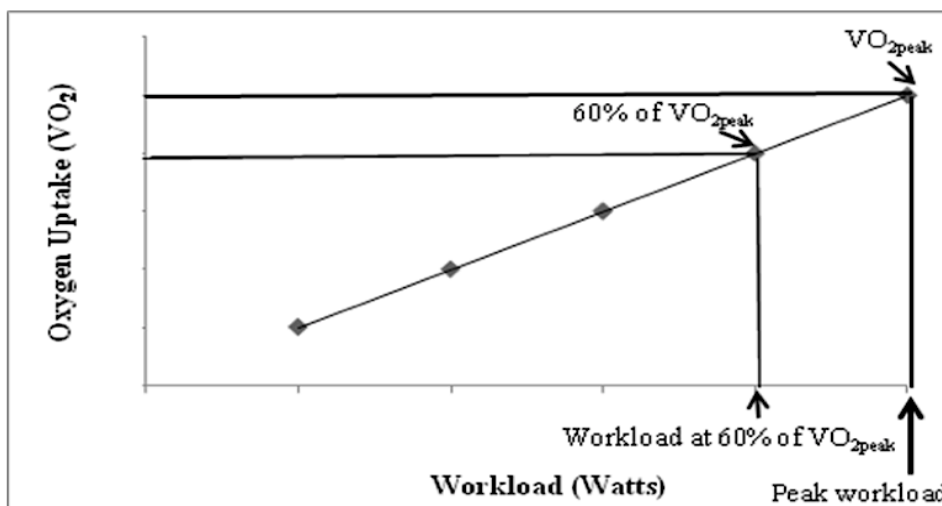
<u>Visit 2: Acute Aerobic Exercise Session</u>

An acute bout of intermittent, moderate exercise was performed by all subjects on a cycle ergometer for 30 minutes at an intensity corresponding to 60% of each subject's VO_{2peak}. A discontinuous exercise protocol was used to ensure that all subjects would be capable of completing the exercise session. Subjects underwent 30 minutes of exercise in a 43.5-minute period, alternating ten 3-minute intervals of exercise with 1.5 minutes of rest.

All subjects rested in the supine position for approximately 20 minutes at the beginning of visit 2, while a catheter was inserted into an antecubital vein in the arm for blood sampling. The three-syringe technique was used for blood sampling. A small amount of blood was drawn for the first blood draw to remove "waste," such as saline, from the catheter. The second blood draw contained the actual blood sample. Finally, 1.5 mL of sterile saline was injected to keep the catheter patent between blood sampling.

Pre-exercise (baseline) blood samples were drawn into K_3EDTA Vacutainer tubes for the measurements of NK cell counts and markers of inflammation. Subjects sat quietly on a cycle ergometer for 3 minutes after the pre-exercise blood sample was taken, while resting metabolic data was collected. All subjects then warmed up with light pedaling at about 30 Watts on a cycle ergometer for 4-5 minutes. Subjects were also able to stretch the lower body in a comfortable manner before the 30-minute moderate-intensity exercise bout began. Heart and RPE were documented at the end of every 3-minute period of exercise, while expired gases were recorded during the first, third, seventh, and tenth exercise intervals. Workload was modified if needed in order

to make sure each subject was exercising as close to 60% VO_{2peak} as possible. Subjects were informed to carefully dismount from the cycle ergometer immediately at the end of the exercise bout and return to the supine position. Blood sampling for the immediately-post exercise time period was carried out in the same nature as described before. Subjects then rested comfortably in the laboratory until a final blood sample during visit 2 was obtained at 2 hours post-exercise using the same techniques as the two previous blood samples. Subjects were allowed to drink water during the 2-hour resting period, but no food or other beverages could be ingested. At the end of visit 2, each subject's catheter was removed followed by bandaging of the arm.

Visit 3: 24-hour Follow-up Session

A resting blood sample was taken from each subject 24 hours after the acute aerobic exercise bout. Using a standard venipuncture technique, the sample of blood was drawn from an antecubital vein.

Calculation of Plasma Volume Shifts

Plasma volume shifts were measured in order to account for the effect of exercise and posture on fluid shifts throughout the study, which may affect concentrations of leukocytes and markers of inflammation. Plasma volume shifts were calculated using the equation by Dill and Costill (Dill and Costill, 1974). The calculation of plasma volume shifts used the hematocrit and hemoglobin values obtained from the whole blood samples taken pre-exercise, immediately post-exercise, 2 hours post-exercise, and 24 hours post-exercise. Hematocrit and hemoglobin were measured from complete blood count data using an automated hematology analyzer.

Determination of Inflammatory Markers

        The subjects' plasma samples stored at an -80 degree Celsius freezer from the subjects who participated in Dr. Evans dissertation study will be used for the analyses of cytokines in this study. Markers of inflammation were measured from the stored plasma via enzyme-linked immunosorbent assay (ELISA) procedures. To ensure quality control, all samples were measured in duplicate. C-reactive protein was measured using a sandwich enzyme immunoassay kit with a sensitivity of 28.6 pg/mL. The manufacturer reported mean intra-assay coefficient of variation for C-reactive protein was 2.73%, and the reported mean inter-assay coefficient of variation was 4.33%. TNF-α was measured using a sandwich enzyme immunoassay kit with a sensitivity of 5.0 pg/mL. The manufacturer reported mean intra-assay coefficient of variation for TNF-α was 7.70%, and the reported mean inter-assay coefficient of variation was 8.10%. IL-10 was measured using a sandwich enzyme immunoassay kit with a sensitivity of 1.0 pg/mL. The manufacturer reported mean intra-assay coefficient of variation for IL-10 was 3.20%, and the reported mean inter-assay coefficient of variation was 5.60%. All manufacturer reported values were obtained from the Abnova Corporation, 2013. The microplate reader used for the ELISA assays had a manufacturer reported accuracy of ± 1% and a coefficient of variability <0.5% with a reported spectral range from 340-750nm. The manufacturer reported values for the Finstruments 347 microplate reader were gathered from MTX Lab Systems, Inc. (2013).

<u>Determination of Natural Killer cell counts</u>

NK cell counts were analyzed using flow cytometry. Whole blood samples were stained using fluorescently-labeled monoclonal antibodies for the cell surface markers CD3, CD16, and CD56. Fluorescent staining was done in order to identify the proportion of lymphocytes that express the CD3-CD16+CD56+ phenotype, which is characteristic of NK cells. Samples were fixed in a 1% paraformaldehyde solution and were analyzed within 24 hours of preparation using a flow cytometer. Absolute NK cell counts were calculated by multiplying total lymphocyte counts by the NK cell proportions.

Data Analysis

<u>Sample Size Calculation</u>

For this study, the desired sample size was the number of subjects per group needed to detect statistical significance between markers of inflammation with a level of power at .80 and an alpha level of 0.05. Estimation of sample size for the current study was based on the evaluation of IL-10 response to an acute maximal exercise test from pre-exercise (baseline) to immediately post-exercise in patients with major depressive disorder (Boettger et al., 2010). A power and sample size calculation (PS) program was used to calculate the current study's estimated sample size (Dupont and Plummer, PS version 3.0, 2009). The sample size calculation was used to examine the number of subjects needed to reach statistical significance in the response of inflammatory markers to an acute bout of exercise based on the results of the study conducted by Boettger et al. (2010). The sample size calculation was focused on a continuous response variable from matched pairs of study subjects. Prior data

indicates that the difference in the response of matched pairs is normally distributed with standard deviation of 1. If the true difference in the mean response of matched pairs was 0.8, the current study needed 14 pairs of subjects (14 subjects paired against one another) to be able to reject the null hypothesis that this response difference was zero with a power of .80. A sample size of 14 pairs of subjects was most likely an underestimate of the number of subjects needed due to the use of mean data instead of raw data in the calculation of sample size. Therefore, the current study was "under-powered" due to there only being 9 pairs of subjects included.

Statistical Analysis

All data in the current study was analyzed using SPSS Statistics version 20.0. The alpha level was set *a priori* for all statistical analyses at 0.05. Independent samples t-tests were used in order to analyze the comparison of physical parameters (age, height, weight, BMI, and percent body fat) between the two groups. Comparison of VO_{2peak}, peak workload, submaximal VO_2, and submaximal workload between the two study groups was also performed using independent samples t-tests. Paired-samples t-tests were used to compare the estimated submaximal VO_2 and workload to the actual submaximal VO_2 and workload that each study group performed during the acute aerobic exercise bout. A 2 x 3 mixed model ANOVA was used in order compare plasma volume shifts between groups across the three post-exercise time periods.

The markers of inflammation as well as NK cell counts were analyzed using multiple 2 x 4 mixed-model ANOVAs. The two independent variables of group and time were chosen in order to compare the markers of inflammation and NK cell counts between the two groups across the four time periods throughout the study. Post-hoc

analyses were run using a Tukey HSD test in order to see where differences exist within groups across time. Spearman rho correlation analyses were used to determine the relationship between markers of inflammation (independent variable) in relation to NK cell count (dependent variable) for both study groups. Delta scores were calculated for markers of inflammation and NK cell count from pre-exercise (baseline) to each of the three post-exercise time periods (immediate post, 2 hours post, 24 hours post) for both study groups. Delta scores included immediate post – baseline, 2 hours post – baseline, and 24 hours post – baseline. The delta scores for the markers of inflammation and NK cell count were used for the correlation analyses.

CHAPTER IV

RESULTS

The purpose of this study was to investigate the effect of one acute bout of

moderate intensity intermittent exercise on selected markers of inflammation in post-

treatment breast cancer survivors. A secondary purpose was to examine the

relationship between selected markers of inflammation and natural killer cell count.

Subjects

This study included a total of 18 subjects: 9 subjects in the breast cancer

survivor group and 9 subjects in the control group. Physical characteristics for the 18

subjects are presented in Table 1. Descriptive statistics are presented as mean ±

standard deviation (SD). Due to low sampling volume obtained during the blood draw

procedure, 2 subjects in the breast cancer survivor group had missing values. One

subject had a missing value immediately post-exercise and another subject at 2 hours

post-exercise. Therefore the mean substitution using the percent change of the mean

across time was imputed as a substitute value for these missing values. Age was the

only characteristic for which there was a statistically significant difference between

groups, where the average age of the control group was approximately 9 years older

than the average age of the breast cancer survivor group (p = 0.002). It should be noted

that the subject percent body fat was very high for both groups (~42%).

Table 1. Subject characteristics (mean ± SD)

Characteristic	Breast Cancer Survivor Group (n = 9)	Control Group (n = 9)
Age (years)	$50 \pm 6^*$	$59 \pm 5^*$
Race (# of women)	Caucasian (8) African American (1)	Caucasian (9)
Height (cm)	164.7 ± 5.8	163.8 ± 5.9
Weight (kg)	76.9 ± 12.6	77.7 ± 13.3
Body Mass Index (kg/m^2)	28.4 ± 5.0	28.9 ± 4.6
Percent Body Fat (%)	41.6 ± 4.5	42.1 ± 4.0
VO_{2peak} (ml/kg/min)	18.1 ± 2.7	18.5 ± 5.1
Peak Workload (W)	107 ± 19	106 ± 17

$^*p < 0.05$ for comparing age between groups

Table 2. Cancer treatments received by breast cancer survivor group (n = 9)

Treatment	Number of Subjects Receiving Treatment
Surgery	
Mastectomy	4
Lumpectomy	5
Radiation Therapy	9
Chemotherapy	
Adriamysin	6
Cytoxan	7
Taxol/Taxotere	9
Carboplatin	3
Hormonal Therapy	
Tamoxifen	5
Femara	1
Other	
Herceptin	2
Lapatinib	1
Bevacizumab	1

Metabolic Responses

The submaximal VO_2 and workloads that were used during the aerobic exercise session are presented in Table 3. Submaximal VO_2 and workload were compared to determine if there were any differences in exercise intensity between the groups during the aerobic exercise session. Estimated submaximal VO_2 and workload were calculated from the results of the VO_{2peak} test. Actual submaximal VO_2 and workload were the results from the subjects actually performing during the exercise session. Estimated and actual submaximal VO_2 were similar between groups ($p > 0.05$) as were estimated and actual submaximal workload ($p > 0.05$). Within the breast cancer survivor group, actual submaximal VO_2 was significantly higher than estimated submaximal VO_2 ($p = 0.016$). Within the control group, there was no significant difference between estimate and actual submaximal VO^2 ($p = 0.376$).

Table 3. Metabolic responses during the submax aerobic exercise session (mean ± SD)

Parameter	Breast Cancer Survivor Group	Control Group
Estimated VO_2 (ml/kg/min)	10.9 ± 1.6*	11.1 ± 3.1
Actual VO_2 (ml/kg/min)	12.1 ± 1.3*	11.1 ± 1.8
Estimated Workload (W)	63 ± 12	66 ± 11
Actual Workload (W)	59 ± 9	62 ± 11

*$p < 0.05$ for estimated vs. actual VO_2

Inflammatory Marker and Immune Response Changes

Descriptive statistics for analyses performed on TNF-α, CRP, IL-10, and NK cell counts are presented in Table 4. There was no significant group by time interaction

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effect observed for any inflammatory/immune marker. When comparing groups, IL-10

was significantly lower in the breast cancer survivor group at 24 hours post-exercise (p

= 0.044). There were no significant differences across time for any inflammatory

marker in both groups (p > 0.05). There were significant changes observed in NK cell

counts across multiple study time points in both groups.

Table 4. Inflammatory marker and immune parameter changes across time (mean ± SD)

Parameter	Breast Cancer Survivor Group	Control Group
TNF-α (pg/ml)		
Pre-exercise	1.31 ± 0.92	1.59 ± 1.02
0h post-exercise	1.37 ± 0.91	1.60 ± 0.98
2h post-exercise	1.34 ± 0.91	1.55 ± 0.97
24h post-exercise	1.32 ± 0.91	1.59 ± 0.99
CRP (mg/L)		
Pre-exercise	2.51 ± 2.70	3.10 ± 2.45
0h post-exercise	3.23 ± 3.52	3.50 ± 2.88
2h post-exercise	3.86 ± 6.02	3.72 ± 2.98
24h post-exercise	3.25 ± 4.10	3.22 ± 2.50
IL-10 (pg/ml)		
Pre-exercise	6.35 ± 2.09	16.46 ± 17.77
0h post-exercise	6.00 ± 1.47	6.88 ± 1.73
2h post-exercise	6.44 ± 1.99	10.17 ± 9.81
24h post-exercise	6.25 ± 1.36[a]	8.55 ± 2.85[a]
NK Cells (cells/uL)		
Pre-exercise	70.27 ± 37.86	108.88 ± 51.71
0h post-exercise	213.77 ± 131.96*	357.48 ± 171.09*
2h post-exercise	91.76 ± 62.40**	131.42 ± 92.36**
24h post-exercise	106.30 ± 66.78[x]	143.93 ± 74.41^

*p < 0.05 for pre-exercise vs. 0h post-exercise
**p < 0.05 for 0h post-exercise vs. 2h post-exercise
^p < 0.05 for 0h post-exercise vs. 24h post-exercise
[x]p < 0.05 for 2h post-exercise vs. 24h post-exercise
[a]p < 0.05 for comparing 24h post-exercise between groups

Hypotheses

Hypothesis 1: From pre-exercise to immediately post-exercise both TNF-α and CRP will be significantly reduced in both groups. The results of the ANOVA models showed no significant effect over time for TNF-α (p = 0.251) and CRP (p = 0.168), meaning there was no difference from pre-exercise to immediately post-exercise in both the breast cancer survivor group and the control group.

Hypothesis 1a: From pre-exercise to immediately post-exercise IL-10 will be significantly increased in both groups. The results of the ANOVA model showed no significant effect over time for IL-10 (p = 0.126), meaning there was no difference from pre-exercise to immediately post-exercise in both the breast cancer survivor group and the control group.

Hypothesis 2: From pre-exercise to 2 hours post-exercise both TNF-α and CRP will be significantly reduced in both groups. The results of the ANOVA models showed no significant effect over time for either TNF-α (p = 0.881) or CRP (p = 0.119), meaning there was no difference from pre-exercise to 2 hours post-exercise in both the breast cancer survivor group and the control group.

Hypothesis 2a: From pre-exercise to 2 hours post-exercise IL-10 will be significantly increased in both groups. The results of the ANOVA model showed no significant effect over time for IL-10 (p = 0.421), meaning there was no difference from pre-exercise to 2 hours post-exercise in both the breast cancer survivor group and the control group.

Hypothesis 3: From pre-exercise to 24 hours post-exercise both TNF-α and CRP will be significantly reduced in both groups. The results of the ANOVA models showed no significant effect over time for either TNF-α (p = 0.851) or CRP (p = 0.229), meaning there was no difference from pre-exercise to 24 hours post-exercise in both the breast cancer survivor group and the control group.

Hypothesis 3a: From pre-exercise to 24 hours post-exercise IL-10 will be significantly increased in both groups. The results of the ANOVA model showed no significant effect over time for IL-10 (p = 0.221), meaning there was no difference from pre-exercise to 24 hours post-exercise in both the breast cancer survivor group and the control group. However, a significant difference was found in IL-10 between the BCS group and the control group at 24 hours post-exercise (p = 0.044).

As a secondary purpose (hypotheses 4 and 5), relationships for changes in inflammatory markers and NK cell count were explored across each of the four time points (pre-exercise, immediately post-exercise, 2 hours post-exercise, and 24 hours post-exercise). No significant associations were observed between NK cell change and any of the inflammatory markers evaluated in this study (p > 0.05). Spearman rho correlation coefficients between changes in inflammatory markers and changes in NK cell count in both study groups are presented in Table 5 below.

Table 5. Relationships between NK cell count and inflammatory markers

	TNF-α	CRP	IL-10
Pre-exercise to 0h post	BCS: ρ = -0.168 Con: ρ = -0.500	BCS: ρ = -0.084 Con: ρ = 0.317	BCS: ρ = 0.333 Con: ρ = -0.550
Pre-exercise to 2h post	BCS: ρ = 0.333 Con: ρ = -0.433	BCS: ρ = 0.267 Con: ρ = 0.067	BCS: ρ = 0.250 Con: ρ = -0.633
Pre-exercise to 24h post	BCS: ρ = -0.435 Con: ρ = -0.310	BCS: ρ = -0.400 Con: ρ = -0.383	BCS: ρ = -0.042 Con: ρ = -0.150

Hypothesis 4: There will be a significant inverse correlation between changes in NK cell count and changes in both TNF-α and CRP from pre-exercise to immediately post-exercise in both groups. No significant relationship was found between changes in NK cell count and changes in TNF-α ($\rho = -0.168$, $p = 0.666$ and $\rho = -0.500$, $p = 0.170$ for the BCS group and control group, respectively) or CRP ($\rho = -0.084$, $p = 0.831$ and $\rho = 0.317$, $p = 0.406$ for the BCS group and control group, respectively) from pre-exercise to immediately post-exercise.

Hypothesis 4a: There will be a significant inverse correlation between changes in NK cell count and changes in both TNF-α and CRP from pre-exercise to 2 hours post-exercise in both groups. No significant relationship was found between changes in NK cell count and changes in TNF-α ($\rho = 0.333$, $p = 0.381$ and $\rho = -0.433$, $p = 0.244$ for the BCS group and control group, respectively) or CRP ($\rho = 0.267$, $p = 0.488$ and $\rho = 0.067$, $p = 0.865$ for the BCS group and control group, respectively) from pre-exercise to 2 hours post-exercise.

Hypothesis 4b: There will be a significant inverse correlation between changes in NK cell count and changes in both TNF-α and CRP from pre-exercise to 24 hours post-exercise in both groups. No significant relationship was found between changes in NK cell count and changes in TNF-α (ρ = -0.435, p = 0.242 and ρ = -0.310, p = 0.417 for the BCS group and control group, respectively) or CRP (ρ = -0.400, p = 0.286 and ρ = -0.383, p = 0.308 for the BCS group and control group, respectively) from pre-exercise to 24 hours post-exercise.

Hypothesis 5: There will be a significant positive correlation between changes in NK cell count and changes in IL-10 from pre-exercise to immediately post-exercise in both groups. No significant relationship was found between changes in NK cell count and changes in IL-10 (ρ = 0.333, p = 0.381 and ρ = -0.550, p = 0.125 for the BCS group and control group, respectively) from pre-exercise to immediately post-exercise.

Hypothesis 5a: There will be a significant positive correlation between changes in NK cell count and changes in IL-10 from pre-exercise to 2 hours post-exercise in both groups. No significant relationship was found between changes in NK cell count and changes in IL-10 (ρ = 0.250, p = 0.516 and ρ = -0.633, p = 0.067 for the BCS group and control group, respectively) from pre-exercise to 2 hours post-exercise.

Hypothesis 5b: There will be a significant positive correlation between changes in NK

cell count and changes in IL-10 from pre-exercise to 24 hours post-exercise in both

groups. No significant relationship was found between changes in NK cell count and

changes in IL-10 (ρ = -0.042, p = 0.915 and ρ = -0.150, p = 0.700 for the BCS group and

control group, respectively) from pre-exercise to 24 hours post-exercise.

CHAPTER V

DISCUSSION

The purpose of the study was to examine the effect of one acute bout of moderate intensity intermittent exercise on specific markers of inflammation in post-treatment breast cancer survivors. A secondary purpose was to investigate the relationship between selected markers of inflammation and natural killer cell count to explore if the potential mediation of the immune response was also influenced by the inflammatory response due to exercise in breast cancer survivors.

There is still much to learn about whether breast cancer survivors have similar exercise-induced inflammatory responses when compared to healthy individuals. Currently, there are general exercise prescription guidelines for breast cancer survivors, and these guidelines are not that different from those designed for healthy populations (Schmitz et al., 2010). Even though breast cancer survivors appear to tolerate exercise and very few adverse effects have been reported due to exercise training, the literature lacks critical information necessary for the development of more specific exercise guidelines for breast cancer survivors. Questions regarding the immune and inflammatory responses due to acute and chronic exercise are among those that must be answered in order to advance the science of prescribing the most appropriate exercise interventions that can safely promote health and maximize improvements in physiological systems impacted by anti-cancer treatment.

The majority of studies examining the inflammatory response due to exercise in the breast cancer population have been a result of long-term exercise interventions. Even though studies evaluating the long-term immune and inflammatory adaptation to exercise training are important, understanding an acute response to one bout of exercise is critical for the development of safer and efficacious exercise prescriptions for breast cancer survivors. In addition, continuing to explore the response during subsequent bouts of exercise (i.e. during short training microcycles) mimicking the current recommended exercise guidelines will also be essential for the advancement of more precise exercise programs for breast cancer survivors. This knowledge would enhance exercise prescription guidelines by minimizing the risk of exercise to promote further immune suppression and to better define the time course needed between exercise sessions for full recovery. As a result, a more specific exercise prescription would potentially assist breast cancer survivors to benefit the most from a tailored exercise training plan aimed to address some of the side-effects of cancer treatment, improve overall health, and reduce the risk of a potential recurrence.

As previously stated, the research in this area has been focused on examining pre- and post- levels of inflammatory markers in breast cancer survivors over the course of exercise training interventions. The current literature consists of mixed findings, with some studies resulting in positive changes in inflammatory markers due to exercise interventions in this population, while others have found no changes at all over time. Breast cancer survivors have been shown to have higher levels of circulating cytokines and receptors when compared to healthy populations, and a significant association has been found between increased concentrations of certain inflammatory

markers and reduced overall survival (Seruga et al., 2008; Pierce et al., 2009a). It has been speculated that appropriate amounts of physical activity may promote the up-regulation of anti-inflammatory cytokines while simultaneously down regulating the expression of certain pro-inflammatory cytokines (Battaglini et al., 2012). The exact mechanisms explaining this shift in markers of inflammation with exercise are currently unknown but may be occurring indirectly through improved endothelial function, reductions in body weight, or increased insulin sensitivity (Pierce et al., 2009b). Repeated bouts of exercise have resulted in cumulative anti-inflammatory effects in healthy populations, but the beneficial effects of exercise in cancer survivors remain to be determined (Gomez et al., 2011).

Some studies have found no significant changes in inflammatory markers over the course of training interventions consisting of various modes of exercise and program length (Fairey et al., 2005a; Gomez et al. 2011; Hutnick et al., 2005). On the other hand, a couple of studies have found significant reductions in pro-inflammatory markers over time, including CRP and IL-6, as a result of training interventions (Fairey et al., 2005b; Jones et al., 2012). Potential explanations for the discrepancy on the results of the studies that have examined the effects of exercise training in breast cancer patients may be due to different ways inflammation was assessed (i.e. time periods), heterogeneity of the cancer populations studied, and most importantly, the varied training protocols used (i.e. different exercise intensities, exercise session duration, frequency of training, and overall length of the interventions) (Mills et al., 2000; Pedersen and Hoffman-Goetz, 2000; Pedersen, 2000).

A better understanding is needed of how exercise influences the inflammatory response during and after (recovery) one bout of acute exercise performed at different intensities, modes, and durations of exercise as well as its potential influence on the immune system response. Until this knowledge is obtained, exercise prescriptions for breast cancer survivors will continue to be general and perhaps not as efficacious and safe as the prescriptions could potentially be.

This study was designed to begin the examination of the inflammatory response to an acute bout of moderate intensity intermittent exercise in breast cancer survivors. Comparisons between the breast cancer survivor group response and a healthy sedentary control group was made to allow for this initial understanding of how an exercise bout performed at moderate intensity impacted the inflammatory response of a cancer patient in comparison to their matched healthy control subject. In the current study, all subjects underwent a 30-minute bout of intermittent exercise and had blood samples taken pre-exercise, immediately post-exercise, 2 hours post-exercise, and 24 hours post-exercise. Biomarkers of interest included TNF-α, CRP, and IL-10. NK cell count was also analyzed previously, and these values were used in the current study in order to investigate the relationship between inflammation and the immune response to exercise.

Pre-exercise to Immediately Post-exercise

Exercise has been shown to lower circulating levels of pro-inflammatory markers while actually increasing levels of anti-inflammatory markers in a healthy population of recreational athletes (Bruunsgaard, 2005). The results of the current study were somewhat surprising in that there was no significant interaction effect

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found for each inflammatory marker over time in either the cancer or control group. Also, there was no significant change in TNF-α and CRP from pre-exercise to immediately post-exercise in either group. These inflammatory markers responded differently than what was hypothesized where it was expected that TNF-α and CRP would be significantly reduced in both groups from pre-exercise to immediately post-exercise.

Very few studies have examined the inflammatory response due to acute moderate-intensity exercise, and inconsistent findings have been reported for TNF-α and CRP (Ostrowski et al., 1999). The results of the current study are in agreement with previous studies where non-significant changes in inflammatory markers due to acute moderate-intensity exercise were observed (Nieman et al., 2005; Markovitch et al., 2008). These non-significant changes may be due to the nature of the acute exercise bout. The exercise intensity and duration may be important factors in determining the inflammatory response to exercise (Markovitch et al., 2008). Also, the intermittent protocol for the current study could have resulted in a non-significant inflammatory response since the subjects never underwent continual exercise. Even though TNF-α was not significantly increased from pre-exercise to immediately post-exercise, this marker followed a similar upward trend as was found for CRP in both groups. The current study's sample size could also explain the lack of significant findings across time for the selected markers of inflammation due to variability in the values of biomarkers within groups analyzed across the study time points. As a result, this study may have been underpowered and thus could have affected the data's statistical significance.

Since there was no significant interaction effect observed in TNF-α (p = 0.407) or CRP (p = 0.409) in both groups from pre-exercise to immediately post-exercise, the two groups underwent similar changes in levels of these inflammatory markers. An interesting finding of this study was that the control group had higher resting levels at pre-exercise and immediately post-exercise of both TNF-α and CRP when compared to the breast cancer survivor group. In healthy individuals, elevated levels of CRP may be associated with increased body fat and sedentary lifestyles (Pierce et al., 2009b). Since the control group consisted of healthy women who were sedentary, this may provide a possible explanation for why they had higher resting values. However, the majority of the subjects in the breast cancer survivor group were also sedentary, and both groups had similar VO$_{2peak}$ values. The differences in TNF-α and CRP between groups at both pre-exercise and immediately post-exercise were not significant, but these findings came as a surprise since breast cancer survivors have been shown to have higher resting concentrations of certain pro-inflammatory markers, such as TNF-α and CRP (Pierce et al., 2009a; Seruga et al., 2008).

The anti-inflammatory cytokine IL-10 was not significantly changed from pre-exercise to immediately post-exercise in either group (p = 0.126). There was also no significant group by time interaction effect (p = 0.154) observed even though the control group had more than twice the concentration of IL-10 in pg/ml at pre-exercise compared to the breast cancer survivors. This was most likely due to the variability in the IL-10 data for both groups. Some duplicate determinates had high variability and therefore, the lower singlet values of the duplicates, more in line with the rest of the samples, were selected for statistical analyses. Exercise has been shown to produce

acute increases in various anti-inflammatory mediators (Kasapis and Thompson, 2005).

However, the current study resulted in a non-significant anti-inflammatory response

due to exercise from pre-exercise to immediately post-exercise. Both groups in this

study surprisingly experienced a downward trend in IL-10 levels from pre-exercise to

immediately post-exercise, meaning there was no anti-inflammatory response

experienced as a result of the acute bout of moderate intensity exercise.

Pre-exercise to 2 Hours Post-exercise

There was no significant effect over time for either TNF-α ($p = 0.881$) or CRP ($p = 0.119$) from pre-exercise to 2 hours post-exercise in both the breast cancer survivor

group and the control group. Even though the results did not match what was

hypothesized, a non-significant change in inflammatory markers was somewhat

expected from pre-exercise to 2 hours post-exercise based on the current research

literature in healthy populations. There was no interaction effect for TNF-α ($p = 0.506$)

and CRP ($p = 0.551$) between the groups from pre-exercise to 2 hours post-exercise,

indicating both groups responded similarly.

Several studies have examined the effect of an acute bout of moderate intensity

exercise on the inflammatory response in healthy populations. These studies have

found non-significant changes in the levels of certain inflammatory markers up to

several hours post-exercise (Nieman et al., 2005; Markovitch et al., 2008). It appears

that moderate intensity exercise may not stimulate a prolonged inflammatory response

such as that found with more vigorous activities (Pedersen and Hoffman-Goetz, 2000;

Markovitch et al., 2008). It should be noted, however, that CRP continued to trend

upward in both groups at 2 hours post-exercise. This could be an indication that levels of certain pro-inflammatory markers continually increase after acute exercise.

IL-10 concentration was not significantly changed over time from pre-exercise to 2 hours post-exercise in either the breast cancer survivor group or control group (p = 0.421). There was also no significant interaction effect observed between the two study groups over time from pre-exercise to 2 hours post-exercise (p = 0.408). Along with the changes in TNF-α and CRP, both groups exhibited similar changes in IL-10 at 2 hours post-exercise. The current study may be scrutinized in the handling of subject blood samples. Numerous freeze-thaw cycles have been shown to result in the damage of antibodies or antigens in blood samples (Zhou et al., 2010). Even though there were several freeze-thaw cycles, this process was performed universally for all samples in both groups.

It appeared that the moderate intensity intermittent bout of acute exercise was insufficient in inducing a significant pro- or anti-inflammatory response up until 2 hours post-exercise. Even though there were non-significant changes, specific inflammatory markers, such as CRP, continued to increase up until the 2 hour post-exercise assessment time. This continual increasing trend in CRP was not clinically significant with the minute change observed up until 2 hours post-exercise. Therefore, it appears that after 2 hours post moderate intensity intermittent exercise, the non-increase in the systemic inflammatory response represented by CPR, may indicate that the acute bout of exercise was not tasking the subjects enough to produce clinically relevant levels of inflammation. The breast cancer survivors did respond similarly to the healthy sedentary controls from pre-exercise to 2 hours post-exercise, suggesting

they may recover in a comparable manner to acute exercise as their healthy

counterparts up until 2 hours post-exercise.

<u>Pre-exercise to 24 Hours Post-exercise</u>

There was no significant effect over time for either TNF-α (p = 0.851) or CRP (p

= 0.229) from pre-exercise to 24 hours post-exercise in both the breast cancer survivor

group and the healthy control group. The results observed at this point in time support

the fact that the moderate intensity exercise bout may not have been enough stimulus

to cause a prolonged response in inflammatory markers. For both groups TNF-α levels

were very similar at 24 hours post-exercise compared to the subjects' pre-exercise

resting values. In the healthy controls, CRP concentration in the blood essentially

remained the same from pre-exercise to 24 hours post-exercise while CRP levels in the

breast cancer survivor group remained elevated at 24 hours post-exercise when

compared to pre-exercise levels. However, CRP concentration did decrease from 2

hours to 24 hours post-exercise, indicating a full recovery was taking place in the breast

cancer survivors due to the acute bout of exercise. There was also no interaction effect

between the two groups over time from pre-exercise to 24 hours post-exercise in both

TNF-α (p = 0.821) and CRP (p = 0.379). These results imply that both groups had

similar minute changes in the concentrations of TNF-α and CRP from pre-exercise to 24

hours post-exercise.

No significant difference was found for IL-10 from pre-exercise to 24 hours post-

exercise in either group (p = 0.221). In addition, there was no significant interaction

effect observed between the two groups from pre-exercise to 24 hours post-exercise (p

= 0.233). IL-10 responded similarly compared to the pro-inflammatory markers in that

all of these markers were not significantly altered over time up until 24 hours post-exercise in both study groups. However, there was a significant difference found in IL-10 between the breast cancer survivor group and the healthy control group at 24 hours post-exercise. The healthy control subjects had significantly higher levels of IL-10 compared to the breast cancer survivors at 24 hours post-exercise ($p = 0.044$). This finding may be important when examining the more prolonged anti-inflammatory response due to exercise in breast cancer survivors. It is known that breast cancer survivors, along with the cancer population in general, have lower concentrations of certain anti-inflammatory markers such as IL-10 compared to healthy populations of a similar age (Seruga et al., 2008). The current finding may be misinterpreted that a longer recovery is needed from an acute bout of exercise for breast cancer survivors. Even though there was a statistically significant difference between the study groups, the breast cancer survivors returned to their pre-exercise resting level of IL-10 at 24 hours post-exercise. It is possible that this population has lower resting levels of anti-inflammatory markers compared to healthy populations, but they respond similarly to an acute bout of moderate intensity exercise. There were no other significant differences observed between the two groups at any other time point in the study for any inflammatory markers. This makes it apparent that the breast cancer survivor group responded similarly as the healthy control group to the exercise bout.

Since there were no differences between the two groups in CRP ($p = 0.918$) and TNF-alpha ($p = 0.591$) over time, breast cancer survivors may potentially have a similar pro-inflammatory response to exercise as their healthy counterparts. This is exciting because breast cancer survivors may be able to respond similarly and undergo exercise

prescriptions designed for healthy populations. This study lends support to the idea

that current exercise prescriptions designed for healthy populations and used for

breast cancer survivors may be safe and be able to promote benefits. However, these

results should be analyzed with caution and further research needs to be done to

explore the current study's findings.

Relationship between markers of inflammation and the immune response

There is a known relationship between markers of inflammation and immune

parameters. Inflammatory markers are considered the principal mediators of

communication between cells of the immune system, functioning as key contributors to

immunity and inflammation (Abbas, 2005). Cytokines are released from immune cells

in a signaling fashion, and they have strong roles in the up-regulation of particular

immune parameters (Pedersen, 2000). These soluble proteins allow communication

between immune cells with coordination of inflammatory responses (Zhou et al., 2010).

The current study resulted in no significant relationships found between

changes in NK cell count and inflammatory markers across time. This was an

unexpected finding. There were significant changes observed in NK cell count over time

in both groups, but there were no corresponding changes in the markers of

inflammation. The lack of associations between markers of inflammation and the

immune response in this study may be attributed in part to the intensity and nature of

the exercise used. Since breast cancer patients were not able to perform 30 minutes of

moderate intensity aerobic exercise uninterruptedly in a pilot study conducted prior to

the beginning of the study, the adoption of the intermittent nature of the exercise was

an attempt to reproduce the duration and intensity known to provoke an immune and

inflammatory response. Considering the acute bout of exercise was not continuous, the

recurrent 1.5 minutes of rest for every 3 minutes of exercise could have lead to the non-

significant inflammatory response observed, and therefore, resulted in the lack of

mediation of the immune response.

A major finding of this investigation was that acute moderate intermittent

exercise led to inflammatory and NK cell count responses that were similar in both

study groups, and that neither group experienced significant post-exercise values in

inflammation or NK cell count relative to pre-exercise values. This is an important

finding as it suggests this particular physical activity, which is commonly used in

prescriptive exercise for breast cancer survivors, caused a typical response as

previously seen in other healthy individuals (Ostrowski et al., 2003; Steensberg et al.,

2003; Nieman et al., 2005).

Inflammatory and immune response due to an acute bout of exercise

The mechanisms through which physical activity influences the inflammatory

process are not well understood (Ford, 2002). Potential mechanisms include:

reductions in body mass, positive changes in lipid and glycemic profiles, and improved

endothelial function (Markovitch et al., 2008; Pierce et al., 2009b). The results seen in

the current study are most likely due to the exercise session itself, since there were

similar non-significant changes found in both groups.

While some previous studies have found moderate-intensity exercise to elicit

changes in certain inflammatory markers, other studies have found no effect (Mills et

al., 2000; Nieman et al., 2005; Plaisance et al., 2007). There are several possible

explanations for these variable results on cytokines response in relation to exercise

including: 1) the intensity and duration of the exercise, as well as the type of physical activity; 2) the specificity and the sensitivity of the assays used in particular studies (Pedersen and Hoffman-Goetz, 2000; Pedersen, 2000). The current study resulted in no significant inflammatory marker changes in the breast cancer survivor group and the healthy control group, so it appeared that both groups responded similarly to the acute bout of moderate intensity intermittent exercise. Since moderate intensity exercise was not able to significantly change inflammatory markers in both groups, there may be a specific intensity or duration threshold that must be achieved in order to elicit an acute change in biomarkers such as TNF-α, CRP, and IL-10 in breast cancer survivors and sedentary healthy women. The magnitude of the increases found in markers of inflammation may be closely related to the duration of the exercise (Pedersen and Hoffman-Goetz, 2000). It is possible that longer durations of exercise may produce a more significant inflammatory response (Pedersen, 2000).

Exercise has the ability to produce acute and long-term increases in various anti-inflammatory mediators, and several studies have found increases in anti-inflammatory markers after acute bouts of exercise (Kasapis and Thompson, 2005; Starkie et al., 2003). These anti-inflammatory effects of acute exercise are very prominent in studies examining vigorous intensity exercise as well as longer durations of exercise in healthy populations (Starkie et al., 2003; Petersen and Pedersen, 2005). The current study did not result in the up-regulation of anti-inflammatory pathways, such as a positive IL-10 response elicited by an acute bout of exercise that has been thoroughly reported in the literature. This could be due to cancer treatments suppressing the inflammatory response in the breast cancer survivors. The reason for this response not occurring in

the current study may be speculated to be a result of the acute bout of exercise implemented, especially since both groups experienced a similar non-significant inflammatory response. It may be that moderate intensity exercise is simply not demanding enough to stimulate the anti-inflammatory effects commonly seen at more vigorous intensities of exercise. Markovitch et al. (2008) similarly found that acute moderate intensity exercise had no effect on pro- or anti-inflammatory responses. The results from this study may suggest that the long-term anti-inflammatory effects that were previously reported with exercise of moderate-intensity must be explained by something other than a net anti-inflammatory response to each exercise bout (Markovitch et al., 2008). Other mechanisms such as changes in body mass, lipid profiles, and glycemic profiles have been shown to be responsible for changes in inflammatory markers due to exercise over time (Okita et al., 2004; Shojaee-Moradie et al., 2007). In addition, there may be a certain threshold that needs to be met during exercise in order to stimulate a significant change in markers of inflammation. Once again, this is currently all speculation, and more research needs to be done in order to support or refute these findings.

The intermittent nature of the acute bout of moderate intensity exercise implemented in the current study could have contributed to non-significant changes in inflammatory markers across time. A discontinuous exercise protocol was used to ensure that all subjects would be capable of completing the exercise session, and subjects underwent 30 minutes of exercise in a 43.5-minute period, alternating ten 3-minute intervals of exercise with 1.5 minutes of rest. Since the acute bout was not continuous moderate intensity, this could have lead to a further delay in a significant

inflammatory response.

NK cell count was significantly changed as measured conclusively in both groups. The magnitude of change in both NK cell count and inflammatory markers was similar between groups. This suggests that the exercise bout elicited a response in the breast cancer survivors that paralleled the healthy controls who had never experienced cancer treatment. However, no significant changes were observed when values were compared back to baseline in any of the inflammatory markers. The intensity and/or duration of the acute exercise bout may be considered the major reason for these non-significant changes occurring in the current study, and a specific threshold may need to be obtained in order to stimulate the inflammatory response. Interestingly, the majority of studies finding significant changes over time have included recreationally active healthy populations (Ostrowski et al., 1999; Starkie et al., 2003; Steensberg et al., 2003; Kasapis and Thompson, 2005). This study had a healthy sedentary population as the control group with similar activity levels to the breast cancer survivors. The mediation of the immune system response due to exercise may not be attributable to markers of inflammation, especially at lower intensities of exercise. In addition, the acute inflammatory response commonly seen as a result of exercise may occur to a lesser extent in sedentary individuals. In order to improve immune response and receive positive anti-inflammatory benefits from exercise, more than one acute bout may be needed. Multiple acute bouts may be more likely to stimulate the inflammatory mediation of the immune response in sedentary populations.

Conclusion

In a group of breast cancer survivors and healthy controls, no significant changes were observed between groups across time due to an acute bout of moderate intensity exercise in levels of TNF-α, CRP, and IL-10. Both groups responded similarly to the acute bout of exercise in regards to markers of inflammation and NK cell count, indicating there is potential that exercise prescriptions for healthy populations may be able to be implemented for breast cancer survivors. There were significant changes over time in the immune parameter of NK cell count in both groups, while inflammatory markers in these groups did not significantly change. The bout of acute exercise appeared to be less intense than needed to stimulate the mediation of the immune response by inflammatory markers. Whether this is due to the intensity or duration, we currently do not know. The exact mechanisms explaining the changes in both the inflammatory and immune response to acute exercise are currently not well understood. Further research needs to be done in order to support or refute this study's findings.

Recommendations for future research

Based on the results of the current study, future studies should include larger sample sizes with more time points for the analysis of the inflammatory response due to acute exercise. Increasing the number of blood sampling time points would allow a better understanding of the recovery process due to exercise, but may not be very practical. Future research should focus on whether a certain intensity or duration threshold is needed to elicit a significant inflammatory response from an acute bout of exercise, since it has been commonly reported that lower levels of exercise have

resulted in non-significant changes in the inflammatory response in both healthy and clinical populations. Different durations and intensities of acute exercise should be compared in order to see if the bout of exercise itself is a limiting factor in changes in markers of inflammation in breast cancer survivors. Also, investigation of cumulative acute bouts of exercise versus single acute bouts of exercise should be explored. Since there is a known anti-inflammatory effect that occurs with chronic exercise training in healthy physically active individuals, it would be interesting to investigate the effects of multiple acute bouts of exercise in cancer patients. A training program reproducing current exercise guidelines for cancer patients could be carried out to see if in fact the cumulative effects of training is what produces a favorable response in relation to inflammation (i.e. increase in anti-inflammatory markers).

APPENDIX A: SAMPLE SIZE CALCULATION

Sample size calculation using PS software program, 2009

PS log entry:

PS logging enabled 3/5/2013 11:02:43 AM
Version 3.0.43

Suggested citation:
Dupont WD, Plummer WD: 'Power and Sample Size Calculations: A Review and Computer Program', Controlled Clinical Trials 1990; 11:116-28.
    Or
Dupont WD, Plummer WD: 'Power and Sample Size Calculations for Studies Involving Linear Regression', Controlled Clinical Trials 1998; 19:589-601.

Type of study: T-test
Requested output: Sample Size
Design: Paired
        alpha=0.05 power=0.8 DIFF=0.8 SIGMA=1 M=0
        Sample size=14

We are planning a study of a continuous response variable from matched pairs of study subjects. Prior data indicate that the difference in the response of matched pairs is normally distributed with standard deviation 1. If the true difference in the mean response of matched pairs is 0.8, we will need to study 14 pairs of subjects to be able to reject the null hypothesis that this response difference is zero with probability (power) 0.8. The Type I error probability associated with the test of this null hypothesis is 0.05.

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