Effects of an Acute Bout of Resistance Training on Leukocyte and Leukocyte Subset Response in Breast Cancer Survivors

Rachel Graff

A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Masters of Arts in the Department of Exercise and Sport Science (Exercise Physiology).

Chapel Hill
2013

Approved By:
Claudio L. Battaglini, PhD
Anthony C. Hackney, PhD, DSc
Eric D. Ryan, PhD
Elizabeth S. Evans, PhD
Abstract

RACHEL GRAFF: Effects of an acute bout of resistance training on leukocyte and leukocyte subset response in breast cancer survivors
(Under the direction of Dr. Claudio Battaglini)

The purpose of this study was to examine the immune response to a single bout of moderate-intensity resistance training in breast cancer survivors (BCS). BCS (n=4) and healthy controls (n=8) completed an exercise session consisting of 3 sets of 10 repetitions at 70% of one repetition-maximum on leg press, lateral pull down, leg extension, and seated row performed in a circuit fashion. Blood samples taken at baseline, immediately post (0h post), 2-hours post (2h post), and 24-hours post (24h post) exercise were assessed for total leukocyte, granulocyte, lymphocyte, and monocyte cell counts. Percent change (%Δ) scores from baseline-0h post, baseline-2h post, and baseline-24h post were compared using mixed-model ANOVAs between groups and indicated that there were no significant differences between total leukocytes (p=0.560), granulocytes (p=0.239), lymphocytes (p=0.257), or monocytes (p=0.721) at any point. Results suggest BCS and controls exhibit similar immune responses to an acute bout of resistance training.
Acknowledgements

I would like to take this opportunity to thank all of those that made this thesis project possible. First and foremost, I would like to thank Dr. Claudio Battaglini and Dr. Anthony Hackney, who invested so much time and effort into virtually all aspects of the study and who put up with my endless amount of questions. Without their expertise, guidance, and mentorship, this thesis would not have been possible. I have thoroughly enjoyed working with and learning from both of them on a professional as well as a personal level. I would also like to thank Jacob Allen, as he served as my ‘partner-in-crime’ throughout the duration of the thesis process from beginning to end and an integral member of our research team. This project was definitely a team effort and these individuals are certainly all-stars!

I would like to extend a special thanks to Dr. Beth Evans, as the ‘back-bone’ of this project came from her doctoral dissertation and she served as an invaluable resource throughout all stages of the research process. Also, I would like to thank Dr. Eric Ryan, the final member of my thesis committee, for investing his time and effort into making this thesis the best it can be.

To my friends and family, without your unrelenting support and encouragement, I would not have been able to succeed in any of my endeavors. I owe my livelihood and optimistic outlook on life to all of you.
Table of Contents

List of Figures ................................................................................................................................. viii

List of Tables ........................................................................................................................................ ix

Chapters

I. Introduction.......................................................................................................................................... 1

   Statement of Purpose ......................................................................................................................... 4

   Hypotheses (H) ................................................................................................................................. 5

   Definition of Terms ......................................................................................................................... 5

   Assumptions ................................................................................................................................. 6

   Limitations ......................................................................................................................................... 7

   Delimitations ..................................................................................................................................... 7

   Significance ....................................................................................................................................... 8

II. Review of the Literature ................................................................................................................ 9

   Breast Cancer Prevalence and Treatments ....................................................................................... 9

   Physical Activity and Breast Cancer ............................................................................................... 10

   Resistance Exercise in Cancer Patients ............................................................................................ 12

   Relationship Between Cancer and the Immune System ............................................................... 14

   Acute Exercise and the Immune Response ....................................................................................... 16
<table>
<thead>
<tr>
<th>Appendix</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix C</td>
<td>69</td>
</tr>
<tr>
<td>Appendix D</td>
<td>74</td>
</tr>
<tr>
<td>Appendix E</td>
<td>77</td>
</tr>
<tr>
<td>Appendix F</td>
<td>81</td>
</tr>
<tr>
<td>Appendix G</td>
<td>83</td>
</tr>
<tr>
<td>Appendix H</td>
<td>85</td>
</tr>
<tr>
<td>Appendix I</td>
<td>86</td>
</tr>
<tr>
<td>References</td>
<td>87</td>
</tr>
</tbody>
</table>
List of Figures

1. Protocol Timeline………………………………………………………………31
2. Blood Draw Timeline …………………………………………………………31
List of Tables

1. Table 1: Subject Characteristics ................................................................. 33
2. Table 2: Subject Cancer Treatment Characteristics ................................. 34
3. Table 3: Leukocyte, Leukocyte Subset, and Cortisol Values ...................... 35
4. Table 4: Mean Hematocrit (Hct) and Hemoglobin (Hb) Values ............ 36
5. Table 5: Percent Change Scores for Total Leukocytes, Granulocytes, Lymphocytes, and Monocytes .......................................................... 37
Chapter I

Introduction

In the year 2012, more than 200,000 people in the United States alone are expected to be diagnosed with invasive breast cancer (American Cancer Society, 2012). Of these, 90% are likely to survive for at least five years after diagnosis (ACS, 2012). Many of these individuals will have to undergo surgery, radiation, chemotherapy, hormonal therapies, or some combination of one or more of these treatments in order to survive the disease (ACS, 2012). Cancer and its treatments have profound impacts on the physiology of its patients, drastically altering physical, psychological, and functional capacity and quality of life (Battaglini et al., 2006). Therefore, attempts to improve or reverse these negative physiological alterations that result from cancer and its treatments are paramount.

Among many side-effects commonly experienced by cancer patients, severe fatigue, reduction in cardiopulmonary function, negative alterations in body composition, reduction in strength and physical functionality, along with psychosocial complications including depression, anxiety, and a decline in self-esteem all have been shown to significantly reduce the overall functionality of patients significantly compromising their overall quality of life (Al-Majid & Waters, 2008; Courneya, 2003; Fairey et al., 2002; Loescher et al., 1989). This constellation of side effects often leads to a reduction in daily physical activity levels. Reduced daily activity may serve to exacerbate the effects of treatments that have adverse effects on many different physiological systems, including the musculoskeletal and
cardiovascular systems (Battaglini et al., 2006; Loescher et al., 1989; Schneider et al., 2007). Loss of skeletal muscle, in particular, is a very frequent and undesirable effect of cancer and its treatments, which can negatively impact various aspects of life in cancer survivors (Al-Majid & Waters, 2008; Courneya, 2003; Schneider et al., 2007).

In the last couple of decades, alternative interventions, often referred to complementary alternative medicine (CAM) have been explored in oncology patients in an attempt to attenuate the severe side-effects commonly experienced by cancer patients during and post-completion of cancer treatments. One intervention that has gained a lot of attention the past few years is exercise training due its efficacious ability to positively impact many physiological systems that are altered during cancer treatment, its non-invasive nature, and relatively affordable price. Since the mid 1980s, studies have been conducted examining the effects of exercise on different physiological systems as well as in different cancer populations, with most of the studies being conducted in breast cancer patients. Most of these initial studies focused on aerobic forms of exercise (Courneya et al, 2003; Courneya et al. 2006; Decker et al. 1989; Dimeo et al., 1997; MacVicar et al., 1986). It wasn’t until 2002 that other modes of exercise training began to be explored. Kolden et al. (2002) was the first research team to examine the effects of a combined aerobic and resistance exercise training intervention on multiple physiological parameters. In 2004, Drake and colleagues were one of the first groups to examine the effects of resistance training alone in cancer patients. This intervention was called for due to the negative effects of cancer treatments on body composition, an example of which is the progressive loss of muscle mass (Al-Majid & Waters, 2008; Cheema, et al., 2008; Schneider et al., 2007). These negative alterations in muscle mass are believed to be directly influenced by the effects of cancer treatments on
muscle cells and metabolism, often leading to reductions in protein synthesis. Resistance exercise, however, has shown to attenuate and even reverse the loss of muscle mass that is associated with cancer and its treatments (Courneya et al., 2007; Drake et al., 2004; Schmitz et al. 2005; Schwartz, 2007, 2009; Segal et al., 2009).

The body of research regarding the specific role of progressive resistance training in cancer patients and survivors is relatively small. However, studies are demonstrating that resistance training holds promise in improving physical, psychosocial, and functional aspects in cancer patients (Cheema et al., 2007; Courneya et al., 2008; Schmitz et al., 2005; Segal et al., 2009). Even though these first generation studies provide important information on patient acceptability and tolerability for exercise training, safety and precise information on optimum exercise training regimens for cancer patients have yet to be determined.

A well functioning immune system plays an integral role in maintaining overall health and optimal physiological function. However, cancer and its treatments are associated with immunodeficiency, which often lasts well beyond the completion of treatments (Fairey et al., 2005; Hutnick et al., 2005). The adaptive immune system is particularly influenced by the disease, and is often slow to recover (Hutnick et al., 2005). Therefore, cancer survivors often experience an increased risk of secondary infection as well as a risk of cancer recurrence as a result of their immunosuppressed condition (Hutnick et al., 2005). Some research has shown that exercise may boost immune function in cancer patients, improving overall health and potentially enhancing longevity (Fairey et al., 2002; Fairey et al., 2005; Hutnick et al., 2005).

To date, few studies have examined the effects of exercise on the immune system in breast cancer patients (Fairey et al. 2005; Nieman et al., 1995; Peters et al., 1994).
Furthermore, most of these studies have looked at aerobic exercise modes and have only examined the chronic effects of exercise. Even though these studies have shown promising results, which demonstrate the potential that exercise training may have on improving the immune system of breast cancer survivors, nothing is currently known about the effects of one bout of exercise on the immune response of these patients. Examining markers of immunity such as total leukocyte and leukocyte subset counts may serve as an important initial step for the development of the body of knowledge regarding the response of the immune system to an acute bout of exercise, since these markers are routinely part of hematological profile analyses in cancer patients.

In order to maximize the benefits of exercise for cancer patients, understanding their immune response to different intensities and modes of exercise is a paramount initial step for the development of future exercise prescription guidelines for optimum exercise training regimens. Since the use of resistance training has grown in popularity among the scientific community due to its potential to alleviate many of the negative side-effects associated with body composition and overall functionality in cancer patients, examining the effects of an acute bout on the immune response would allow for the development of more precise future interventions aimed not only at maximizing training response, but also at improving the safety of patients engaging in this mode of exercise training.

Statement of Purpose

The purpose of this study was to compare the effects of an acute bout of moderate intensity resistance training on changes in leukocyte and leukocyte subset response between breast cancer survivors and apparently healthy controls, which were matched by age and physical activity level. Exploratory analyses examined the cortisol response to an acute bout
of moderate intensity resistance training, as well as the relationship between the cortisol and leukocyte and leukocyte subset response in both the breast cancer survivors and control groups.

**Hypotheses (H)**

H1. There will be a significantly greater difference in leukocyte, granulocyte, lymphocyte, and monocyte percent cell count changes from baseline to immediate post-exercise in the control group compared to the breast cancer survivor group.

H2. There will be a significantly greater difference in leukocyte, granulocyte, lymphocyte, and monocyte percent cell count changes from baseline to 2 hours post-exercise in the breast cancer survivor group compared to the control group.

H3. There will be a significantly greater difference in leukocyte, granulocyte, lymphocyte, and monocyte percent cell count changes from baseline to 24 hours post-exercise in the breast cancer survivor group compared to the control group.

**Definition of Terms**

**Leukocytes**: White blood cells that help protect the body via involvement in immune and inflammatory responses (Marieb & Hoehn, 2010).

**Leukocytosis**: An increase in the number of leukocytes (Marieb & Hoehn, 2010).

**Lymphocytes**: Agranular leukocytes that form within the bone marrow and become functionally mature in the lymphoid organs (Marieb & Hoehn, 2010).

**Lymphocytopenia**: A reduction in the number of lymphocytes.

**Lymphocytosis**: An increase in the number of lymphocytes.
Granulocytes: a subset of leukocytes that includes neutrophils, basophils, and eosinophils (Marieb & Hoehn, 2010).

Granulocytosis: An increase in the number of granulocytes.

Neutrophil: Most abundant type of leukocyte (makes up about 60% of total leukocytes). A phagocytic cell that often serves as the body’s first line of defense against infection (Marieb & Hoehn, 2010; Walsh et al., 2011).

Neutrophilia: An increase in the number of neutrophils.

Monocyte: Large leukocyte that makes up 3-8% of total leukocytes (Marieb & Hoehn, 2010).

Monocytosis: An increase in the number of monocytes.

Breast Cancer Survivors: Individuals diagnosed with stage I, II, or III breast cancer who have completed their major treatment(s), including chemotherapy, radiation, and/or surgery within the last 6 months and who may or may not be currently receiving hormonal therapy.

Progressive Resistance Training: A training program where muscles work against gradually increasing resistance; involving implementation of the overload principle (Powers & Howley, 2009).

1 Repetition Maximum (1-RM): The heaviest load that an individual can lift through a full range of motion one time (Tan, 1999).

Assumptions

- All participants in both groups adhered to the pre-assessment guidelines prior to experimental sessions.
- All participants were honest in their responses to the medical history form.
- The amount of time since treatment completion (1 month vs. 6 months, vs. 12 months) did not influence the patients’ immune and hormonal responses.
• Subjects did not change their diet between the familiarization (1-RM testing) and acute bout.

Limitations

• Subjects may not have complied with the dietary and exercise guidelines. To encourage participant compliance, subjects were asked upon arrival to verify that they have not eaten within the last 2 hours nor exercised within the last 12 hours.

• The training status of the subjects prior to their enrollment in the Get REAL & HEEL breast cancer program was not controlled for. Therefore, training status may confound or interact with the results of the resistance training exercise bout.

• The various anti-cancer treatments underwent by the subjects were not accounted for during analysis.

• Diurnal rhythms of cortisol were not accounted for.

Delimitations

• All subjects in the breast cancer survivor group had completed chemotherapy and/or radiation treatments within 12 months of the experimental sessions.

• Subjects in the breast cancer survivor group were not taking any medications that may influence the response of the immune system, other than hormonal therapy (i.e., Tamoxifen).

• All subjects in the control group were healthy with no contraindications to participating in resistance exercise, and not taking any medications that may influence the response of the immune system.

• All subjects in both groups did not have any range of motion restrictions that impaired their ability to participate in the resistance exercise session.
Significance

It is necessary for exercise science professionals to account for the effects of exercise training sessions on immune function when designing training regimens for their clients. This includes being aware of the extent to which the immune system is suppressed following certain types of training, and making certain to allocate adequate recovery time before implementing another training session. In athletes, the continuous implementation of consecutive training bouts with inadequate recovery time is suggested to facilitate immunosuppression and the onset of the Overtraining Syndrome (Gleeson, 2002). While breast cancer survivors are not likely to be performing exercise at the same level or intensity as high-level athletes, it remains important that the implementation of exercise training in this population does not result in further chronic immunosuppression in addition to that caused by the various anti-cancer treatments.

To ensure that the suppression of the immune systems of breast cancer survivors is not being exacerbated by exercise training programs, it is essential for the timeline of immune recovery following a single exercise session be known. A secondary training session should not be implemented until the potential immunosuppressive effects of the primary training session recover fully, so as to avoid prolonged impairment of the immune system (Gleeson, 2001; Koch, 2010). As the immune system responds somewhat differently to the various types of training (i.e. endurance versus resistance training), it is necessary that the timeline of recovery be known for both in order to facilitate a more precise program design that will enhance the immune function of this population as opposed to inhibiting it (Koch, 2010).
Chapter II

Review of the Literature

Introduction

This review of the literature is divided into 6 sections. Section 1 will discuss breast cancer and its treatments; section 2 will review physical activity in breast cancer patients; section 3 will discuss the current research on resistance exercise in cancer patients; section 4 will introduce the relationship between cancer and the immune system; section 5 will focus on the relationship between acute exercise and the immune response; and section 6 will summarize the potential implications of exercise, particularly resistance exercise, in breast cancer survivors.

Breast Cancer Prevalence and Treatments

The prevalence of breast cancer within the United States has continually increased over the last number of years. Today, 1 out of 8 women will be diagnosed with breast cancer at some point in her lifetime (American Cancer Society, 2012). Surgery, radiation, chemotherapy, and hormonal therapies comprise the list of the various treatments that these individuals may have to endure, and they are each accompanied by a vast array of side effects, which can drastically influence physiological factors and quality of life (ACS, 2012; Courneya, 2003).

Surgery may be accompanied by infection, decreased function, reduced range of motion (ROM), diarrhea, shortness of breath, pain, numbness, and lymphedema (Courneya,
Radiation treatment is associated with potential toxicity to irradiated normal tissue, pain and blistering, reductions in ROM, fatigue, diarrhea, lung fibrosis, and damage to cardiac myocytes (Courneya, 2003). Chemotherapy is often coupled with side effects such as fatigue, reduced appetite, nausea, anemia, reduction in neutrophil counts, peripheral neuropathy, ataxia, and cardiotoxicity (Courneya, 2003). Finally, hormonal therapy typically results in weight gain, sarcopenia, muscle weakness, increases in adiposity (particularly within the torso and face), bone loss, fatigue, hot flashes, and immunosuppression (Courneya, 2003). Following a breast cancer diagnosis, individuals will often undergo one or more of these treatments, which clearly exhibit monumental influences upon various aspects of human physiology.

**Physical Activity and Breast Cancer**

Until recently, participation in regular physical activity for individuals undergoing and recovering from cancer treatments was contraindicated by medical professionals (Battaglini et al., 2006). Within the last several years, however, there has been a growing body of evidence to suggest that participation in a regular exercise program can be extremely beneficial in mediating and even reversing many of the aforementioned side effects associated with cancer treatments both during and following cancer treatments. Although the first study to incorporate resistance training in cancer patients was conducted in 2002, successive studies over the past 10 years have demonstrated that resistance training shows promise in enabling the improvement in a wider range of treatment-related side effects than cardiovascular training alone (Kolden et al., 2002).

Skeletal muscle loss is a common problem in cancer patients both during and after the completion of various cancer treatments (Al-Majid & Waters, 2008; Schneider et al., 2007).
In addition to metabolic effects, the time period following a cancer diagnosis is often coupled with an increase in inactivity, which contributes to further muscle loss (Al-Majid & Waters, 2008; Schneider et al., 2007). Metabolically, individuals diagnosed with cancer typically experience a decrease in protein synthesis and a subsequent increase in protein degradation (Al-Majid & Waters, 2008; Schneider et al., 2007). In other words, cancer patients’ bodies are unable to re-build skeletal muscle at the same rate at which it is being broken down. It is suggested that the reduction in protein synthesis may be due to one or more of the following: increased levels of tumor-released proteolysis-inducing factor, angiotensin II, disruption of the mTOR pathway, and a reduction in daily physical activity levels as a result of fatigue and muscle weakness (Al-Majid & Waters, 2008). It is also possible that the reduction in physical activity can play a role in exacerbating the metabolic effects on the reduction in protein synthesis (Al-Majid & Waters, 2008).

In addition, the increase in skeletal muscle protein degradation may be attributed to an activation of various proteolytic mechanisms. One such mechanism is the non-lysosomal calcium-dependent protease system that is responsible for the release of calpains, which break down the myofibrils that comprise skeletal muscle fibers. Another, is the ubiquitin-proteasome system, which breaks down damaged proteins in healthy individuals, but shows increased activity within the skeletal muscles of cancer patients. A third, is an increase in the amounts of pro-inflammatory cytokines such as tumor necrosis factor- alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and interferon-gamma (IFN-γ) within the bodies of individuals with cancer, which are suggested to indirectly activate the ubiquitin-proteasome system (Al-Majid & Waters, 2008; Schneider et al., 2007). The combination of these factors, which contribute to the reduction in protein synthesis and an increase in protein degradation
within the skeletal muscles of individuals with cancer serve to comprise the cachexia, or skeletal muscle wasting, experienced by cancer patients.

**Resistance Exercise in Cancer Patients**

Progressive resistance training in healthy individuals is responsible for providing a stimulus to increase the amount of skeletal muscle protein synthesis. This may be due, in part, to the anti-inflammatory effect of resistance training. Resistance training has demonstrated a reduction in pro-inflammatory cytokine levels, which as previously mentioned, are suggested to indirectly activate the ubiquitin-proteasome system, contributing to skeletal muscle breakdown (Al-Majid & Waters, 2008). By reducing the release of pro-inflammatory cytokines and thereby the indirect stimulation of the ubiquitin-proteasome system, protein degradation may be prevented. Further, resistance training has been shown to affect the mTOR pathway, which as previously mentioned, is another mechanism of protein synthesis that is oftentimes disrupted as a result of cancer and its treatments. In healthy individuals, resistance training significantly stimulates the mTOR pathway within skeletal muscle cells, thereby promoting skeletal muscle protein synthesis (Al-Majid & Waters, 2008; Spiering et al., 2008). Therefore, resistance exercise may be beneficial in cancer patients by opposing several cancer-related mechanisms that contribute to skeletal muscle loss.

To date, the majority of the research studies involving exercise and cancer patients have been done on individuals with breast cancer (Galvao & Newton, 2005). Further, most of the exercise interventions have prescribed either aerobic only, or combined aerobic and resistance training interventions, with very few examining the effects of resistance training alone (Cheema et al., 2008). Overall, however, progressive resistance training in cancer patients is continuously proving to be safe and beneficial in individuals recovering from
cancer and its treatments, as it can improve various aspects of physiology and quality of life without the occurrence of any serious adverse effects (Cheema, et al., 2008; Drake et al., 2004; Galvao et al., 2006; Galvao et al., 2008; Schwartz et al, 2007; Schwartz & Winters-Stone 2009).

In a study by Segal et al., (2009), men currently receiving radiation treatment for prostate cancer were divided into resistance exercise, aerobic exercise, and non-exercise control groups. The exercise groups each trained 3 times a week for 24 weeks with progression as participants became more physically fit. Results indicated that the resistance exercise group resulted in greater improvements in cancer-specific quality of life, muscular strength, triglycerides, and body composition than the aerobic training group. Both resistance and aerobic training groups had a positive effect on reducing levels of fatigue and in improving aerobic fitness. Even though this study was conducted in men with prostate cancer who were undergoing radiation treatment, it suggests that resistance exercise could potentially play an important role in individuals with other types of cancer who have undergone radiation therapy.

A different study by Schneider et al. (2007) examined the effects of combined aerobic and resistance training on breast and prostate cancer patients. Participants exercised 2-3 times per week over the course of 6 months. At the end of the training period, the group that had completed treatment prior to the exercise intervention demonstrated increased muscular endurance, significantly reduced depression, and increased quality of life. Along the same lines, De Backer et al., (2007) exercised 57 cancer survivors using high intensity resistance training and aerobic interval training over the course of 18 weeks. After the training period, results showed significant increases in muscular strength, maximal oxygen consumption
(VO$_{2\text{max}}$), and health-related quality of life. In sum, these studies indicated that moderate to high intensity combined resistance and aerobic exercise training in cancer patients and survivors can improve muscular strength, performance, and quality of life as well as the functionality of cancer survivors.

Schmitz et al. (2005) and Courneya et al. (2007) both employed the use of resistance training in isolation in breast cancer survivors and breast cancer patients undergoing chemotherapy, respectively. Both studies found significant improvements in body composition and muscular strength, and were well tolerated by the participants. Schmitz et al. (2005) noted significant reductions in body fat percentages over the 6-12 month training period, which has important implications in preventing cancer recurrence. Courneya et al., (2007) reported that in addition to the improvements in body composition and muscular strength, resistance training was in fact superior to aerobic training in increasing self esteem and chemotherapy completion rate in participants over the course of the training intervention. Although research on resistance training alone in breast cancer patients and survivors is limited, it appears to have positive influences on many of the ailments caused by a cancer diagnosis and its subsequent treatments.

**Relationship Between Cancer and the Immune System**

Cancer and its treatments are linked to deviations in immune function from that of healthy individuals. During and following treatment, cancer patients typically experience immunosuppression and hence, an increase in their susceptibility to secondary infection as well as cancer recurrence (Courneya, 2003; Fairey et al., 2005; Hutnick et al., 2005). This immunosuppression can be observed in both the innate and the adaptive immune cells (Fairey et al., 2005; Hutnick et al., 2005).
The innate immune system is typically the body’s first line of defense against invading pathogens. The cellular components of the innate response include neutrophils (a subset of granulocytes, which comprise about 60% of all circulating leukocytes), macrophages (which come from the maturation of monocytes), natural killer (NK) cells (a subset of lymphocytes), and dendritic cells (Koch, 2010; Walsh et al., 2011). These cells provide non-specific protection of the host against any foreign or non-self components that exist within the body (Walsh et al., 2011).

The cellular components of the adaptive immune system consist primarily of the various lymphocyte subpopulations with the exception of NK cells, which are part of the innate immune system. The basic lymphocyte subpopulations are divided into T-helper cells (CD4+), T-cytotoxic cells (CD8+), and B-cells, which can be further divided into additional subsets (Walsh et al., 2011). These cellular components are typically activated by the innate immune response to a particular antigen or abnormal body cell and undergo a cascade of events to enable the body to more effectively fight off the foreign invader or abnormal cell via antibody production or apoptosis (programmed cell death). Therefore, the duration of the adaptive response may take much longer than that of the innate response. Once the antigen or non-self component is effectively removed from the body, memory T-cells and B-cells remain so that upon secondary exposure, the body can respond more quickly and efficiently to wipe out the invader (Marieb & Hoehn, 2010).

In breast cancer survivors specifically, fatigue is a major problem experienced by many individuals for months or even years after the completion of treatment (Bower et al., 2002; Servaes, Verhagen, & Bleijenberg, 2002). A study by Bower et al. (2002) examined some of the cellular immune markers in fatigued breast cancer survivors who had completed
all cancer treatments aside from tamoxifen and found that these individuals had lower proportions of NK cells and T-helper cells than those who were not fatigued. It is suggested that these lymphocyte subsets are particularly slow to recover following cancer treatment, and may be affected for up to five years after chemotherapy and radiation treatments (Bower et al., 2002; Hutnick et al., 2005).

The cellular components of the innate and adaptive immune systems have immense implications for cancer patients and survivors. The cells of both immune systems have important roles in protecting the body from tumor formation as well as infection from outside elements (Marieb & Hoehn, 2010; Walsh et al., 2011). Both groups of cells are affected by cancer and anti-cancer therapies. Cancer and its treatments have been shown to reduce both the number and the functionality of lymphocytes (T-cells, B-cells, and NK cells), neutrophils, and macrophages (Fairey et al., 2005). Even after the completion of treatment, many of these components are unable to recover in a timely fashion (Fairey et al., 2005; Hutnick et al., 2005). Therefore, these individuals are more likely to develop additional infections or cancer recurrence. However, exercise has shown to play a mediating role in immune regulation and may potentially serve as a form of immunotherapy to help improve the immune systems of cancer survivors (Fairey et al., 2005; Hutnick et al., 2005).

**Acute Exercise and the Immune Response**

It is suggested that one’s level of physical activity plays a role in overall immune function and susceptibility to infection. This can be depicted by a “J-shaped curve” indicating that moderate levels of daily physical activity result in enhanced immune function, whereas a sedentary lifestyle and extremely high levels of physical activity are suggested to impair various immune parameters (Nieman, 1997). This can be further explained by the “open
window” theory, which portrays the immune response to a single bout of acute exercise (Pederson, 1999). According to this theory, a single exercise bout can result in an initial up-regulation in immune parameters followed by a subsequent down-regulation of these parameters, which can last up to 72 hours post-exercise (Pederson et al., 1999). In other words, following a single exercise bout, immune function may increase immediately following the exercise session and then may decrease below baseline, thereby increasing susceptibility to infection. The magnitude of this immunosuppression is directly proportional to the intensity and duration of the exercise performed (Pedersen et al., 1999).

The majority of the research to date is focused on the effects of endurance training on the immune system. Therefore, much less is known about the impact of resistance training (Koch, 2010; Walsh et al., 2011). As a result of endurance exercise, an immediate and significant increase in neutrophils, a subset of granulocytes, is observed. This immediate neutrophilia is due primarily to catecholamine release and the shear stress exerted by an increase in cardiac output against the blood vessel walls that occur during exercise (Walsh et al., 2011). Several hours following the acute exercise bout, a secondary increase in circulating neutrophils is typically observed. This is suggested to be mediated by cortisol, which stimulates neutrophil release from the bone marrow (Walsh et al., 2011).

A single bout of aerobic exercise has substantial influence on the levels of circulating lymphocytes as well. During and immediately following moderate or intense exercise, there is an up-regulation in lymphocyte concentrations, which can then plummet below baseline levels during recovery (Nieman et al., 1991; Pedersen & Hoffman Goetz, 2000). This initial increase in circulating lymphocytes is explained by the recruitment of all lymphocyte subpopulations (T-helper, T-cytotoxic, B-cells, and NK cells) into the circulation, possibly
from the spleen, lymph nodes, and gastrointestinal tract (Pedersen & Hoffman-Goetz, 2000). This recruitment is thought to be a response to increases in catecholamine levels as well as increases in cardiac output that typically accompany exercise sessions (Koch, 2010).

However, cortisol, which is often released during prolonged or intense exercise is associated with immunosuppression (Neves Jr. et al., 2009). It has an inhibitory effect on the proliferative response of lymphocytes, suggesting that cortisol impairs the ability of lymphocytes to respond to antigens (Nieman, 1997). The inhibitory effects of cortisol coupled with a redistribution of lymphocytes to respective body tissues are likely explanations for the decrease in circulating lymphocytes observed after acute exercise (Gleeson, 2007; Pedersen & Hoffman-Goetz, 2000). An intensity threshold of 40% to 60% of $VO_2\text{max}$ has been established as the aerobic intensity threshold for initiation of lymphocytopenia, or the reduction in circulating lymphocytes following the cessation of exercise (Koch, 2010).

Levels of circulating monocytes are also affected by acute endurance exercise (Pedersen & Hoffman-Goetz, 2000). However, increase in monocyte concentration is typically not observed until about 2 hours post-exercise (Walsh et al., 2011). This monocytosis is suggested to be mediated by either plasma volume shift, exercise-induced cortisol release, exercise-induced catecholamine release, or some combination of the three (Walsh et al., 2011). While monocytes are relatively immature cells, they are responsible for the release of a variety of cytokines which can exacerbate systemic inflammation (Smith, 2000). However, monocytes also serve as an integral component of innate immunity. They mature into macrophages, which play a major phagocytic role in ridding the body of foreign
substances (Marieb & Hoehn, 2010). Prolonged elevation in active macrophages can be indicative of chronic inflammation (Marieb & Hoehn, 2010).

As a result of moderate intensity endurance exercise, the leukocytosis observed immediately post-workout is often less pronounced than following intense endurance exercise (Koch, 2010). Further, the lymphocytopenia, or the reduction in circulating lymphocytes below baseline levels, is also of a smaller magnitude following moderate-intensity endurance exercise compared to high-intensity endurance exercise (Koch, 2010). It is suggested that the immune system responds similarly to resistance exercise as well, however, the lymphocyte response to resistance exercise has not been clearly established (Koch, 2010).

It appears that the immunosuppression following an acute bout of resistance exercise is less pronounced than that of the response following aerobic exercise (Neves Jr. et al., 2009). In the study by Neves Jr. et al. (2009), older women experienced an increase in circulating leukocytes following an acute bout of resistance training with no immunosuppression observed during the recovery period. In fact, this study observed elevated lymphocyte levels at 3 hours post-exercise. The absence of observable immunosuppression was suggested to be due to a lack of cortisol elevation during the resistance exercise protocol, as cortisol is associated with initiating many of the immunosuppressive effects observed following endurance-type exercise sessions.

Because cortisol is suggested to be such a prominent mediating factor in exercise-induced immunosuppression (particularly within the lymphocyte subpopulations) typically observed in the recovery period following exercise, resistance training can have profound implications regarding exercise program design with the intent of improving immune
function (Neves Jr. et al., 2009). In a study by Kraemer et al. (1996), subjects underwent two identical high-intensity resistance training exercise sessions, with the only difference between the two being the rest period between subsequent sets. One protocol required 3 minutes of rest in between sets, whereas the other limited recovery to 1 minute between sets. Results indicated that while cortisol was significantly elevated within the 1 minute rest protocol, it was not significantly elevated within the 3 minute rest protocol even though the absolute work performed during both protocols was the same. The authors further observed that in the immediately post-exercise blood sample, both protocols initiated a similar leukocytosis. This is important because it suggests that the increase in circulating leukocytes is caused by factors unrelated to cortisol release during exercise, and that manipulating the rest period between subsequent sets of heavy resistance training exercises can influence whether or not cortisol is significantly elevated.

It is clear that exercise has a significant influence on the levels of circulating leukocytes. These effects seem to vary with differing training variables, such as intensity, duration, and mode of exercise. Even though the research exploring the influence of resistance training on these immune parameters is somewhat limited, the manipulation of various resistance training variables may have important implications in enabling the maximization of immune function, especially for those clinical populations, like cancer patients, to whom optimal immune function is essential to enhancing quality of life and preventing cancer recurrence.

**Exercise and Immune Function: Implications for Breast Cancer Survivors**

The majority of the research regarding immune function in cancer survivors has focused on the effects of training programs on immune parameters (Fairey et al., 2002).
These programs included either aerobic only training, or a combined aerobic and resistance training regimen, with little emphasis placed on resistance-training only interventions (Fairey et al., 2002). Overall, training studies involving cancer survivors indicate that exercise training can have a positive effect on immune function in these individuals (Fariey et al., 2002).

In addition, there are limited established exercise program design parameters for cancer populations (Schmitz et al., 2010). The American College of Sports Medicine has published a consensus regarding exercise guidelines for cancer survivors. However, large gaps remain in the prescription of certain training variables, such as frequency, duration, and intensity, particularly when pertaining to resistance training (Schmitz et al., 2010). Hence, the training intervention designs between studies are rarely homogenous, and consequently difficult to compare. Therefore, criteria for exercise prescription to improve immune function in cancer survivors must be derived from the limited data available, and often from studies involving alternate populations.

A study conducted by Fairey et al. (2005) examined the effects of aerobic exercise performed 3 times per week on immune function in breast cancer survivors. Results showed that at the end of the 15 week intervention, NK cell function was significantly greater than non-exercise control group. The results are important because optimal NK cell function is associated with disease free, overall survival rates, while poor NK function is indicative of an increased risk of cancer recurrence (Fairey et al., 2005). Even though this was an aerobic intervention and a training study, it is important because it demonstrates the role exercise can play in improving immune function in this population.
Taking this one step further, Hutnick et al. (2005) examined the effects of a mixed aerobic and resistance training intervention on lymphocyte function in breast cancer survivors. Before the initiation of the training program, all of the participants had mean lymphocyte subset values below the interquartile range for healthy individuals. After the 3 to 6 month training period, results indicated that circulating levels of T-helper cells had increased proliferative capacity following the training period in the exercise group, but not in the non-exercising control group. The authors suggest that exercise training may emerge as a sort of immunotherapy for breast cancer survivors, aiding them in the prevention of secondary infections as well as cancer recurrence.

Ramel et al. (2003) examined the immune response to an acute bout of resistance exercise in healthy men. Subjects performed a resistance exercise circuit consisting of 10 exercises focusing on the major muscle groups at 75% of 1 RM with 1 minute of rest in between each exercise and completed the 10-exercise circuit 2-3 times each. Results indicated that total leukocytes and leukocyte subset (NK cells, neutrophils, total lymphocytes, and monocytes) values were increased during and immediately following the exercise bout. While neutrophils and monocytes remained significantly elevated throughout the 120 minute recovery period, lymphocyte values dropped below baseline after the cessation of exercise, returning to above baseline values at 120 minutes into recovery. The changes in lymphocyte concentrations were negatively associated with cortisol levels, indicating that cortisol played a mediating role in this process of altered immune function. However, the authors stressed that the hormonal response of noradrenaline and cortisol to resistance exercise is moderate when compared with endurance exercise.
These conclusions suggest that resistance exercise may play less of an inhibitory role in immune function in healthy males, particularly that of lymphocytes whose fluctuations appear to be directly related to the magnitude of cortisol release. As lymphocyte levels and function appear to be sub-optimal in breast cancer survivors following cancer treatments, the implementation of resistance exercise may be beneficial in improving lymphocyte function in these individuals.

**Summary and Significance**

The current research in exercise science points to positive effects on the immune system as just one of the many benefits to be obtained from exercise. Knowledge regarding the influence of exercise on the immune system is integral to creating optimal program design for specific populations. For example, athletes, who typically employ the use of prolonged, high intensity training bouts must allow adequate time for their immune systems to recover before implementing a second intense training bout. Failure to allot adequate time for the recovery of the immune system may result in further immunosuppression with additional training and may eventually culminate in the overtraining syndrome, in which the athlete’s performance will regress to sub-optimal levels despite additional athletic training (Gleeson, 2002). Likewise, it is necessary for exercise professionals to be aware of the timeline in which breast cancer survivors’ immune systems recover from acute bouts of exercise when coming up with an exercise prescription for this population. This knowledge would allow for the establishment of guidelines regarding recovery periods between consecutive exercise sessions that would not inhibit, and would perhaps enhance the immune functions of these individuals over time. It would provide vital information concerning
precautions that should be taken against training these individuals to the point where their immune function may be further depressed by subsequent training bouts.

Breast cancer survivors, who are already immunosuppressed following anti-cancer treatments, comprise a population that can benefit immensely from regular participation in resistance exercise training. In addition to the variety of benefits initiated by resistance training on physiological variables including increased functional capacity, increased muscle mass, improved body composition, increased quality of life, reductions in muscle wasting and sarcopenia, and decreased risk of osteoporosis, resistance training is also showing promise as a means of potentially enhancing various aspects of the immune system. As such, implementing regular resistance exercise in the breast cancer survivor population may reduce the risk of secondary infection, reduce the risk of cancer recurrence, and ultimately improve length and quality of life.
Chapter III

Methodology

Subjects

Participants of this study consisted of 4 female early stage breast cancer (stage I-III) survivors and 8 healthy controls. The breast cancer survivor inclusion criteria consisted of: being a female between 40 and 72 years old, being within 4-12 months post breast cancer treatment (having undergone chemotherapy treatment at minimum), not taking any anti-inflammatory medications, and having clearance from their oncologists to participate in the present study. Patients receiving adjuvant hormonal therapy or adjuvant trastuzumab were also eligible to participate in the study.

Inclusion criteria for the control group consisted of: apparently healthy, 40 to 70 year old females, no history of cancer diagnosis or treatment, no limitations in range of motion that would impede upon the ability to perform the prescribed resistance training exercises safely, not taking any anti-inflammatory medications, and clearance from their primary physician to participate in the study for subjects older than 65 years of age. All control subjects were matched by age (within 5 years) with the breast cancer group. Also, all attempts were made to match control subjects with the breast cancer group based on physical activity level.

Exclusion criteria for all subjects was determined by a screening based on the ACSM guidelines set forth to determine contraindications to exercise participation (Whaley,
Brubaker, & Otto, 2006). Information regarding the exclusion criteria was obtained from each subject based on their responses to their medical history and from a medical and physical screening.

**Recruitment**

Participants for the breast cancer survivor group were recruited from UNC Hospital’s Breast Cancer Care Clinic and from the Get REAL & HEEL Breast Cancer Program at the University of North Carolina, Chapel Hill. Participants for the control group were recruited by the posting of flyers at various locations throughout the campus of the University of North Carolina, Chapel Hill, via informational emails sent out to the campus community at the University of North Carolina, Chapel Hill, as well as through word of mouth.

**Instrumentation**

*Anthropometric Measures:* Resting cardiac function was assessed using a GE CASE Cardiosoft V. 6.6 ECG diagnostic system (Palatine, IL). Height was taken using a Perspective Enterprises Stadiometer (Portage, MI). Weight was obtained via a Detecto Scale (Webb City, MO). A Discovery Dual Energy X-Ray Absorption (DEXA) scanner (Bedford, MA) was used to assess body composition.

*Exercise Testing:* Blood pressure was assessed before and after the exercise protocol using a sphygmomanometer (American Diagnostics Corporation, Hauppauge, NY) and a Littmann® Stethoscope (St. Paul, MN). Heart rate before and throughout the exercise protocol was assessed using a Polar telemetry system (Lake Success, NY). The warm-up period was performed on a Lode electronically-braked cycle ergometer (Gronigen, The Netherlands). The exercise components were performed on a seated leg press (Cybex,
Blood Analyses: Blood was taken at 4 time intervals throughout the study. The first three were done using a 22 gage Protectiv Plus catheter (Ethicon Endo-Surgery Inc., Cincinnati, OH). The fourth was done using standard venipuncture techniques. Blood was collected in 10.8 mg K$_3$EDTA BD Vacutainers ® (Franklin Lakes, NJ). Total leukocyte, granulocyte, lymphocyte, and monocyte counts were determined using a Coulter AC T diff Hematology Analyzer (Block Scientific, Inc., Bohemia, NY). Blood cortisol values were determined using a cortisol ELISA Kit, KA 0981, Abnova Version 2, (Tapei City, Taiwan).

General Procedures

The general timeline for the experimental protocol is depicted in Figure 1.

Familiarization Session: Medical history forms and pre-assessment guidelines were administered to subjects via email correspondence prior to their first visit to the lab for experimental testing. Once they arrived, these forms were reviewed to confirm that all subjects met the inclusion criteria for the present study. Subjects then signed an informed consent form detailing the experimental protocol and potential risks and benefits associated with participation in the study. Next, subjects underwent a medical and physical screening to determine if there were any contraindications to exercise by a member of the research team who is certified/authorized by the UNC school of medicine to perform physicals for exercise testing. After receiving clearance to participate in the study, adherence to pre-assessment guidelines was reviewed. Pre-assessment guidelines consisted of: no eating for at least 2 hours prior to the lab visit; no exercise for at least 12 hours prior to the lab visit; drink water
throughout the day to stay hydrated; No caffeine use for at least 12 hours prior to the lab visit; No alcohol use for at least 48 hours prior to the lab visit.

Height and weight were then taken, and subjects underwent a body composition assessment using the DEXA. Following this, they were fitted with a heart rate monitor and rested in the seated position for 10 minutes, at which time resting heart rate and blood pressure were recorded. Subjects then underwent a 5-10 minute warm-up consisting of unloaded cycling on a cycle ergometer, while keeping their heart rates around 40 - 50% of heart rate reserve (HRR), as calculated by the Karvonen formula.

After the warm-up, subjects were familiarized with the resistance training equipment that was used in the experimental protocol by undergoing approximately 10 repetitions of each exercise using minimal resistance. Following initial exposure to each of the resistance exercise machines in the same order in which they were used during the experimental protocol, each subject’s 1 RM values were obtained on the leg press, lateral pull down (LPD), leg extension, and seated row exercises using the protocol as described by Levinger et al. (2009). The order of exercises during the experimental protocol was as follows: leg press, lateral pull down, leg extension, and seated row. Therefore, 1 RM values were taken in that order as well to account for any neuromuscular fatigue obtained from the preceding exercise. Heart rate was monitored continuously throughout the exercise session.

Upon the completion of the familiarization trial, subjects were asked to rest for 10 minutes, after which heart rate and blood pressure were taken again. If these measures were abnormally elevated, subjects were asked to remain seated until the values decreased. If within normal values, subjects were released and asked to return to the lab for their second lab visit appointment.
Experimental Session: The second lab visit took place between 1.5 and 14 days following the familiarization session. Upon arrival, subjects put on a heart rate monitor and rested in the seated position, during which time adherence to the pre-assessment guidelines was reviewed. A Psychological Stress Scale (PSS) was also administered to determine if the subject was experiencing psychological stress, which could affect the release of the stress hormones which were measured during the study. A resting heart rate and blood pressure were taken. Again, if either of these values were not within normal range for the individual, the subject was asked to return to the lab on a different day.

The subjects then underwent catheter placement into an antecubital vein in the arm for blood sampling by a faculty member in the Department of Exercise and Sport Science who has been trained and certified in phlebotomy techniques. After this, subjects rested in the seated position for approximately 10 minutes, and then the first pre-exercise blood sample was collected. A 5 minute warm-up consisting of unloaded cycling on a cycle ergometer followed, again keeping subjects’ heart rates around 40 - 50% of HRR. Subjects then performed several repetitions of each of the four resistance exercises with minimal weight to ensure that their muscles were adequately prepared for the resistance protocol. After the warm up, the resistance training protocol began. Subjects performed 10 repetitions at 70% of 1-RM of each exercise (leg press, lateral pull down, leg extension, and seated row, respectively) with 30 to 45 seconds of rest between subsequent exercises, and 1 minute and 30 seconds to 2 minutes of rest between subsequent circuits. This circuit was completed three times. Heart rate and RPE were taken following each set to ensure subject safety and monitor their conditions. Subjects were encouraged to consume water at any point during and following the experimental protocol.
Directly upon completion of the third circuit of exercises, subjects were seated and a blood sample was taken immediately post-exercise. Subjects were then sequestered for the next 2 hours, during which time they were encouraged to consume plenty of water and to relax. They were allowed to perform a brief series of light stretches. Following this 2 hour period, the 2 hour post-exercise blood sample was taken, after which the subjects were encouraged to eat a snack of their choice. The catheter was removed, any necessary bandaging was performed, and the subjects was reminded not to perform any kind of strenuous activity over the next 24 hours, and to adhere, once again, to the pre-assessment guidelines before their third and final lab visit.

24 Hour Blood Draw: Twenty-four hours following the completion of the resistance exercise session, subjects returned to the lab for their 24 hour post-exercise blood draw. There was a 1-hour window at which this blood sample will be taken. In other words, subjects may come in for their third visit 30 minutes prior to the time of exercise completion session on the previous day, or 30 minutes after the time of completion of the exercise session on the previous day. Adherence to the pre-assessment guidelines was reviewed, and the blood sample was taken by a certified phlebotomist using a 22 gage needle. After obtaining the blood sample, subjects have completed the experimental procedure. The blood-draw timeline is depicted in Figure 2.

All data was adjusted to account for plasma volume shift (the shift of fluid out of the vascular system that occurs as a result of exercise) prior to statistical analysis using the Dill and Costill method (Dill & Costill, 1974). Plasma volume shifts are reported in order to account for the influence of exercise on fluid shift, which may in turn influence the concentrations of leukocytes, leukocyte subsets, and cortisol.
Figure 1: Protocol Timeline

Lab Visit 1:
• Informed Consent
• Body Composition
• Familiarization
• 1-RM Testing

1.5 – 14 Days

Lab Visit 2:
• Resistance Training Protocol

24 Hours

Lab Visit 3:
• 24-hour post-exercise blood draw

Figure 2: Blood Draw Timeline

Exercise 2 hours 24 hours

Pre-Exercise Blood Sample

Immediately Post-Exercise Blood Sample

2 Hrs Post-Exercise Blood Sample

24 Hrs Post-Exercise Blood Sample
**Statistical Analysis:**

Descriptive statistics are presented in the form of means and standard deviations. The independent variables for this analysis include subject condition (breast cancer survivor and healthy control) and the acute bout of resistance training. The dependent variables are the total leukocyte, granulocyte, lymphocyte, and monocyte counts at the various time intervals (immediately pre-exercise, immediately post-exercise, 2 hours post-exercise, and 24 hours post-exercise). All data was analyzed using SPSS version 20.0 (Chicago, IL). Statistical significance was set \textit{a priori} at an alpha-level of $p \leq 0.05$.

Hypotheses 1, 2, and 3 were analyzed using 2 x 3 mixed model ANOVAs. The between subjects factor is subject condition (breast cancer survivor or healthy control). The within subjects factor is the percent change of cell counts ($\%$ change = immediately post-exercise count – pre-exercise count/ pre-exercise count x 100; $\%$ change = 2 hours post-exercise count – pre-exercise count / pre-exercise count x 100; and $\%$ change = 24 hours post-exercise count – pre-exercise count / pre-exercise count x 100, respectively) for each dependent variable (leukocytes, granulocytes, lymphocytes, and monocytes cell count).

Exploratory analyses were conducted to evaluate the cortisol response in both the breast cancer survivor group and the control group to the acute bout of moderate-intensity resistance training by using a Mann Whitney U analysis to test for between group (breast cancer survivor compared to healthy control) differences in cortisol response, and a Friedman test for each of the conditions (breast cancer survivor or healthy control) to examine within group differences at baseline, immediately post-exercise, 2 hours post exercise and 24 hours post exercise.
Chapter IV

Results

The primary purpose of this study was to compare the effects of an acute bout of resistance training on percent cell count changes in leukocyte and leukocyte subset values in breast cancer survivors and healthy controls. A secondary purpose was to conduct exploratory analyses on cortisol to examine the cortisol response to an acute bout of moderate intensity resistance training in both the breast cancer survivors and control groups, since cortisol has been shown to be a potential mediator of some cellular immune responses. Participants consisted of 4 breast cancer survivors and 8 healthy controls. Descriptive characteristics are presented in Table 1 below.

Table 1: Subject Characteristics and 1-RM Strength Assessment Results. No significant differences exist between the BCS and Control groups.

<table>
<thead>
<tr>
<th></th>
<th>BCS (n=4) (mean ± SD)</th>
<th>Controls (n=8) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong> (yrs)</td>
<td>60 ± 12</td>
<td>56 ± 7</td>
</tr>
<tr>
<td><strong>Height</strong> (cm)</td>
<td>168.6 ± 3.1</td>
<td>163.7 ± 6.8</td>
</tr>
<tr>
<td><strong>Weight</strong> (kg)</td>
<td>75.6 ± 13.4</td>
<td>67.2 ± 6.3</td>
</tr>
<tr>
<td><strong>Body Fat</strong> (%)</td>
<td>37.6 ± 10.3</td>
<td>37.6 ± 4.5</td>
</tr>
<tr>
<td><strong>1-RM Leg Press</strong> (kg)</td>
<td>81.6 ± 20.0</td>
<td>79.5 ± 14.5</td>
</tr>
<tr>
<td><strong>1-RM LPD</strong> (kg)</td>
<td>33.2 ± 5.5</td>
<td>35.8 ± 6.8</td>
</tr>
<tr>
<td><strong>1-RM Leg Ext.</strong> (kg)</td>
<td>58.4 ± 15.1</td>
<td>60.3 ± 12.5</td>
</tr>
<tr>
<td><strong>1-RM Seated Row</strong> (kg)</td>
<td>34.1 ± 5.3</td>
<td>38.8 ± 3.9</td>
</tr>
</tbody>
</table>
Means and standard deviations for total leukocyte, granulocyte, lymphocyte, and monocytes cell counts at each of the four measurement points (baseline, 0h post-exercise, 2h post-exercise, and 24h post-exercise) are presented below in Table 3. Immune parameter data at 0h post and 24h post-exercise for one control, and at 2h post-exercise for one BCS and one control subject were not obtained during the study due to technical measurement errors (i.e. blood clotting, catheter malfunction, inability to perform the venipuncture procedure, or inability of a subject to return to the lab for the 24h post-exercise blood draw). Mean substitution procedures for the missing measurement data were performed for all variables of these subjects. Exploratory analyses on the effects of the acute bout of resistance exercise on cortisol were conducted and are also presented in Table 3. Similarly, mean substitution was performed for 2 control subjects at all four time points, and one breast cancer survivor at the 24h post time point. All reported cell and cortisol values have been adjusted for plasma volume shift (PV shift), which is depicted in Table 4.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Subjects (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery, Chemotherapy, Hormonal Therapy</td>
<td>2</td>
</tr>
<tr>
<td>Surgery, Chemotherapy, Radiation</td>
<td>1</td>
</tr>
<tr>
<td>Surgery, Chemotherapy, Radiation, Hormonal Therapy</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 3: Leukocyte, Leukocyte Subset, and Cortisol Values Adjusted for Plasma Volume Shift.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>BCS (n=4)</th>
<th>Controls (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
</tr>
<tr>
<td>Total Leukocytes</td>
<td>Baseline</td>
<td>5575.0 ± 1358.0</td>
<td>5166.7 ± 795.3</td>
</tr>
<tr>
<td>(cells/uL)</td>
<td>0h Post</td>
<td>6498.3 ± 1460.6*</td>
<td>5785.1 ± 934.6</td>
</tr>
<tr>
<td></td>
<td>2h Post</td>
<td>6753.1 ± 1619.5*</td>
<td>5760.4 ± 686.8</td>
</tr>
<tr>
<td></td>
<td>24h Post</td>
<td>5700.5 ± 1003.7</td>
<td>5426.1 ± 785.6</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>Baseline</td>
<td>3704.2 ± 1054.4</td>
<td>3410.4 ± 476.7</td>
</tr>
<tr>
<td>(cells/uL)</td>
<td>0h Post</td>
<td>4255.3 ± 1040.3*</td>
<td>3819.4 ± 734.2*</td>
</tr>
<tr>
<td></td>
<td>2h Post</td>
<td>4640.3 ± 1193.1*</td>
<td>3691.2 ± 652.6</td>
</tr>
<tr>
<td></td>
<td>24h Post</td>
<td>3833.6 ± 607.3</td>
<td>3609.3 ± 624.2</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Baseline</td>
<td>1479.2 ± 151.2</td>
<td>1410.4 ± 434.7</td>
</tr>
<tr>
<td>(cells/uL)</td>
<td>0h Post</td>
<td>1845.5 ± 356.9</td>
<td>1640.8 ± 371.3</td>
</tr>
<tr>
<td></td>
<td>2h Post</td>
<td>1788.0 ± 433.7</td>
<td>1745.8 ± 501.8</td>
</tr>
<tr>
<td></td>
<td>24h Post</td>
<td>1310.1 ± 243.5</td>
<td>1418.6 ± 273.4</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Baseline</td>
<td>395.8 ± 141.7</td>
<td>354.2 ± 152.4</td>
</tr>
<tr>
<td>(cells/uL)</td>
<td>0h Post</td>
<td>398.8 ± 204.9</td>
<td>328.2 ± 146.9</td>
</tr>
<tr>
<td></td>
<td>2h Post</td>
<td>325.0 ± 23.2</td>
<td>318.1 ± 118.0</td>
</tr>
<tr>
<td></td>
<td>24h Post</td>
<td>589.7 ± 168.2*†</td>
<td>400.0 ± 108.2</td>
</tr>
<tr>
<td>Cortisol**</td>
<td>Baseline</td>
<td>94.36 ± 18.05</td>
<td>139.65 ± 67.42</td>
</tr>
<tr>
<td>(ng/mL)</td>
<td>0h Post</td>
<td>65.18 ± 8.49</td>
<td>87.95 ± 36.17</td>
</tr>
<tr>
<td></td>
<td>2h Post</td>
<td>47.58 ± 11.35*</td>
<td>126.45 ± 108.43</td>
</tr>
<tr>
<td></td>
<td>24h Post</td>
<td>55.31 ± 14.60</td>
<td>127.16 ± 82.05</td>
</tr>
</tbody>
</table>

Paired-samples and independent samples T-tests were performed for the evaluation of comparisons between post-exercise values and baseline values within as well as between the BCS and control groups at each assessment time. *Denotes statistical significance (p ≤ 0.05) when compared to baseline. †Denotes statistical significance (p ≤ 0.05) between BCS and control groups. •Indicates normal cell ranges under resting conditions (Marieb & Hoehn, 2010). **For the cortisol analyses, only data for 6 control and 4 BCS subjects is presented.

Non-parametric analyses were conducted on the cortisol data, since the results were unequally distributed. A Mann-Whitney U analysis was conducted to test for between group differences, and no statistically significant differences were found (p=0.3938; p=0.6698; p=0.0550; p=0.5224 for baseline, 0h post-exercise, 2h post-exercise, and 24h post-exercise, respectively). A Friedman test was used to evaluate within-group differences, and a significant difference in cortisol response was found within the breast cancer survivor group between baseline and 2h post-exercise (p = 0.0129).
Table 4: Mean Hematocrit (Hct) and Hemoglobin (Hb) values with corresponding plasma volume changes (Δ % changes) from baseline to 0h post-exercise; baseline to 2h post-exercise; and baseline to 24h post-exercise.

<table>
<thead>
<tr>
<th></th>
<th>HCT (%)</th>
<th>Hb (g/dL)</th>
<th>%Δ Baseline to 0h Post</th>
<th>%Δ Baseline to 2hr Post</th>
<th>%Δ Baseline to 24hr Post</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>0h Post</td>
<td>2hr Post</td>
<td>24hr Post</td>
<td>Baseline</td>
</tr>
<tr>
<td>BCS</td>
<td>38.08</td>
<td>39.33</td>
<td>38.00</td>
<td>38.44</td>
<td>11.05</td>
</tr>
<tr>
<td>Controls</td>
<td>37.92</td>
<td>40.86</td>
<td>38.31</td>
<td>38.83</td>
<td>10.70</td>
</tr>
</tbody>
</table>

A mixed-model ANOVA for each of the dependent variables (total leukocytes, granulocytes, lymphocytes, and monocytes) using the percent change (% Δ) scores from baseline – 0h post-exercise, baseline – 2h post-exercise, and baseline – 24h post-exercise was conducted to examine the differences between changes in the breast cancer survivor group and changes in the control group. For subjects missing data points, a mean substitution for the % Δ scores of the group was used. Percent Δ scores were calculated as follows: (% Δ = 0h post-exercise count – baseline count/ baseline count x 100; % Δ = 2h post-exercise count – baseline count / baseline count x 100; and % Δ = 24h post-exercise count – baseline count / baseline count x 100, respectively). Results are presented in Table 5 below:
Table 5: Percent change (\(\% \Delta\)) scores for total leukocytes, granulocytes, lymphocytes, and monocytes depicting the \(\% \Delta\) from baseline-0h post-exercise, baseline-2h post-exercise, and baseline-24h post-exercise.

<table>
<thead>
<tr>
<th></th>
<th>(% \Delta) Baseline-0h Post (mean ± SD)</th>
<th>(% \Delta) Baseline-2h Post (mean ± SD)</th>
<th>(% \Delta) Baseline-24h Post (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monocytes</strong></td>
<td>BCS: -3.77 ± 21.41 Controls: 44.35 ± 159.66</td>
<td>BCS: -9.07 ± 33.02 Controls: 7.29 ± 47.00</td>
<td>BCS: 66.28 ± 18.55 Controls: 61.83 ± 125.19</td>
</tr>
</tbody>
</table>

No significant differences between the breast cancer survivor group and the control group were found between the \(\% \Delta\) scores for any of the dependent variables (total leukocytes: \(p = 0.560\); granulocytes: \(p = 0.239\); lymphocytes: \(p = 0.257\); monocytes: \(p = 0.721\)), indicating that the breast cancer survivor and control groups experienced similar percent cell count changes from baseline at 0h, 2h, and 24h following the exercise bout.
Chapter V

Discussion

The examination of the effect of an acute bout of resistance training on the immune system’s response in breast cancer survivors can provide researchers with important information for exercise prescription in a breast cancer population. Since resistance exercise has been shown to be a feasible and efficacious method for improving a plethora of physiological and psychological parameters in individuals with breast cancer (Cheema et al., 2008; Courneya et al., 2007; Schmitz et al., 2005; Segal et al., 2009), it is of great importance for researchers to determine an exercise protocol designed to optimize these results. Further, it is essential that exercise programs for individuals with cancer do not impair the immune system because sub-optimal immune function can increase the risk of secondary malignancy and illness susceptibility (Hutnick et al., 2005). Although the body of research is small, current research in exercise oncology suggests that exercise may serve as a means of enhancing immune function in cancer patients, which can improve the chances of disease-free survivorship (Fairey et al., 2002; Fairey et al., 2005; Hutnick et al., 2005).

Knowing how the immune systems of breast cancer patients respond to a single bout of exercise is imperative to program design for this population. It is essential that secondary training sessions are not implemented until the immune system has fully recovered from the primary bout. Consistent failure to allot adequate recovery time between subsequent training
sessions in exercising individuals is suggested to result in chronic immunosuppression and impairment of overall health (Gleeson, 2001; Koch, 2010). Because breast cancer survivors often already have impaired immune function as a result of cancer treatments, further immunosuppression must be avoided. Since the acute immune response differs between the mode, intensity, and duration of exercise bouts (Koch, 2010), the aim of this study was to determine how the immune system in breast cancer survivors responded to a single bout of moderate-intensity resistance training.

To the authors’ knowledge, this is the first study to look at the immune response to a single bout of resistance training in a breast cancer population. The only other study to examine the effects of an acute bout of resistance exercise in cancer patients was a training study by Galvao and colleagues in 2008, where the immune response in prostate cancer patients was measured during and following a 20 week resistance training program. It is important to note that the acute response was not the main outcome variable of this study, as the authors’ primary objective was to evaluate the effect of chronic resistance training on various endocrine and immune parameters. The results of this study indicated that immediately following an acute bout of resistance training, total leukocyte, neutrophil, lymphocyte, and monocyte cell counts in the prostate cancer patients responded similarly to those of healthy individuals. However, blood samples were not taken into the recovery period following the exercise bout. Therefore, the present study is the first to examine the timeline of immune response and recovery to an acute resistance training session in cancer patients.

It should also be noted that the vast majority of the literature reporting the immune response to acute exercise is describing the effects of a bout of endurance training, as opposed to resistance training. The differences in the immune response to the various
exercise modalities even in healthy individuals have not been clearly established (Koch, 2010).

**Immune Response to Resistance Training in Breast Cancer Survivors**

The following discussion will be broken down into 6 sections. The first section will focus on the response from baseline to 0h post-exercise, the second will discuss the response from baseline to 2h post-exercise, the third will address the response from baseline to 24h post-exercise, the fourth will outline the limitations of the present study, the fifth will present concluding statements, and the sixth will provide recommendations for future research.

**Baseline – 0h Post-Exercise**

The immune response from baseline to 0h post-exercise in the BCS group is relatively consistent with the current literature in exercise immunology (Gleeson, 2007; Pedersen & Hoffman-Goetz, 2000; Walsh et al., 2011). Results indicated a significant increase in total leukocytes and granulocytes immediately following the exercise bout. Although the subset of granulocytes is comprised of neutrophils, basophils, and eosinophils, the majority of circulating granulocytes are made up of neutrophils, which are highly responsive to acute exercise (Walsh et al., 2011). Further, neutrophils and lymphocytes comprise approximately 60% and 25%, respectively, of all circulating leukocytes, so transient changes in these cells will likely be reflected by the overall change in total leukocyte count as well (Gleeson, 2007; Marieb & Hoehn, 2010; Pedersen & Hoffman-Goetz, 2000; Walsh et al., 2011).

The neutrophilia observed at 0h post-exercise is likely due to increases in cardiac output and sympathetic activity that occurs as a result of exercise. It is widely hypothesized that the increases in shear stress that occur as a result of increased cardiac output, coupled
with the increased catecholamine secretion that happens upon engaging in physical activity cause a demarginalization of neutrophils into the circulation, thereby increasing the numbers of circulating neutrophils (Koch 2010; Pedersen & Hoffman-Goetz, 2000, Walsh et al., 2011). It is known that neutrophils contain a $\beta$-adrenergic receptor, which provides the basis for support that catecholamines play a partial role in mediating the neutrophil response to acute exercise (Pedersen & Hoffman-Goetz, 2000). Although the magnitude of catecholamine release was not measured in this study, it is widely accepted that participation in physical activity is accompanied by sympathetic nervous system activation and the secretion of epinephrine and norepinephrine into the circulation, which is likely a contributing factor to the observed neutrophilia.

The current research available on the immune response to resistance training is in accordance with the present findings, indicating that an acute bout of resistance training results in an increase in total leukocyte (Kraemer et al., 2006; Neves Jr. et al., 2009) and neutrophil counts (Galvao et al., 2008; Koch, 2010; Ramel et al., 2003) immediately following completion of the exercise bout. Although exercise has been shown to increase neutrophil mobilization into the circulation, research has suggested that these cells show a reduction in their responsiveness to bacterial lipopolysaccharide, which can last for several hours and is indicative of impaired functionality (Gleeson 2007; Nieman 1997). Neutrophil function was not measured in this study, therefore the capacity of these cells to respond to external stimuli following an acute bout of resistance training cannot be commented upon.

The influence of exercise on lymphocytes in breast cancer patients is of particular interest to a variety of researchers, as it has been shown that chemotherapy and radiation treatments negatively influence optimal lymphocyte functioning in virtually of the
lymphocyte subsets and that optimal lymphocyte function, particularly that of NK cells, is related to overall survivorship (Hutnick et al., 2005; Fairey et al., 2002). No significant differences were observed in lymphocyte counts from baseline to 0h post-exercise in either the BCS or the control group. This is likely due to the nature of the exercise bout performed (moderate-intensity, relatively short duration; Nieman, 2003). Although lymphocyte counts did not increase significantly in either group, there were modest increases in the number of circulating lymphocytes immediately following the exercise bout in both BCS and control groups, which likely served as a contributing factor to the increase in total leukocytes observed immediately post-exercise. According to the literature, a post-exercise lymphocytosis is observed due to the mobilization of these cells from a variety of organs, including the spleen, lymph nodes, and gastrointestinal tract (Pedersen & Hoffman-Goetz, 2000). Post-exercise lymphocytosis is depicted to occur in proportion to exercise intensity and duration, with intensity serving as the more prominent mediator of the lymphocyte response (Walsh et al., 2011).

Like neutrophils, T, B, and NK lymphocytes contain β-adrenergic receptors, suggesting that these cells are responsive to catecholamine secretion, which is released as a result of exercise onset (Pedersen & Hoffman-Goetz, 2000; Walsh et al., 2011). This is demonstrated by the fact that NK cells contain the greatest concentration of β-adrenergic receptors, followed by CD8+ T-cells and B-cells, and finally CD4+ T-cells. It is known that NK cells are the most responsive lymphocyte subpopulation to an acute bout of exercise, followed by CD8+ cells and B-cells, and that CD4+ cells are the least responsive, which suggests the powerful role of catecholamines in influencing the lymphocyte response to exercise (Pedersen & Hoffman-Goetz, 2000; Walsh et al., 2011). It is speculated that
catecholamines initiate a down-regulation in adhesion molecule expression on endothelial cell walls (Walsh et al., 2011). As neither catecholamines or the specific lymphocyte subsets were measured as part of the present study, the magnitude of catecholamine secretion and its potential effect on the lymphocyte response cannot be commented upon.

Although derived primarily from studies examining the immune response to aerobic exercise, the upregulation of lymphocyte counts is supported in the study by Simonson and Jackson in 2004. This study demonstrated that the transient increase in the concentration of circulating lymphocytes immediately following an acute bout of resistance training was due primarily to increases in NK cell count, with less pronounced upregulation of CD8+ and CD4+ subsets. Conversely, Kraemer et al. (1996) and Neves Jr et al. (2009) did not report a significant increase in the numbers of circulating lymphocytes immediately following a single bout of resistance training in either healthy men or older women, respectively. This disparity in the lymphocyte response to resistance training may be related to the rest intervals allotted between subsequent exercises, but more research is needed to explore this possibility.

Therefore, the lymphocyte response to resistance training specifically remains controversial.

No significant difference in monocyte concentration was noted from baseline to 0h post-exercise in either the BCS or control groups. This appears to contrast with the current literature, which reports increases in monocyte counts in response to a single bout of resistance training (Galvao et al., 2008; Simonson & Jackson, 2004). The mechanism for post-exercise monocyteosis is suggested to be in line with that of neutrophils and lymphocytes, with increases in cardiac output, hemodynamic alterations, and catecholamines playing a mediating role. It is speculated that these factors result in a demargination of
monocytes into the circulation partially due to manipulation of the monocyte-endothelial interaction (Woods et al., 2000; Walsh et al., 2011).

Further, it is known that exercise affects the phenotype, cytokine expression, and cell surface proteins of circulating monocytes. Monocytes are responsible for secreting a variety of cytokines into the circulation. Also, monocytes can be classified as classical or as pro-inflammatory, which differ in their physiological function (Gleeson et al., 2011; Simpson et al., 2009). It has been shown that aerobic exercise results in an increase of the pro-inflammatory type of monocytes into the circulation (Gleeson et al., 2011; Simpson et al., 2009). However, the clinical significance of exercise-induced changes in circulating monocyte counts has yet to be established, as monocytes are immature cells that ultimately become tissue macrophages (Walsh et al., 2011). It has not yet been determined whether changes in monocyte characteristics reflect changes within bodily tissues, nor whether they respond similarly to resistance training (Koch, 2010; Walsh et al., 2011).

Existing literature on the monocyte response to an acute bout of resistance training is relatively scarce. In the study by Simonson and Jackson (2004), there was a significant increase in monocytes immediately post-exercise. Further, Galvao and colleagues in 2008 saw a non-significant increase in monocytes following an acute bout of resistance training in prostate cancer patients at the halfway point of their 20-week exercise intervention, but a significant increase in circulating monocyte count following a single bout of resistance training at week 20. The properties of the monocytes in circulation before and following exercise in these, as well as the present, studies were not determined; therefore the importance of these exercise-induced changes in overall monocyte counts cannot be inferred.
It is known that cancer and its treatments interfere with the function of the hypothalamic-pituitary-adrenal axis (HPA axis; Bower, 2007). Therefore, many breast cancer survivors experience a disruption in the diurnal variations of cortisol even several years after completing treatment. Specifically, breast cancer survivors exhibit blunted diurnal variations in cortisol when compared to healthy counterparts, as their daily cortisol fluctuations are much less pronounced even at rest (Bower, 2007). As both BCS and controls experienced a modest, non-significant reduction in cortisol at 0h post-exercise, it is therefore assumed that the exercise session was not of a great enough intensity or duration to stimulate cortisol secretion.

**Baseline – 2h Post-Exercise**

Two hours into the recovery period following the acute bout of resistance training, total leukocytes and granulocytes were significantly elevated from baseline in the BCS group only, and not the control group. At the 2h post-exercise time point, the BCS group continued to undergo an upregulation in total leukocytes and granulocytes, whereas the control group began to plateau, or drop closer to baseline. The continued increase in granulocytes 2h into recovery, which is likely reflected by increases in neutrophils, is somewhat surprising as delayed secondary neutrophilia following an acute bout of exercise is typically attributed to an exercise-induced rise in cortisol (Walsh et al., 2011). However, cortisol actually decreased as a result of the exercise bout and continued to decrease 2h into recovery, reaching a significant reduction from baseline in the BCS group at the 2h post-exercise time point. This suggests that the delayed neutrophilia observed in the BCS group may be either a continued response to the exercise / catecholamine induced primary neutrophilia or that it may be attributed to an alternate mechanism, perhaps specific to a breast cancer survivor population.
There were no significant changes from baseline and 2h post-exercise lymphocyte counts as a result of the single bout of resistance training in either the BCS or the control groups. However, the depiction of the lymphocyte response indicates a trend towards 2h post-exercise lymphocytosis in the control group, but a blunted response, or a slight reduction in lymphocytosis in the BCS group. The literature, the majority of which focuses on the effects of endurance training on the acute immune response, consistently reports a reduction in circulating lymphocytes below baseline when exercising individuals enter the recovery period, which can last up to several hours post-exercise (Gleeson, 2007; Nieman et al., 1991; Pedersen & Hoffman-Goetz, 2000; Walsh et al., 2011). Interestingly, in the study by Nieman and colleagues (1991), where healthy female subjects walked on the treadmill at the moderate intensity of 60% of VO$_{2\text{max}}$, lymphocytes increased significantly immediately post-exercise, dropped slightly below baseline values (though non-significantly) at 1.5h post-exercise, and then returned to slightly elevated levels at both 3h and 5h into the recovery period. While this differs from the present study in exercise modality (aerobic vs. resistance), the moderate intensity of the exercise performed is similar between the studies. Because in the present study bloods were sampled at 0h and 2h post-exercise it is possible that due to infrequency of sampling, we might have missed the biphasic drop in lymphocytes that was seen in the study by Nieman (1991) where more frequent blood sampling took place.

The response of the control group mirrors that of participants in the study by Neves Jr. et al. (2009) whereby older women performed a single bout of resistance training at either 50% of 1-RM or 80% of 1-RM and both groups experienced a significant lymphocytosis 3h after the completion of the exercise bout. In the present study, although the control group
appeared to exhibit an upwards trend in the number of circulating lymphocytes at 2h into the recovery period, the lymphocytosis was non-significant.

In the BCS group, circulating lymphocytes appear to peak at 0h post exercise, and begin to decline at 2h post exercise. Typically, post-exercise lymphocytopenia is attributed to the migration of lymphocytes from the circulation into the tissues and/or increases in apoptosis resulting in lymphocyte reduction via programmed cell death (Koch, 2010; Walsh et al., 2011). Because the literature on the immune response to resistance training is so scarce, possible mechanisms for prolonged or blunted lymphocytosis without lymphocytopenia into the recovery period from exercise are unknown. Further, the function and proliferative capacity of these cells several hours following resistance training are also unknown.

Monocytes in both BCS and control group appear to be reduced (though non-significantly) at 2h into the recovery period, which appears to contrast with the present literature, which depicts a 2h post-exercise monocytes (Walsh et al., 2011). The clinical significance of this change is not known, as it has been established that exercise alters cell surface receptor expression in monocytes, which affect the role that these cells play in immunity (Simpson et al., 2009). Without knowing the properties of these cells, the implications for breast cancer survivors cannot be inferred.

The BCS group experienced a further, significant reduction of cortisol compared to baseline values 2h into the recovery period, whereas the cortisol concentration in the control group moved back towards baseline. Because the HPA axis appeared not to be stimulated to initiate cortisol secretion as evidenced by the absence of cortisol elevations 0h post-exercise, it is likely that the response of cortisol in both groups was due to diurnal variation. As
previously discussed, the diurnal rhythm of cortisol in breast cancer survivors is oftentimes altered from that of healthy women (Bower, 2007), which serves as a possible explanation for the significant reduction in cortisol within the BCS group 2h post-exercise. However, diurnal variation of cortisol was not controlled for in the present study.

**Baseline – 24h Post-Exercise**

The 24h post-exercise blood sample served as a major component in the present study to assist future researchers in establishing more optimal program design for breast cancer survivors. While the results of this study indicated no significant difference from baseline in any of the measured immune and hormonal parameters, it provided us with insight into certain trends that future research should attempt to explore. The return of immune parameters to baseline values 24h following the acute bout of resistance training would suggest that the immune systems of the participants had adequately recovered from the primary bout, and that from an immunological standpoint, it would be physiologically safe to implement a secondary training bout 1 day following the first.

However, upon closer look, there are several factors that should be examined further before such conclusions can be drawn. It appears that the peripheral blood mononuclear cells (lymphocytes and monocytes) in the BCS group differ slightly from that of the controls in their pattern of response to the resistance exercise bout. The mean %Δ score from baseline – 24h post-exercise in the BCS group was -11.62%, whereas it was +10.13 for the control group, indicating that at 24h post exercise, the lymphocyte count was below baseline in the BCS group and above baseline in the control group. Although not statistically significant, this disparity between the groups may be of clinical significance. The fact that the lymphocyte count in the BCS group is below baseline at 24h post-exercise while the healthy
controls are above baseline suggests that perhaps the BCS group may not have sufficiently
recovered from the primary exercise bout, though a larger sample size is needed to support or
refute this statement.

Similarly, monocyte counts 24h post-exercise in the BCS group are both significantly
higher than baseline values, and they are significantly greater than that of the controls. As
previously discussed, monocyte cells can be further broken down into classical monocytes, or
pro-inflammatory monocytes, which determines the effect that these cells exert on the living
host (Gleeson et al., 2011; Simpson et al., 2009). The type of monocytes in the circulation of
the BCS groups at 24h post-exercise was not determined and therefore cannot be addressed
in this discussion. However, it may be of importance to note that while the clinical
significance of significantly elevated monocytes 24h post-exercise in the BCS group was not
determined, the results suggest that this subset of circulating leukocytes is significantly
deviated from baseline values, adding to the possibility that the BCS group had not fully
recovered from the exercise bout.

Current consensus in exercise immunology suggests that the period of
immunosuppression, or a reduction in the number and/or function of circulating immune
parameters can last anywhere between 3 and 72 hours following an acute bout of exercise
(Pedersen 1999). Further, it is suggested that moderate-intensity exercise results in a positive
immune response, observed by the absence of significant stress hormone release and a lack
of pro-inflammatory cytokine secretion (Nieman, 2003). The immune response to moderate-
intensity exercise, as compared to high intensity exercise, is thought to exhibit transient
upregulations in the various immune parameters in an absence of significant
immunosuppression of these parameters (Nieman, 2003). The trend towards lymphocyte
suppression in the BCS group 24h post-exercise suggests that breast cancer survivors, who are thought to have altered immune parameters as a result of cancer and its treatments may respond differently to moderate-intensity exercise than healthy, age-matched controls. It is possible that even moderate-intensity exercise for a cancer population is perceived as a greater physiological stressor than in a healthy individual of the same age.

**Limitations of the Study**

There were some limitations to the present study. Perhaps the most prominent is the small sample size that was recruited for participation, consisting of 8 control subjects and only 4 BCS. Additionally, it is possible that some subjects who participated in the study were more physically fit than others, and training status is known to influence the acute immune response to exercise (Koch, 2010). The immune and inflammatory response to resistance exercise in individuals who are trained is known to be less pronounced than in those who are untrained, particularly upon secondary exposure to a similar resistance exercise routine (known as the ‘repeated bout effect’; Koch, 2010). However, this particular influence was likely minimized as all participants within this study were unfamiliar with the 4 resistance exercises used for the resistance training protocol. Another potential limitation to the present study is the time since treatment completion for subjects in the BCS group. The time since chemotherapy completion ranged from 4 months up to 1 year within the 4 BCS subjects. It is possible that the participants closer to treatment completion were experiencing more prominent treatment-related immune effects that those farther out from treatment (Fairey et al., 2002). It is a possibility that these differences were exacerbated by the small sample size.
Conclusion

It is known that cancer and its treatments are accompanied by negative alterations in the immune system, which can persist several years following the completion of treatment (Hutnick et al., 2005). However, it is possible that exercise may serve as a means of enhancing immune function in cancer survivors (Fairey et al., 2005; Hutnick et al., 2005). Exercise programs designed around the complete recovery of immune parameters from a primary exercise bout before the implementation of secondary exercise bout serve to ensure that participants do not experience chronic immunosuppression that is associated with overtraining (Gleeson, 2002). In a breast cancer survivor population, this concept is important in program design, as exercise programs geared towards this population are aimed at improving functional capacity, overall quality of life, and survivorship.

The results of this study suggest that breast cancer survivors and healthy age-matched controls respond similarly to an acute bout of resistance training. In other words, the changes in total leukocyte, granulocyte, lymphocyte, and monocyte cell counts from baseline to 0h post-exercise, baseline to 2h post-exercise, and baseline to 24h post-exercise in the BCS and healthy control groups were not statistically different from each other. However, this response warrants further investigation before conclusions can be made. Regardless, the present study serves as the foundation for future research to examine the immune response to an acute bout of resistance exercise in breast cancer patients.

Recommendations for Future Research

Although this was a small-scale study, it was the first one to examine the effects of an acute bout of resistance training on leukocyte and leukocyte subset response in breast cancer
survivors. As such, the results of the present study generated a wide array of questions that future research should aim to answer.

Firstly, the present study should be repeated with a larger sample size to confirm the nature of the immune response in both the BCS and the healthy age-matched controls. Secondly, future studies should aim to recruit a more homogenous sample in regards to time since last chemotherapy treatment in the BCS group and physical activity level in both groups. Thirdly, future research should aim to stratify the various leukocyte subsets further, to get a better idea as to how the exercise affected the cellular immune response, particularly that of the lymphocytes and monocytes, which can be classified into cells with drastically different phenotypes. Once these have been done, if the results (a similar immune response in BCS and healthy controls) hold true, future studies should aim to examine the effects of multiple exercise bouts over the course of several days on immune parameters in breast cancer patients and healthy controls.

Finally, the functionality of the circulating cells should be examined to determine the influence of resistance exercise on the ability of the immune system to respond to external stimuli and prevent infection. Future research should attempt to explore the clinical significance of alterations in the circulating immune parameters, as many immune components act outside of the circulation in the lymph and bodily tissues.
Appendix A

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects: Breast Cancer Survivor Group
Biomedical Form

IRB Study # 11-1405
Consent Form Version Date: January 10, 2013

Title of Study: The Impact of Acute Aerobic and Resistance Exercise on Natural Killer Cell, Catecholamine, and Cortisol Responses in Breast Cancer Survivors

Principal Investigator: Claudio L. Battaglini, PhD
UNC-Chapel Hill Department: Exercise & Sport Science
UNC-Chapel Hill Phone number: 843-6045
Email Address: claudio@email.unc.edu

Co-Investigators: Elizabeth S. Evans, MA; Anthony C. Hackney, PhD, DSc; Robert G. McMurray, PhD; Hyman B. Muss, MD; Scott H. Randell, PhD; Rachel Graff, BS; Jacob Allen, BA; Miles Bartlett, MA; Nathan Berry, MA; Jamie Pearson, BS; Charlotte Shatten MA; Ryan Vanhoy, BS; Jeremiah Boles, MD; Mingqing Li, MD; Amy Lucas, MD; Autumn Mcree, MD; Payal Desai, MD; Christine Lin, MD; Marshall Mazepa, MD; Micah Mooberry, MD; Ryan Raddin, MD; Tyler Buckner, MD; Andrea Dean, MD; Satish Gopal, MD; Emily Jenkins, MD; Adam Kuykendal, MD; Keeran Sampat, MD

Funding Source and/or Sponsor: NA

Study Contact telephone number: (516) 633-5235 or (919) 621-1322
Study Contact email: rgraff1@email.unc.edu or jmallen@email.unc.edu

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.
Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

**What is the purpose of this study?**
The purpose of this research study is to learn about the effect of one session of moderate intensity aerobic or resistance exercise on the immune system and endocrine system of breast cancer survivors. The immune system helps to protect the body from getting sick. The endocrine system includes the hormones that the body makes, some of which can affect the immune system. We will also be examining how the same type of aerobic or resistance exercise affects age-matched women who have never experienced cancer treatment.

Over the past 20 years, regular aerobic and resistance exercise has been shown to improve the health and well-being of breast cancer survivors in many different ways. Regular participation in exercise can help decrease feelings of fatigue, anxiety, and depression. Regular aerobic and resistance exercise can also help improve physical fitness, build strong bones and muscles, and lower the amount of fat stored in the body. Regular moderate aerobic or resistance exercise may also improve the immune system’s ability to fight infections, and it may also lower the body’s production of stress hormones. However, researchers still have many questions to answer regarding how aerobic exercise and resistance exercise affect the immune system and stress hormone levels in breast cancer survivors, especially when compared to healthy age-matched women who have never experienced treatment for cancer.

The primary aim of this study is to examine the effects of a moderate-intensity, 30-minute aerobic exercise bout or a moderate-intensity resistance exercise bout on the natural killer (NK) cell. The NK cell is an important part of the immune system that helps to destroy viruses and tumor cells. Secondly, the affect of these exercise bouts on the stress hormones epinephrine, norepinephrine, and cortisol will be examined. Thirdly, the relationships between the response of the NK cell and the response of the stress hormones will be measured. The levels of these markers will be measured immediately before exercise, immediately after exercise, 2 hours post-exercise, and 24 hours post-exercise via blood samples.

**Why are you being asked to participate in this study?**
You are being asked to be in the study because you are a woman who has been diagnosed with breast cancer, you have completed surgery, chemotherapy, and radiation therapy within the past 3-6 months, and you are between the ages of 40 and 70 years. The full list of inclusion criteria for participation in this study are listed below:

3.1.1. Confirmed diagnosis of Stage I, II, or III invasive breast cancer;
3.1.2. Must have received chemotherapy;
3.1.3. Must have completed surgery, chemotherapy, and radiation therapy 3-6 months prior to enrollment;
3.1.4. Patients receiving adjuvant hormonal therapy or adjuvant trastuzumab are eligible;
3.1.5. No presence of metastatic disease;
3.1.6. Female, between 40 and 70 years of age;
3.1.7. Not involved in regular organized physical activity for at least 1 year prior to enrollment, meaning that you exercise less than 3 times per week and that you do not engage in activities of moderate or vigorous intensity.
3.1.8. Not taking any anti-inflammatory medications;
3.1.9. Clearance from your oncologist to participate in exercise.

**Are there any reasons you should not be in this study?**
You should not be in this study if you have any type of disease that affects your heart, lungs, bones, or muscles which would make it very difficult for you to exercise. You should not be in this study if you have uncontrolled diabetes or thyroid disease. You should not be in this study if you have had a fever, body aches, and/or swollen glands within the past 6 weeks or if you have a chronic infectious disease such as hepatitis or AIDS. You should not be in this study if you have abnormally low values for certain blood markers, including potassium, magnesium, white blood cells, hematocrit, and platelets. Also, you should not be in this study if you are currently using an anti-inflammatory medication such as non-steroidal anti-inflammatory drugs (NSAIDs) or glucocorticoids, or you have any other mental or physical impairment that would prevent you from being able to exercise.

**How many people will take part in this study?**
If you decide to be in this study, you will be one of approximately 40 people in this research study.

**How long will your part in this study last?**
You will be asked to come to the laboratory for 3 separate visits. Laboratory Visit 1 will last approximately 2 hours, Laboratory Visit 2 will last approximately 3 hours, and Laboratory Visit 3 will last approximately 30 minutes. The overall length of time that you will participate in the study will be approximately 1-2 weeks, but may be shorter.

As this study involves drawing blood, some of your blood samples may be frozen and stored indefinitely for future analyses. You will be given a separate consent form describing storage of the blood samples.

**What will happen if you take part in the study?**
Participation in this study will require you to report to the laboratory for 3 separate visits. All Laboratory Visits will take place in the Integrative Exercise Oncology Research Laboratory (IEORL), which is located in Fetzer Hall, in the Department of Exercise and Sport Science at UNC-Chapel Hill. All study procedures are a requirement of participation in the study.

**Laboratory Visit 1: Orientation / Familiarization to the Study and 1-RM Assessment (Resistance Exercise Group Only)**
The purpose of Laboratory Visit 1 is to provide you with an overview of the study, give you an opportunity to ask questions about your participation, sign this informed consent, gather some demographic information about you, and to assess your muscular strength. The specific procedures that will be performed are listed below:
1. Sign informed consent documents
2. Fill out a medical history questionnaire
3. Undergo a brief medical and physical screening, including a resting electrocardiogram (ECG) to measure heart function
4. Undergo body composition analysis to assess percent body fat using a DEXA
5. Familiarization with equipment used during exercise testing
6. Undergo an exercise test to assess muscular strength

The purposes of items 2, 3, and 4, are to determine if it is safe for you to participate in exercise and to gain some demographic information about you, including your age, height, weight, percent body fat, race, physical activity participation over the past year, and type of cancer treatments you have received and are currently receiving. The machine that will be used to measure your percent body fat in item 4 is called a DEXA. DEXA stands for Dual Energy X-ray Absorptiometry. The DEXA uses two x-ray beams to measure differences in the composition of different tissues in your body, such as bones, soft tissue, and fat. The scan is performed while you rest on your back, and takes approximately 6 minutes.

The purpose of item 6 is to perform an exercise assessment that will allow the researchers to determine your muscular strength, or the greatest amount of weight that certain muscle groups can lift one time. This exercise assessment will involve performing 4 different exercises. They are: leg press, lateral pull down, leg extension, and seated row. The exercise assessment will involve a series of one-repetition trials with increasing workloads until you decide that the given weight is the most that you can lift with proper form. This will be done for each of the 4 exercises. The duration of the exercise assessment will likely last no more than 30 minutes.

Laboratory Visit 2: Acute Resistance Exercise Session (Resistance Exercise Group Only)
Laboratory Visit 2 will occur between 7-14 days of Laboratory Visit 1. During this Visit, you will be performing 1 session of moderate-intensity resistance exercise on the same 4 pieces of equipment used in Laboratory Visit 1 (leg press, lateral pull down, leg extension, and seated row). This will be the exercise bout that will be used to elicit a response in your immune cells, inflammatory markers, and stress hormones. The specific procedures that will be performed are listed below:

1. Fill out a psychological stress questionnaire
2. Pre-exercise blood sample
3. Moderate-intensity resistance exercise session
4. Post-exercise blood sample (immediately post-exercise)
5. Post-exercise blood sample (2 hours post-exercise)

The purpose of item 1 is to determine if you are feeling any emotional or psychological stress on the day of testing. This is important information as the levels of stress hormones in your blood may be influenced by emotional or psychological stress.

During item 3, you will be performing an approximately 30-minute bout of moderate-intensity resistance exercise on the leg press, lateral pull down, leg extension, and seated row. A moderate intensity level of exercise will make you feel like your muscles are working, but
you are able to carry on a conversation. You will alternate between each of the four resistance exercises, performing 10 repetitions of each followed by 1.5 minutes of rest before continuing on to the next exercise. You will move from the leg press to the lateral pull down to the leg extension to the seated row, with the same number of repetitions and amount of rest until you have completed each exercise three times.

The 3 blood samples to be obtained during items 2, 4, and 5 will be performed by using a small catheter inserted into a vein in your arm. This blood sampling procedure is very similar to a blood sampling procedure that is used in routine medical clinic settings. Approximately 1 tablespoon of blood will be collected for each item. Some of this blood will be used during the course of this study to measure the number of NK cells in your blood, the activity of the NK cells (i.e., how well they destroy other cells), and the levels of stress hormones and cytokines in your blood.

**Laboratory Visit 3: 24-hour Follow-up Session**

Laboratory Visit 3 will occur 24 hours after Laboratory Visit 2. During this Visit, you will answer the same psychological stress questionnaire as you did in the previous laboratory visit. You will also have 1 blood sample drawn, which will also be used to the number of NK cells in your blood, the activity of the NK cells (i.e., how well they destroy other cells), and the levels of stress hormones and cytokines in your blood. The procedure for obtaining the blood sample is the same as described above, except that the blood will be drawn through a small needle instead of a catheter.

**What are the possible benefits from being in this study?**

Research is designed to benefit society by gaining new knowledge. The benefits to you from being in this study may be the opportunity to have your various aspects of your health assessed during Laboratory Visit 1. This would include looking at how well your heart is working by using the ECG and by listening to your heart with a stethoscope. Additionally, you will have your muscular strength assessed, as well as your percent body fat. This information may be useful if you are thinking of beginning an exercise program, and you may elect to provide this information to an exercise specialist who could construct and supervise an individualized exercise program for you.

**What are the possible risks or discomforts involved with being in this study?**

The risk of physiological and psychological harm is very minimal and would not cost you physical or emotional loss. However, any research study does carry with it some potential for risk or discomfort, listed below:

1. During Laboratory Visit 1, you will be performing an exercise test to determine your muscular strength. The test will start with an easy warm-up and movement rehearsal for your muscles. As the one repetition maximum trials begin, you will find that the assessment will become more difficult. It is possible that increased stress will be placed on your musculoskeletal system. To ensure your safety, your heart rate and your lifting technique will be monitored continuously throughout the various trials to verify that there are no movement compensations. Your heart rate will continue to be
monitored for several minutes after the conclusion of the one repetition maximum assessments. At the conclusion of the test, you will be allowed to cool down, stretch, and drink water at liberty.

2. During Laboratory Visit 1, you will be exposed to radiation during the DEXA scan. However, the scan itself is very safe, and the amount of x-ray radiation that you will receive during the scan is 0.8 mrems, which is extremely small. To put this in perspective, this amount of radiation is about the same amount of radiation that you would receive from natural background sources in one day.

3. During Laboratory Visits 1 and 2, it is possible that you could experience slight muscle or joint soreness after the exercise. However, this soreness would not be any worse than after doing any other type of exercise. To minimize the amount of soreness you might feel, you will be allowed to stretch before and after exercise, and you will be allowed to do a light warm-up and cool-down before and after exercise.

4. During Laboratory Visits 2 and 3, it is possible that you could experience some mild bruising or discomfort while having your blood drawn. This discomfort would not be any more significant than having your blood drawn at the doctor’s office. The blood sampling procedures will be performed by research team members who have drawn blood many times before, and proper bandaging will be performed to minimize bleeding.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers.

**What if we learn about new findings or information during the study?**

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

**How will your privacy be protected?**

You will not be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies (for example, the FDA) for purposes such as quality control or safety.

All data obtained from you during this study will be coded using a research ID number, and only the members of the research team will have access to these codes. Study databases and logs will not have any information that will allow for subjects to be identified by anyone other than a research team member.
A copy of this consent form will go into your medical record. This will allow the doctors caring for you to know what study tests you may be receiving as a part of the study and know how to take care of you if you have other health problems or needs during the study.

**What will happen if you are injured by this research?**
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from being in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. The University of North Carolina at Chapel Hill has not set aside funds to pay you for any such reactions or injuries, or for the related medical care. However, by signing this form, you do not give up any of your legal rights.

**What if you want to stop before your part in the study is complete?**
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, you become ill, you use a medication that may affect the outcome of the study variables being measured, you are not able to complete the exercise testing sessions, you refuse to have your blood drawn, you miss a Laboratory Visit, or because the entire study has been stopped. If you decide to withdraw from the study, all data collected from you will be destroyed and will not be used in any data analyses that may result from this study.

**Will you receive anything for being in this study?**
You will be provided with a written report of your fitness level and percent body fat, both of which will be measured during Laboratory Visit 1.

**Will it cost you anything to be in this study?**
It will not cost you anything to participate in this study.

**What if you are a UNC student?**
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

**What if you are a UNC employee?**
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

**What if you have questions about this study?**
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

**What if you have questions about your rights as a research subject?**
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

Title of Study: The Impact of Acute Aerobic and Resistance Exercise on Natural Killer Cell, Catecholamine, and Cortisol Responses in Breast Cancer Survivors

Principal Investigator: Claudio L. Battaglini, PhD

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_________________________________________________ __________________
Signature of Research Subject Date

_________________________________________________
Printed Name of Research Subject

_________________________________________________
Signature of Research Team Member Obtaining Consent Date

_________________________________________________
Printed Name of Research Team Member Obtaining Consent
Appendix B

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects: Healthy Control Group
Biomedical Form

IRB Study # 11-1405
Consent Form Version Date: January 10, 2013

Title of Study: The Impact of Acute Aerobic and Resistance Exercise on Natural Killer Cell, Catecholamine, and Cortisol Responses in Breast Cancer Survivors

Principal Investigator: Claudio L. Battaglini, PhD
UNC-Chapel Hill Department: Exercise & Sport Science
UNC-Chapel Hill Phone number: 843-6045
Email Address: claudio@email.unc.edu
Co-Investigators: Elizabeth S. Evans, MA; Anthony C. Hackney, PhD, DSc; Robert G. McMurray, PhD; Hyman B. Muss, MD; Scott H. Randell, PhD; Rachel Graff, BS; Jacob Allen, BA; Miles Bartlett, MA; Nathan Berry, MA; Jamie Pearson, BS; Charlotte Shatten MA; Ryan Vanhoy, BS; Jeremiah Boles, MD; Mingqing Li, MD; Amy Lucas, MD; Autumn Mcree, MD; Payal Desai, MD; Christine Lin, MD; Marshall Mazepa, MD; Micah Mooberry, MD; Ryan Raddin, MD; Tyler Buckner, MD; Andrea Dean, MD; Satish Gopal, MD; Emily Jenkins, MD; Adam Kuykendal, MD; Keeran Sampat, MD

Funding Source and/or Sponsor: NA

Study Contact telephone number: (516) 633-5235 or (919) 621-1322
Study Contact email: rgraff1@email.unc.edu or jmallen@email.unc.edu

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.
Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

**What is the purpose of this study?**
The purpose of this research study is to learn about the effect of one session of moderate intensity aerobic or resistance exercise on the immune system and endocrine system of breast cancer survivors. The immune system helps to protect the body from getting sick. The endocrine system includes the hormones that the body makes, some of which can affect the immune system. We will also be examining how the same type of aerobic or resistance exercise affects age-matched women who have never experienced cancer treatment.

Over the past 20 years, regular aerobic and resistance exercise has been shown to improve the health and well-being of breast cancer survivors in many different ways. Regular participation in exercise can help decrease feelings of fatigue, anxiety, and depression. Regular aerobic and resistance exercise can also help improve physical fitness, build strong bones and muscles, and lower the amount of fat stored in the body. Regular moderate aerobic or resistance exercise may also improve the immune system’s ability to fight infections, and it may also lower the body’s production of stress hormones. However, researchers still have many questions to answer regarding how aerobic exercise and resistance exercise affect the immune system and stress hormone levels in breast cancer survivors, especially when compared to healthy age-matched women who have never experienced treatment for cancer.

The primary aim of this study is to examine the effects of a moderate-intensity, 30-minute aerobic exercise bout or a moderate-intensity resistance exercise bout on the natural killer (NK) cell. The NK cell is an important part of the immune system that helps to destroy viruses and tumor cells. Secondly, the affect of these exercise bouts on the stress hormones epinephrine, norepinephrine, and cortisol will be examined. Thirdly, the relationships between the response of the NK cell and the response of the stress hormones will be measured. The levels of these markers will be measured immediately before exercise, immediately after exercise, 2 hours post-exercise, and 24 hours post-exercise via blood samples.

**Why are you being asked to participate in this study?**
You are being asked to be in the study because you are a woman who has never been diagnosed with or treated for cancer, you are post-menopausal, you are sedentary, and you are between the ages of 40 and 70 years. The full list of inclusion criteria for participation in this study are listed below:

3.1.10. Female, between 40 and 70 years of age;
3.1.11. No history of cancer diagnosis or treatment;
3.1.12. No presence of any bone, muscle, or other medical condition that would prevent you from being able to participate in resistance exercise;
3.1.13. Not taking anti-inflammatory medications such as non-steroidal anti-inflammatory drugs (NSAIDs) or glucocorticoids;
3.1.14. Not involved in regular organized physical activity for at least 1 year prior to enrollment, meaning that you exercise less than 3 times per week and that you do not engage in activities of moderate or vigorous intensity.

3.1.15. Clearance from your primary physician, if you are age 65 years or older.

**Are there any reasons you should not be in this study?**
You should not be in this study if you have any type of disease that affects your heart, lungs, bones, or muscles which would make it very difficult for you to exercise. You should not be in this study if you have uncontrolled diabetes or thyroid disease. You should not be in this study if you have had a fever, body aches, and/or swollen glands within the past 6 weeks or if you have a chronic infectious disease such as hepatitis or AIDS. You should not be in this study if you have abnormally low values for certain blood markers, including potassium, magnesium, white blood cells, hematocrit, and platelets. Also, you should not be in this study if you are currently using an anti-inflammatory medication such as non-steroidal anti-inflammatory drugs (NSAIDs) or glucocorticoids, or you have any other mental or physical impairment that would prevent you from being able to exercise.

**How many people will take part in this study?**
If you decide to be in this study, you will be one of approximately 40 people in this research study.

**How long will your part in this study last?**
You will be asked to come to the laboratory for 3 separate visits. Laboratory Visit 1 will last approximately 2 hours, Laboratory Visit 2 will last approximately 3 hours, and Laboratory Visit 3 will last approximately 30 minutes. The overall length of time that you will participate in the study will be approximately 1-2 weeks, but may be shorter.

As this study involves drawing blood, some of your blood samples may be frozen and stored indefinitely for future analyses. You will be given a separate consent form describing storage of the blood samples.

**What will happen if you take part in the study?**
Participation in this study will require you to report to the laboratory for 3 separate visits. All Laboratory Visits will take place in the Integrative Exercise Oncology Research Laboratory (IEORL), which is located in Fetzer Hall, in the Department of Exercise and Sport Science at UNC-Chapel Hill. All study procedures are a requirement of participation in the study.

**Laboratory Visit 1: Orientation / Familiarization to the Study and 1-RM Assessment (Resistance Exercise Group Only)**
The purpose of Laboratory Visit 1 is to provide you with an overview of the study, give you an opportunity to ask questions about your participation, sign this informed consent, gather some demographic information about you, and to assess your muscular strength. The specific procedures that will be performed are listed below:

7. Sign informed consent documents
8. Fill out a medical history questionnaire
9. Undergo a brief medical and physical screening, including a resting electrocardiogram (ECG) to measure heart function
10. Undergo body composition analysis to assess percent body fat using a DEXA
11. Familiarization with equipment used during exercise testing
12. Undergo an exercise test to assess muscular strength

The purposes of items 2, 3, and 4, are to determine if it is safe for you to participate in exercise and to gain some demographic information about you, including your age, height, weight, percent body fat, race, physical activity participation over the past year, and type of cancer treatments you have received and are currently receiving. The machine that will be used to measure your percent body fat in item 4 is called a DEXA. DEXA stands for Dual Energy X-ray Absorptiometry. The DEXA uses two x-ray beams to measure differences in the composition of different tissues in your body, such as bones, soft tissue, and fat. The scan is performed while you rest on your back, and takes approximately 6 minutes.

The purpose of item 6 is to perform an exercise assessment that will allow the researchers to determine your muscular strength, or the greatest amount of weight that certain muscle groups can lift one time. This exercise assessment will involve performing 4 different exercises. They are: leg press, lateral pull down, leg extension, and seated row. The exercise assessment will involve a series of one-repetition trials with increasing workloads until you decide that the given weight is the most that you can lift with proper form. This will be done for each of the 4 exercises. The duration of the exercise assessment will likely last no more than 30 minutes.

Laboratory Visit 2: Acute Resistance Exercise Session (Resistance Exercise Group Only)
Laboratory Visit 2 will occur between 7-14 days of Laboratory Visit 1. During this Visit, you will be performing 1 session of moderate-intensity resistance exercise on the same 4 pieces of equipment used in Laboratory Visit 1 (leg press, lateral pull down, leg extension, and seated row). This will be the exercise bout that will be used to elicit a response in your immune cells, inflammatory markers, and stress hormones. The specific procedures that will be performed are listed below:

6. Fill out a psychological stress questionnaire
7. Pre-exercise blood sample
8. Moderate-intensity resistance exercise session
9. Post-exercise blood sample (immediately post-exercise)
10. Post-exercise blood sample (2 hours post-exercise)

The purpose of item 1 is to determine if you are feeling any emotional or psychological stress on the day of testing. This is important information as the levels of stress hormones in your blood may be influenced by emotional or psychological stress.

During item 3, you will be performing an approximately 30-minute bout of moderate-intensity resistance exercise on the leg press, lateral pull down, leg extension, and seated row. A moderate intensity level of exercise will make you feel like your muscles are working, but you are able to carry on a conversation. You will alternate between each of the four
resistance exercises, performing 10 repetitions of each followed by 1.5 minutes of rest before continuing on to the next exercise. You will move from the leg press to the lateral pull down to the leg extension to the seated row, with the same number of repetitions and amount of rest until you have completed each exercise three times.

The 3 blood samples to be obtained during items 2, 4, and 5 will be performed by using a small catheter inserted into a vein in your arm. This blood sampling procedure is very similar to a blood sampling procedure that is used in routine medical clinic settings. Approximately 1 tablespoon of blood will be collected for each item. Some of this blood will be used during the course of this study to measure the number of NK cells in your blood, the activity of the NK cells (i.e., how well they destroy other cells), and the levels of stress hormones and cytokines in your blood.

Laboratory Visit 3: 24-hour Follow-up Session

Laboratory Visit 3 will occur 24 hours after Laboratory Visit 2. During this Visit, you will answer the same psychological stress questionnaire as you did in the previous laboratory visit. You will also have 1 blood sample drawn, which will also be used to the number of NK cells in your blood, the activity of the NK cells (i.e., how well they destroy other cells), and the levels of stress hormones and cytokines in your blood. The procedure for obtaining the blood sample is the same as described above, except that the blood will be drawn through a small needle instead of a catheter.

What are the possible benefits from being in this study?
Research is designed to benefit society by gaining new knowledge. The benefits to you from being in this study may be the opportunity to have your various aspects of your health assessed during Laboratory Visit 1. This would include looking at how well your heart is working by using the ECG and by listening to your heart with a stethoscope. Additionally, you will have your muscular strength assessed, as well as your percent body fat. This information may be useful if you are thinking of beginning an exercise program, and you may elect to provide this information to an exercise specialist who could construct and supervise an individualized exercise program for you.

What are the possible risks or discomforts involved with being in this study?
The risk of physiological and psychological harm is very minimal and would not cost you physical or emotional loss. However, any research study does carry with it some potential for risk or discomfort, listed below:

5. During Laboratory Visit 1, you will be performing an exercise test to determine your muscular strength. The test will start with an easy warm-up and movement rehearsal for your muscles. As the one repetition maximum trials begin, you will find that the assessment will become more difficult. It is possible that increased stress will be placed on your musculoskeletal system. To ensure your safety, your heart rate and your lifting technique will be monitored continuously throughout the various trials to verify that there are no movement compensations. Your heart rate will continue to be monitored for several minutes after the conclusion of the one repetition maximum
assessments. At the conclusion of the test, you will be allowed to cool down, stretch, and drink water at liberty.

6. During Laboratory Visit 1, you will be exposed to radiation during the DEXA scan. However, the scan itself is very safe, and the amount of x-ray radiation that you will receive during the scan is 0.8 mrems, which is extremely small. To put this in perspective, this amount of radiation is about the same amount of radiation that you would receive from natural background sources in one day.

7. During Laboratory Visits 1 and 2, it is possible that you could experience slight muscle or joint soreness after the exercise. However, this soreness would not be any worse than after doing any other type of exercise. To minimize the amount of soreness you might feel, you will be allowed to stretch before and after exercise, and you will be allowed to do a light warm-up and cool-down before and after exercise.

8. During Laboratory Visits 2 and 3, it is possible that you could experience some mild bruising or discomfort while having your blood drawn. This discomfort would not be any more significant than having your blood drawn at the doctor’s office. The blood sampling procedures will be performed by research team members who have drawn blood many times before, and proper bandaging will be performed to minimize bleeding.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers.

**What if we learn about new findings or information during the study?**
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

**How will your privacy be protected?**
You will not be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies (for example, the FDA) for purposes such as quality control or safety.

All data obtained from you during this study will be coded using a research ID number, and only the members of the research team will have access to these codes. Study databases and logs will not have any information that will allow for subjects to be identified by anyone other than a research team member.

A copy of this consent form will go in to your medical record. This will allow the doctors caring for you to know what study tests you may be receiving as a part of the study and know how to take care of you if you have other health problems or needs during the study.
What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from being in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. The University of North Carolina at Chapel Hill has not set aside funds to pay you for any such reactions or injuries, or for the related medical care. However, by signing this form, you do not give up any of your legal rights.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, you become ill, you use a medication that may affect the outcome of the study variables being measured, you are not able to complete the exercise testing sessions, you refuse to have your blood drawn, you miss a Laboratory Visit, or because the entire study has been stopped. If you decide to withdraw from the study, all data collected from you will be destroyed and will not be used in any data analyses that may result from this study.

Will you receive anything for being in this study?
You will be provided with a written report of your fitness level and percent body fat, both of which will be measured during Laboratory Visit 1.

Will it cost you anything to be in this study?
It will not cost you anything to participate in this study.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

What if you have questions about your rights as a research subject?
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject,
or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

---

**Title of Study:** The Impact of Acute Aerobic and Resistance Exercise on Natural Killer Cell, Catecholamine, and Cortisol Responses in Breast Cancer Survivors

**Principal Investigator:** Claudio L. Battaglini, PhD

**Subject’s Agreement:**

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_________________________________________________ ___ _________________
Signature of Research Subject Date

_________________________________________________
Printed Name of Research Subject

_________________________________________________ __________________
Signature of Research Team Member Obtaining Consent Date

_________________________________________________
Printed Name of Research Team Member Obtaining Consent
Appendix C

MEDICAL HISTORY QUESTIONNAIRE

Department of Exercise and Sport Science
Medical History

Subject: __________________________ ID: ___________ Telephone:______________

Address:___________________________________________

Occupation:___________________________________ Age:______________________

Race:________________________________________

Patient History

1. How would you describe your general health at present?
   Excellent______ Good_______ Fair______ Poor______

2. Do you have any health problems at the present time?   _____

3. If yes, please describe:______________________________

4. Have you ever been told you have heart trouble?     _____

5. If yes, please describe:______________________________

6. Do you ever get pain in your chest?     _____

7. Do you ever feel light-headed or have you ever fainted?   _____

8. If yes, please describe:______________________________

9. Have you ever been told that your blood pressure has been elevated? _____

10. If yes, please describe:____________________________

11. Have you ever had difficulty breathing either at rest or with exertion? _____

12. If yes, please describe:____________________________

13. Are you now, or have you been in the past 5 years, under a doctor’s care for any reason?
   _____

14. If yes for what reason?__________________________________________
   ____________________________________________________________
   ____________________________________________________________

15. Have you been in the hospital in the past 5 years?   _____

16. If yes, for what reason?__________________________________________
   ____________________________________________________________
   ____________________________________________________________

17. Have you ever experienced an epileptic seizure or been informed that you have epilepsy?
   _____
18. Have you ever been treated for infectious mononucleosis, hepatitis, pneumonia, or another infectious disease during the past year? _____ _____
19. If yes, name the disease:____________________________________________________
20. Have you ever been treated for or told you might have diabetes? _____ _____
21. Have you ever been treated for or told you might or low blood sugar? _____ _____
22. Do you have any known allergies to drugs? _____ _____
23. If so, what?______________________________________________________________
24. Have you ever been “knocked-out” or experienced a concussion? _____ _____
25. If yes, have you been “knocked-out” more than once? _____ _____
26. Have you ever experienced heat stroke or heat exhaustion? _____ _____
27. If yes, when?___________________________________________________________
28. Have you ever had any additional illnesses or operations? (Other than childhood diseases) _____ _____
29. If yes, please indicate specific illness or operations:___________________________
30. Are you now taking any pills or medications? _____ _____
31. If yes, please list:_______________________________________________________
32. Have you had any recent (within 1 year) difficulties with your:
   a. Feet _____ _____
   b. Legs _____ _____
   c. Back _____ _____
33. Has anyone in your family (grandparent, father, mother, and/or sibling) experienced any of the following?
   a. Sudden death _____ _____
   b. Cardiac disease _____ _____
   c. Marfan’s syndrome _____ _____
34. Have you ever experienced depression? _____ _____
35. If yes, did you seek the advice of a doctor? _____ _____
36. Have you ever been told you have or has a doctor diagnosed you with panic disorder, obsessive-compulsive disorder, clinical depression, bipolar disorder, or any other psychological disease? _____ _____
37. If yes, please list condition and if you are currently taking any medication.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bone and Joint History
34. Have you ever been treated for Osgood-Schlatter’s disease? _____ _____
35. Have you ever had any injury to your neck involving nerves or vertebrae? _____ _____
36. Have you ever had a shoulder dislocation, separation, or other injury of the shoulder that incapacitated you for a week or longer? _____ _____
37. Have you ever been advised to or have you had surgery to correct a shoulder condition? _____
38. Have you ever experienced any injury to your arms, elbows, or wrists? _____ _____
39. If yes, indicate location and type of injury: ________________________________

40. Do you experience pain in your back? _____ _____
41. Have you ever had an injury to your back? _____ _____
42. If yes, did you seek the advice of a doctor? _____ _____
43. Have you ever been told that you injured the ligaments or cartilage of either knee joint? _____ _____
44. Do you think you have a trick knee? _____ _____
45. Do you have a pin, screw, or plate somewhere in your body as the result of bone or joint surgery that presently limits your physical capacity? _____ _____
46. If yes, indicate where: ________________________________

47. Have you ever had a bone graft or spinal fusion? _____ _____

Activity History
48. During your early childhood (to age 12) would you say you were:
   Very active _____ Quite active_____ Moderately active_____ Seldom active____
49. During your adolescent years (age 13-18) would you say you were:
   Very active _____ Quite active_____ Moderately active_____ Seldom active____
50. Did you participate in:
   a. Intramural school sports? _____ _____
   b. Community sponsored sports? _____ _____
   c. Varsity school sports? _____ _____
   d. Active family recreation? _____ _____
51. Since leaving high school, how active have you been?
   Very active _____ Quite active_____ Active_____ Inactive____
52. Do you participate in any vigorous activity at present? _____ _____
53. If yes, please list:

<table>
<thead>
<tr>
<th>Activity</th>
<th>Frequency</th>
<th>Duration</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

54. How would you describe your present state of fitness?
- Excellent
- Good
- Fair
- Poor

55. Please list the type(s) of work you have been doing for the previous ten years:

<table>
<thead>
<tr>
<th>Year</th>
<th>Work</th>
<th>Indoor/Outdoor</th>
<th>Location (city/state)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Menstrual Cycle History
56. Have you been post-menopausal for the past year, in that you have not experienced a menstrual period for at least 1 year?
- Yes
- No

Emergency Contact Information
57. Whom shall we notify in case of emergency?
- Name: ____________________________
- Phone: (Home) ____________________ (Work) ____________________
- Address: __________________________

58. Name and address of personal physician: __________________________

FOR SUBJECTS IN THE BREAST CANCER SURVIVOR GROUP ONLY
59. Please indicate which type(s) of treatment you received/are receiving for your cancer
- Surgery
- Chemotherapy
- Radiation Therapy
- Hormonal Therapy
- Trastuzumab

60. How long ago did you finish receiving surgery, chemotherapy, and/or radiation therapy?

61. If you received surgery, which type did you receive?
- Mastectomy
- Lumpectomy

62. If you received chemotherapy, please list the names of the drugs that were included in your treatment.
63. If you are receiving hormonal therapy, please list the names of the medications that you are taking and how long you have been taking them.

________________________________________________________________________

________________________________________________________________________

64. If you are receiving trastuzumab, please list how long you have been taking this medication.

________________________________________________________________________

65. Please list any other medication(s) that you have taken/are currently taking that is/are directly related to your cancer treatment.

________________________________________________________________________

________________________________________________________________________

All of the above questions have been answered completely and truthfully to the best of my knowledge.

Signature: ______________________________ Date: ________________
Appendix D

University of North Carolina-Chapel Hill
HIPAA Authorization for Use and Disclosure of Health Information for Research Purposes

IRB Study #11-1405

Title of Study: The Impact of Resistance Exercise on Cellular Immune System Responses, Stress Hormones, and Cytokines in Breast Cancer Survivors

Principal Investigator: Claudio L. Battaglini, PhD
Mailing Address for UNC-Chapel Hill Department: Department of Exercise & Sport Science, CB #8700

This is a permission called a “HIPAA authorization.” It is required by the “Health Insurance Portability and Accountability Act of 1996” (known as “HIPAA”) in order for us to get information from your medical records or health insurance records to use in this research study.

1. If you sign this HIPAA authorization form, you are giving your permission for the following people or groups to give the researchers certain information about you (described below):

Any health care providers or health care professionals or health plans that have provided health services, treatment, or payment for you such as physicians, clinics, hospitals, home health agencies, diagnostics centers, laboratories, treatment or surgical centers, including but not limited to the UNC Health Care System, health insurance plans, and government health agencies.

2. If you sign this form, this is the health information about you that the people or groups listed in #1 may give to the researchers to use in this research study any information in your medical records that relates to your participation in this research. These records might include information about your age, race, height, weight, menopausal status, physical activity participation over the past year, breast cancer stage at diagnosis, types of cancer therapies and drugs you have received, medications you may be taking, and presence of any other diseases that could preclude your ability to exercise, including cardiovascular disease, pulmonary disease, orthopedic disease, diabetes, thyroid disease, and chronic infectious disease including hepatitis or AIDS.

3. The HIPAA protections that apply to your medical records will not apply to your information when it is in the research study records. Your information in the research study records may also be shared with, used by or seen by collaborating researchers, the sponsor of
the research study, the sponsor’s representatives, and certain employees of the university or government agencies (like the FDA) if needed to oversee the research study.

HIPAA rules do not usually apply to those people or groups. If any of these people or groups reviews your research record, they may also need to review portions of your original medical record relevant to the situation. The informed consent document describes the procedures in this research study that will be used to protect your personal information. You can also ask the researchers any questions about what they will do with your personal information and how they will protect your personal information in this research study.

4. If this research study creates medical information about you that will go into your medical record, you may not be able to see the research study information in your medical record until the entire research study is over.

5. If you want to participate in this research study, you must sign this HIPAA authorization form to allow the people or groups listed in #1 on this form to give access to the information about you that is listed in #2. If you do not want to sign this HIPAA authorization form, you cannot participate in this research study. However, not signing the authorization form will not change your right to treatment, payment, enrollment or eligibility for medical services outside of this research study.

6. This HIPAA authorization will not stop unless you stop it in writing.

7. You have the right to stop this HIPAA authorization at any time. You must do that in writing. You may give your written stop of this HIPAA authorization directly to Principal Investigator or researcher or you may mail it to the department mailing address listed at the top of this form, or you may give it to one of the researchers in this study and tell the researcher to send it to any person or group the researcher has given a copy of this HIPAA authorization. Stopping this HIPAA authorization will not stop information sharing that has already happened.

8. You will be given a copy of this signed HIPAA authorization.
Signature of Research Subject   Date

___________________________________   _________

Print Name of Research Subject

**For Personal Representative of the Research Participant (if applicable)**

Print Name of Personal Representative: ______________________________

Please explain your authority to act on behalf of this Research Subject:

___________________________________________________ ______

*I am giving this permission by signing this HIPAA Authorization on behalf of the Research Participant.*

___________________________________   _________

Signature of Personal Representative   Date
Appendix E

University of North Carolina at Chapel Hill
Consent for Storing Biological Specimens With Identifying Information

IRB Study # 11-1405
Consent Form Version Date: November 2, 2012

Title of Study: The Impact of Resistance Exercise on Cellular Immune System Responses, Stress Hormones, and Cytokines in Breast Cancer Survivors

Principal Investigator: Claudio L. Battaglini, PhD
Principal Co-Investigator: Anthony C. Hackney, PhD, DSc
UNC-Chapel Hill Department: Exercise & Sport Science
UNC-Chapel Hill Phone number: 843-6045
Email Address: claudio@email.unc.edu
Co-Investigators: Lisa A. Carey; Hyman B. Muss, MD; Eric D. Ryan, PhD; Elizabeth Evans, MA; Rachel Graff BA; Jacob Allen, BA; Dustin Buttars, BS; Robert Mills, BA; Sarah Fultz, BS; and Mary Woessner, B.A.

Funding Source and/or Sponsor: Lineberger Comprehensive Cancer Center

Study Contact telephone number: (516) 633-5235 or (919) 621-1322
Study Contact email: rgraff1@email.unc.edu or jmallen@email.unc.edu

What are some general things you should know about research?
Research is designed to gain scientific information that may help other people in the future. You may not receive any direct benefit from participating. There also may be risks.

You may refuse to take part in research. If you are a patient with an illness, you do not have to be in research in order to receive treatment.

Details are discussed below. It is important that you understand this information so that you can make an informed choice. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this specimen repository or “biobank?”
Research with blood, tissue or body fluids (specimens) can help researchers understand how the human body works. Research can also answer other questions by using specimens. Researchers may develop new tests to find diseases, or new ways to treat diseases. In the future, research may help to develop new products, such as drugs. Specimens are commonly used for genetic research. Sometimes researchers collect and store many specimens together.
and use them for different kinds of research, or share them with other scientists; this is called a specimen repository or “biobank.”

The purpose of this particular repository or biobank is to allow researchers to conduct future analysis of blood samples that will be obtained during the course of this study. For this particular study, blood samples will be collected in order to measure immune and inflammatory markers in the blood in response to one session of moderate-intensity resistance exercise. Additionally, levels of the stress hormone cortisol will be measured. Future analyses that may be performed on these blood samples may include measuring the levels of other hormones as well as the levels of other components of the immune system that may be affected by resistance exercise.

**How will the specimens be collected?**
Blood samples are collected as part of the main study. We would like to store any samples remaining for future use.

**What will happen to the specimens?**
Once your blood samples are collected, they will be frozen and stored in a freezer located in the Applied Physiology Laboratory, Room 25 Fetzer Hall, in the Department of Exercise and Sport Science at UNC-Chapel Hill. The freezer is kept within a self-contained section of the laboratory that is accessible only by a keypad combination on a closed door. The only individuals with access to the freezer are faculty of the Applied Physiology Laboratory and their graduate students. Blood samples will be kept in the freezer indefinitely until it is apparent that an insufficient amount exists needed to perform analysis. The only other investigators besides those on the research team who would have access to your blood samples and accompanying data would be future graduate students of the Principal Investigator.

**What are the possible benefits to you?**
Benefits to you are unlikely. Studies that use specimens from this repository may provide additional information that will be helpful in understanding the effect of acute resistance exercise on hormone responses and immune system responses in breast cancer survivors and healthy women who have never been diagnosed with or received treatment for cancer.

**What are the possible risks or discomforts involved with the use of your specimens?**
The risk of physiological and psychological harm related to specimen collection is very minimal and would not cost you physical or emotional loss. However, any research study does carry with it some potential for risk or discomfort. Additionally, there is a risk of breach of confidentiality; however, your stored blood samples will be coded using a research ID number that will not be linked with any information that would personally identify you.

**Will there be any cost to you for storage of the specimens?**
There will be no cost to you for the storage and use of the specimens for research purposes.

**Will you receive anything for the use of your specimens?**
You will not receive anything for taking part in this research.
**Who owns the specimens?**
Any blood, body fluids, or tissue specimens obtained for this purpose become the exclusive property of the University of North Carolina at Chapel Hill. This organization may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are no plans to compensate you for any future commercial use of these specimens.

**How will your privacy be protected?**
All data obtained from you during this study will be coded using a research ID number, and only the members of the research team will have access to these codes. Study databases and logs will not have any information that will allow for subjects to be identified by anyone other than a research team member.

Information from your medical records may be stored along with your specimens(s). You will be asked to sign a separate form (“HIPAA authorization”) to allow researchers to review your medical records.

You will not be identified in any report or publication about research using your specimens. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

**Will researchers seek approval from you to do future studies involving the specimens?**
By signing this consent form, you are giving your permission for researchers to use your specimens as described above. Current and future research is overseen by a committee called the Institutional Review Board (IRB). The role of the IRB is to protect the rights and welfare of research participants. In some cases, the IRB may require that you be re-contacted and asked for your consent to use your specimens in a specific research study. You have the right, at that future time, not to participate in any research study for which your consent is sought. Refusal to participate will not affect your medical care or result in loss of benefits to which you are entitled.

**Will you receive results from research involving your specimens?**
Most research with your specimens is not expected to yield new information that would be meaningful to share with you personally. There are no plans to re-contact you or other subjects with information about research results.

**Can you withdraw the specimens from the research repository?**
If you decide that you no longer wish for the specimens to be stored, you should contact the researchers on the front page of this form. It is best to make your request in writing.
Any analysis in progress at the time of your request or already performed prior to your request being received by the researcher will continue to be used as part of the research study. Once the researchers have been notified, your remaining specimens would be destroyed. If you do not make such a request, the specimens may be stored forever. The researchers may choose to destroy the specimens at any time.

**What will happen if you are injured by this research?**
NA

**What if you have questions about this research?**
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, you should contact the researchers listed on the first page of this form.

**What if you have questions about your rights as a research subject?**
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously if you wish, the Institutional Review Board at 919-966-3113 or by e-mail to IRB_subjects@unc.edu.

IRB Study # 11-1405: The Impact of Resistance Exercise on Cellular Immune System Responses, Stress Hormones, and Cytokines in Breast Cancer Survivors

**Subject’s Agreement:**

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate. I agree to my specimen(s) being stored with the identifying code(s).

____________________  ___________________
Signature of Research Subject       Date

____________________
Printed Name of Research Subject

____________________  ___________________
Signature of Research Team Member Obtaining Consent       Date

____________________
Printed Name of Research Team Member Obtaining Consent
Appendix F

Laboratory Visit Appointment Reminder

Thank you for agreeing to participate in this study entitled “The Impact of Acute Aerobic and Resistance Exercise on Natural Killer Cell, Catecholamine, and Cortisol Responses in Breast Cancer Survivors.” Your time and participation is greatly appreciated!

This is an appointment reminder for Laboratory Visit (circle one): 1  2  3

Your next Laboratory Visit is scheduled for _________________ at _____________.

Date       Time

Your Laboratory Visit will take place in the Integrative Exercise Oncology Research Laboratory (IEORL), which is room 125 Fetzer Hall (corner of South Rd and Raleigh Rd).

If you are driving into campus, here are the directions for arriving to Fetzer Hall and parking. These directions are written assuming you are coming from the direction of UNC Hospitals.

1. Drive south on Columbia Street until you come to the intersection of South Rd. and Columbia.
2. Turn right onto South Rd.
3. Drive a short distance on South Rd. and turn right onto Stadium Drive. The bell tower will be on your right.
4. Drive a very short distance on Stadium Drive and take your first left. The football stadium will be on your right, and there will be several crosswalk lines painted on the asphalt.
5. You are now in the small visitor parking lot at the junction of Fetzer Hall, the outdoor pool, and the Stallings-Evans Sports Medicine Facility. Park in any of the available visitor parking spaces.
6. One of us (Rachel Graff or Jacob Allen) will meet you in the parking lot with your pink visitor parking pass.

***Please follow these pre-assessment guidelines prior to reporting to your Laboratory Visit***

1. No eating for at least 2 hours prior to your appointment. We do suggest that you eat a little something before you come to the lab, even if it is early in the morning.
2. No exercise for at least 12 hours prior to your appointment
3. Drink water throughout the day to keep yourself hydrated.
4. No caffeine use for at least 12 hours prior to your appointment
5. No alcohol use for at least 48 hours prior to your appointment

What to bring to your Laboratory Visit:

1. Clothing (including shoes) that are comfortable for exercise.
2. Water bottle.
3. Filled-out copies of the informed consents and medical history form.

If you have any questions at any time, please do not hesitate to contact Rachel Graff or Jacob Allen at (516) 633-5235 or (919) 621-1322 or rgraff1@email.unc.edu or jmallen@email.unc.edu.

Again, thank you for your participation!
PERCEIVED STRESS SCALE

Sheldon Cohen

The Perceived Stress Scale (PSS) is the most widely used psychological instrument for measuring the perception of stress. It is a measure of the degree to which situations in one's life are appraised as stressful. Items were designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives. The scale also includes a number of direct queries about current levels of experienced stress. The PSS was designed for use in community samples with at least a junior high school education. The items are easy to understand, and the response alternatives are simple to grasp. Moreover, the questions are of a general nature and hence are relatively free of content specific to any subpopulation group. The questions in the PSS ask about feelings and thoughts during the last month. In each case, respondents are asked how often they felt a certain way.

Evidence for Validity: Higher PSS scores were associated with (for example):
- failure to quit smoking
- failure among diabetics to control blood sugar levels
- greater vulnerability to stressful life-event-elicited depressive symptoms
- more colds


Temporal Nature: Because levels of appraised stress should be influenced by daily hassles, major events, and changes in coping resources, predictive validity of the PSS is expected to fall off rapidly after four to eight weeks.

Scoring: PSS scores are obtained by reversing responses (e.g., 0 = 4, 1 = 3, 2 = 2, 3 = 1 & 4 = 0) to the four positively stated items (items 4, 5, 7, & 8) and then summing across all scale items. A short 4 item scale can be made from questions 2, 4, 5 and 10 of the PSS 10 item scale.

Norm Groups: L. Harris Poll gathered information on 2,387 respondents in the U.S.

<table>
<thead>
<tr>
<th>Norm Table for the PSS 10 item inventory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>18-29</td>
</tr>
<tr>
<td>30-44</td>
</tr>
<tr>
<td>45-54</td>
</tr>
<tr>
<td>55-64</td>
</tr>
<tr>
<td>65 &amp; older</td>
</tr>
<tr>
<td>Race</td>
</tr>
<tr>
<td>white</td>
</tr>
<tr>
<td>Hispanic</td>
</tr>
<tr>
<td>black</td>
</tr>
<tr>
<td>other minority</td>
</tr>
</tbody>
</table>

Copyright © 1994. By Sheldon Cohen. All rights reserved.
**Perceived Stress Scale**

The questions in this scale ask you about your feelings and thoughts *during the last month*. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

Name ___________________________  Date _____________

Age ________  Gender (*Circle*): M F Other ___________________________


<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Almost Never</th>
<th>Sometimes</th>
<th>Fairly Often</th>
<th>Very Often</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please feel free to use the *Perceived Stress Scale* for your research.

**Mind Garden, Inc.**
info@mindgarden.com
www.mindgarden.com

**References**
Appendix H

Data Collection Form: Visit 1

Subject:

ID:__________________________

Height:____________________ (cm)

Weight:____________________ (kg)

Resting HR_________ (BPM)     Heart rate reserve (50%)____________________(HRR)

Resting BP____________ (mmHg)

Body fat (%): __________

One Repetition Max:

Leg Press:_______________ (lbs)          Machine Settings:_______________

Lateral Pull Down:_______________ (lbs)          Machine Settings:_______________

Leg Extension :_______________ (lbs)          Machine Settings:_______________

Seated Row:_______________ (lbs)          Machine Settings:_______________

Post-Exercise Measures:

Final HR____________ (BPM)

Final BP_______________ (mmHg)
Appendix I

Data Collection Form: Visit 2

Subject:

ID: ______________________

Resting HR________ (BPM)  Heart rate reserve (50%)________(HRR)

Resting BP________ (mmHg)

Time of Exercise Onset: ______________

Resistance Training Protocol:

<table>
<thead>
<tr>
<th></th>
<th>Leg Press</th>
<th>Lat Pull Down</th>
<th>Leg Extension</th>
<th>Seated Row</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(lbs)</td>
<td>(lbs)</td>
<td>(lbs)</td>
<td>(lbs)</td>
</tr>
<tr>
<td>1st Set</td>
<td>HR:</td>
<td>HR:</td>
<td>HR:</td>
<td>HR:</td>
</tr>
<tr>
<td></td>
<td>RPE:</td>
<td>RPE:</td>
<td>RPE:</td>
<td>RPE:</td>
</tr>
<tr>
<td>Rest Time</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
</tr>
<tr>
<td>2nd Set</td>
<td>HR:</td>
<td>HR:</td>
<td>HR:</td>
<td>HR:</td>
</tr>
<tr>
<td></td>
<td>RPE:</td>
<td>RPE:</td>
<td>RPE:</td>
<td>RPE:</td>
</tr>
<tr>
<td>Rest Time</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
</tr>
<tr>
<td>3rd Set</td>
<td>HR:</td>
<td>HR:</td>
<td>HR:</td>
<td>HR:</td>
</tr>
<tr>
<td></td>
<td>RPE:</td>
<td>RPE:</td>
<td>RPE:</td>
<td>RPE:</td>
</tr>
<tr>
<td>Rest Time</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
</tr>
</tbody>
</table>

Post-Exercise Measures:

Final HR________ (BPM)

Final BP________ (mmHg)

Time of Exercise Completion: ______________
References


