EXERCISE-INDUCED ALTERATIONS IN IMMUNOGLOBULIN (IgA, IgG, IgM) LEVELS IN CANCER VERSUS NON-CANCER PATIENTS
A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF SCIENCE
BY
LISA K. SELLERS
DR. HEATHER A. BRUNS
BALL STATE UNIVERSITY
MUNCIE, INDIANA
DECEMBER 2008
Acknowledgements

I want to take this opportunity to thank those people who have been crucial to my growth as a student, a scientist, and a person. First, I would like to thank Dr. Heather Bruns for taking on this project and working with me. I have learned new skills that I would have never acquired to otherwise. She is an inspiration and an exceptional mentor. I would not hesitate to work with her again. I would also like to thank Dr. Sue McDowell for her expert contribution with the statistical analysis for this project. She cultivated my ability to think critically and assess all possibilities. Dr. Carole Schneider, University of Northern Colorado, was kind enough to agree to work with me and send her samples for analysis. Without her, this project would be non-existent.

Thank you to Dr. Robert Hammersmith and Dr. C. Ann Blakey for their support and guidance during my time at Ball State. They are extremely intelligent individuals with a great love for genetics. I was able to learn the value of hard work and how to work independently given the right tools.

My family and my boyfriend, Luke, gave me lots of love and support as I wrote my thesis and worked two jobs. Without them I would not be where I am today. I would also like to thank all the graduate students that I have had the pleasure of working with. My sanity would be gone without you.

I would like to acknowledge The Little Red Door Cancer Services of Delaware County and Ball State University for funding this project.
# Table of Contents

**Introduction** .......................................................................................................................... 1  
Benefits of Exercise ..................................................................................................................... 1  
Impact of Exercise on Immune Function .................................................................................... 3  
Function and Description of Immune System ............................................................................. 4  
Health of Cancer Patients ......................................................................................................... 7  
Cancer, Exercise, and Immune Function ................................................................................... 10  
Significance of Project ............................................................................................................... 16  

**Materials and Methods** ......................................................................................................... 18  
Acquisition of Samples .............................................................................................................. 18  
Enzyme-Linked ImmunoSorbent Assay (ELISA) ..................................................................... 19  
Statistical Analysis .................................................................................................................. 21  

**Results** ..................................................................................................................................... 23  
Figure 1 ....................................................................................................................................... 30  
Figures 2 and 3 ............................................................................................................................ 31  
Figures 4 and 5 ............................................................................................................................ 32  
Figures 6 and 7 ............................................................................................................................ 33  
Figure 8 ....................................................................................................................................... 34  

**Discussion** .............................................................................................................................. 35  
Future Direction ......................................................................................................................... 40  

**References** ............................................................................................................................. 41
**Introduction**

The purpose of this study was to determine the effects of an eight week aerobic exercise training program on the mucosal immune system of cancer survivors compared to non-cancer participants. It was hypothesized that the immune system of the cancer patients will positively respond to a moderate exercise program, which can result in a decreased susceptibility to other illnesses, a decrease in fatigue, and enhancement of the quality of life of the survivors. Studies have been done in this general area of investigation, but the results show conflicting reports of benefit and little change. Very few studies to date have compared the effect of exercise on the immune systems of cancer and non-cancer participants. Most studies have focused on cancer survivors in exercise and non-exercise experimental groups. Basic science research is needed in this area to determine which physiological functions are altered by exercise in cancer survivors and if cancer survivors respond differently to exercise compared to non-cancer participants.

**Benefits of Exercise**

Exercise can have a positive effect on the health of an individual if the proper precautions are taken. Before beginning an exercise program a full physical evaluation by a physician is recommended. Benefits of exercise extend to all systems of the body and can decrease mental distress. Regular exercise increases heart strength making it
easier for the muscle to pump blood through the body. As the heart is conditioned, an individual’s resting heart rate decreases therefore putting less strain on the heart to do the same amount of work. Exercise can also increase the body’s ability to get rid of harmful by-products such as carbon dioxide. Muscle fatigue is decreased, body fat lowered, and hormone and cortisol responses are decreased. Exercise can affect many systems of the body and can facilitate balance between them [1].

Exercise can be aerobic, generally lasting at least twenty minutes and uses oxygen in metabolic and energy producing processes, or anaerobic, short bouts of exhaustive exercise where glucose or glycogen is used for energy without the use of oxygen [2]. When exercise is prescribed the frequency, intensity, and duration must be taken into consideration. The frequency of the exercise intervention refers to how many times per week the exercise will occur. General recommendations for frequency are at least three times per week with no more than two days of consecutive rest. The intensity of the exercise program will vary according to the specific goals of the individual [1]. A cancer patient may have the goal to walk up two flights of stairs, but this is an everyday occurrence for most people. Cancer patients will most likely be prescribed a moderate exercise program, but that same program would not benefit an elite athlete training for the Olympics. Elite athletes would need a training program of speed, strength, and endurance goals specific to their sport that is much more intense compared to the exercise program of a recovering cancer patient. Intensity can be measured as a percentage of an individual’s heart rate maximum. Duration is the final aspect of the exercise program to take into consideration. Again, this varies depending on the training goals set for the individual person. ACSM guidelines recommend that a generally healthy person exercise
for twenty to thirty minutes per session. Exercise should be fun and challenging without starting or progressing too aggressively [1].

Impact of Exercise on Immune Function

Exercise generally causes temporary or long-term immunosuppression, measured by a decrease in lymphocyte number, natural killer (NK) cell activity, and IgA levels [3], in healthy individuals if the exercise is too strenuous [3-7]. The innate and adaptive immune systems show signs of suppression after intense activity. Upper respiratory infections are more prevalent in athletes during intense training periods and up to a few weeks after an athletic contest [5]. This finding supports the “open window” theory that suggests there is a window of opportunity for infection to establish during the period of immunosuppression (three to seventy-two hours) that follows exercise [8]. Salivary immunoglobulin levels are thought to return to baseline resting levels one hour or more after exercise. The rate that immunoglobulin levels return to normal could be an indicator of how well the immune system can cope with the stress of exercise [7]. Moderate exercise is thought to have a positive effect on immune function [8], which could narrow the window of opportunity for infection.

Moderate exercise has shown to have varied affects on different measures of immune function, specifically immunoglobulins (IgA, IgG, IgM). Some studies show that IgA is not affected by exercise that is less than exhaustive [4]. Phagocytosis, chemotaxis, and oxidative burst activity have also been shown to be positively influenced by moderate exercise. NK cell activity is generally greater in athletes than individuals who are not active [8]. Active individuals also show an enhanced immune function due
to repeated lymphocyte elevation and an increase in norepinephrine, which could boost antibody production [6].

The type of exercise may also influence immune function [9], but exercise intensity and duration have the most impact on modulating immune parameters. An extended recovery period between exercise sessions is associated with a more complete restoration of immune parameters to baseline levels. One session of moderate exercise has been shown to alter immune parameters up to several hours, but moderate exercise does still show varied immune system alterations. Less is known about the effect on the immune system of several consecutive exercise sessions [4]. Immune function is an important area to study due to its overall affect on the ability of the human body to function at optimal capacity. Since individuals with infections and chronic illnesses are not functioning optimally, the immune system could be the gateway to an improved quality of life.

Function and Description of the Immune System

The immune system is divided into two parts, innate and adaptive. Innate immunity is the non-specific defense system that your body naturally has before exposure to an antigen or outside pathogen. It provides the first defense reaction against the invading molecule. The adaptive immune system involves a response to an antigen or pathogen that is controlled by B and T cells. The adaptive immune system is specific and has a memory response within a week of exposure to the pathogen [10]. The immune system is also responsible for recognizing and killing malignant cells [11].

Natural killer (NK) cells are cytotoxic white blood cells that are components of the innate immune system. These cells kill malignant and infected cells and can also kill
other cell types with the help of antibodies (antibody-dependent cellular cytotoxicity) [10]. NK cells are the type of immune cell to first respond to the development of malignant cells [11, 12] but are not very effective against very large masses of malignant cells [12]. NK cells also significantly increase in number immediately following exercise and resting NK cell activity has been enhanced with exercise. T cells can kill malignant cells but do so with the help of antibodies [11, 12].

There are different types of T cells, which are lymphocytes that mature in the thymus, that can be helper, suppressor, or cytotoxic cells. T helper cells facilitate immune communication by recognizing antigens and secreting cytokines that regulate the strength and extent of an immune response. T suppressor cells repress the humoral and cell-mediated immune function through antigen recognition to prevent unnecessary and harmful autoimmune reactions from occurring. T cytotoxic cells have the ability to directly kill target cells. Cytokines can regulate cells of both the innate immune system and the adaptive immune system [3, 10]. Up-regulation of cytokines such as interleukins, interferons, and tumor necrosis factor-α can cause inflammation, fever, and loss of body fat or muscle [10].

B cells are also lymphocytes, which mature in bone marrow and differentiate into antibody-secreting plasma cells or memory cells. B cells are activated in response to contact with foreign substances and produce antibodies also known as immunoglobulins [6, 10]. Antibodies mark foreign substances for ingestion or destruction by other components of the immune system. Depending on the nature of the foreign substance, B cells switch their antibody production from one class to another [10].
IgA, IgG, and IgM are three of five classes of immunoglobulins, and these are the three which are most likely to be found in secretions (mucosal immune system) and blood (humoral immune system). Immunoglobulins are glycoproteins that can perform a variety of functions in defense of the host. IgA is the immunoglobulin class that is present in large quantities in secretions, particularly saliva [3, 4, 6, 13, 14]. Salivary immunoglobulins have been shown to respond differently than serum immunoglobulins [3, 6] because they are independently regulated [6]. IgA that is in saliva (dimer) is structurally different from IgA in serum (monomer). Importantly, the dimeric form of IgA allows it to bind and sequester pathogens in mucosal tissues and secretions, preventing the invasion and subsequent infection by pathogens [13]. The concentration of IgA is known to more frequently vary in short amounts of time compared to other immunoglobulins, and IgA is a component of the adaptive immune system [14]. IgG is the antibody primarily responsible for the memory immune response and is found in large amounts in blood serum. IgM is the antibody predominantly responsible for the primary immune response and has antibacterial properties [3, 6].

All isotypes of immunoglobulins share a basic common structure of light (MW 25kD) and heavy chains (MW 50-77 kD). The light chains are comparable in all isotypes, but the immunoglobulin structure differs in the formation of the heavy chain. Depending on the isotype, antibodies are responsible for several functions such as binding to an antigen or bacterial toxin and being a messenger in the cascade of molecular events that regulate the immune system. Antibodies also can inhibit bacteria and viruses from entering a cell or facilitate phagocytosis and cytotoxicity through T and
NK cells. The injection of antibodies has also been used as a treatment in patients with a lowered immune function due to disease or infection [6].

**Health of Cancer Patients**

The number of cancer survivors in the United States is increasing each year due to successful prevention, early detection, and more effective treatment options [15-17]. Lifestyle modifications have been shown to be the best way to promote overall health and functional rehabilitation in cancer patients [17]. One study suggests that cancer patients have a lack of information on exercising with cancer. The same study purposes that oncologists could play a more important role in referring patients to the appropriate resources to begin an exercise program [18]. To further aid in the recovery and survival of cancer patients, more effective post-treatment care needs to be developed and implemented based on the health issues of cancer survivors. The post-treatment care should include information and programs supporting moderate exercise, health promoting habits, and any other post-treatment issues that may arise.

Mental distress is one post-treatment issue that cancer survivors may face with little guidance. Treatment for mental distress and depression can include counseling, medication, meditation, and exercise. Exercise has been shown to decrease depression and increase self-esteem in cancer survivors [19]. Patients who exhibit low physical performance are generally associated with a greater susceptibility to depression [20], indeed one study observed a significant reduction in fatigue and mental distress while exercising during chemotherapy treatment [21]. Psychological symptoms can be as influential as physical symptoms in beginning and maintaining an exercise program.
Fatigue and the ability to return to pre-cancer activity levels are two of the most common struggles survivors are faced with today. One study found no evidence linking fatigue and immune function in patients with hematological malignancies post-treatment but did find a relationship between fatigue, increased depression, and a reduced ability to perform physical tasks [22]. Both of these issues have been shown to improve if patients participate in a moderate exercise program [11, 19, 23]. Patients are also faced with a progressive loss of function, decreased muscle performance, skin irritation, and other side-effects from radiation, chemotherapy, and/or surgery. These same struggles must be taken into account when prescribing an exercise program and must be monitored at each session. If the patient shows any signs contradictory to exercise, the patients’ needs must be assessed and the exercise program adjusted. Intestinal disturbances, electrolyte imbalances, fever, low white blood cell count, and low red blood cell count can all contribute to an increase in the risk to infection and make it more difficult to maintain a consistent exercise schedule. Cancer patients must be properly evaluated with a complete medical history and clearance from a physician before a moderate exercise program is prescribed [17, 24]. Cancer patients exhibit a variety of post-treatment health concerns that could be alleviated with moderate exercise and patients who moderately exercise have shown a decreased probability in developing cancer.

Individuals who exercise regularly at moderate intensities have less of a chance to be diagnosed [4] with colon or breast cancer [3, 25]. This could be because exercise decreases body fat and can decrease body weight protecting against obesity. Excess body fat and obesity both carry an additional risk of developing cancer. However, few studies have investigated exercise providing a protective effect against the negative side-
effects of cancer treatment [19]. Aerobic exercise was also found to enhance physical performance and somewhat preserve physical function while patients were undergoing intense chemotherapy and stem cell transplantation [26, 27]. Potential biomarkers for investigation of the molecular events that occur after cancer treatment are NK cells, interleukin-1, tumor necrosis factor α, and white blood cell cytolytic activity [28]. Researchers would look for alterations in biomarkers to determine if exercise facilitates positive changes in immune function during and after treatment. Another possible angle would be to look at biomarkers before treatment, during treatment, and after treatment in exercise and non-exercise groups. Moderate exercise has been shown to facilitate positive physical and psychological changes in cancer patients.

In addition to stimulating immune function to protect against aberrant conditions such as cancer, it has also been demonstrated that moderate exercise is beneficial to the body’s ability to fight infection, while highly intensive exercise can decrease immune system function and cause a greater susceptibility to disease and illness [4, 16, 23, 29]. Cancer survivors already have a lowered immunity due to treatment and the natural course of the disease [30]. Cancer patients have lower blood cell counts, specifically white blood cells [30]. Cancer treatment kills all rapidly dividing cells such as cancer, hair, nail, mouth, intestinal cells, and importantly immune cells. This is responsible for immunosuppression therefore increasing the patients’ susceptibility to infection and disease. Having a normal functioning immune system is important because it protects against any foreign body that can cause illness or increase one’s susceptibility to becoming ill. Antibodies are released by the body to mark substances that are not supposed to be there so that they can be destroyed by the immune system. Low levels of
antibodies mean that the body cannot function or fight infection and disease at an optimal level [29, 31]. It has been shown that patients who undergo a bone marrow transplant and exercise regenerate white blood cells quicker, need fewer red blood cell transfusions, have fewer infections, and are discharged sooner therefore saving the healthcare system money [30]. Exercise may stimulate blood circulation enough to increase the interaction between immune cells and malignant cells [3] creating a relationship between exercise, the immune system, and malignant cells.

Taken together these findings demonstrate that cancer patients need care beyond the treatment phase of the disease due to an increase in mental distress, a decrease in physical function, an increase in fatigue, and a suppressed immune function, which leaves the patient more susceptible to infection. Moderate exercise has been shown to alleviate these problems as well as assist patients in returning to pre-cancer activity levels and protecting them from infection by enhancing immune function.

Cancer, Exercise, and Immune Function

Studies examining immune function, specifically immunoglobulin production, have shown varied results due to the lack of standard methods. Studies differ in the type and duration of exercise, length of exercise intervention, type of cancer, treatment received [16], measure of immune function [32], and there is no standard method for reporting immunoglobulin levels [3, 23, 33, 34]. The type and stage of cancer a patient has and the treatment protocol followed may be a factor because most studies are not grouped accordingly. Different types and treatments of cancer may have a different affect on immune function as well as how the disease has metastasized. Cancer treatment can decrease B cell function, specifically immunoglobulin production, decrease NK cell
activity, and impair T cell function [16]. Since the subject pool is already small due to cancer related complications that result in patients dropping out of a study, it can be a challenge to set up appropriate controls. This generally results in a study with no controls or no matched controls [16, 35]. In measuring immune function, it is important to have age and gender matched individuals due to general differences between male and female individuals or young and old patients. Body mass index and the use of contraceptives and hormone replacement therapy can have an effect on the outcome of measuring immune parameters [32]. Another concern of investigators is the type of physical fitness assessment that is being used because there are several standard protocols that could be administered [16]. Investigators can choose a variety of parameters for a study involving cancer patients, exercise, and immune function, which makes the field less consistent and ever evolving.

As the primary way to assess immune function, B and T cell activity have been investigated by quantifying antibody and cytokine production, respectively, [3] using both human and mouse models. Few immunology studies have been done using a mouse model due to the complex cascade of events that are altered by exercise and cancer in the human body. One study investigated the effect of six weeks of voluntary exercise on a wheel and food restriction on the humoral and mucosal immune system using female C57BL/6 mice. The mice that exercised showed an increase in T cell and mucosal cytokine production but no change in the weight or body fat of the mice. This study demonstrated that the immune system can be altered without significant changes in body weight or body fat [36]. The only other study found involving a mouse model investigated the effect of moderate exercise on the humoral immune system of older
mice. Young (2-4 months) and old (16-18 months) mice were infected with HSV-1 and exercised on a treadmill for eight weeks at moderate intensity to investigate the ability of the mice to fight a viral infection. The young mice showed no effect to the exercise, but the older mice showed signs of an increased immune response seven days after infection. The older mice showed an increase in interferon-γ, interleukin-2, and HSV-1 specific Th2-associated cytokines, but no change was seen in IgM [37]. Some immune components are enhanced by exercise in mice, but they are incomplete because not all questions about a human disease, specifically cancer, can be answered using a mouse model. Thus, studies using a human model are more prevalent because methods to study the immune system are less invasive than other systems and more relevant to the main issue at hand.

Several measures of immune function have been used to quantify exercise-induced immune alterations in humans such as NK cell activity, lymphocyte apoptosis, antibody response to a viral infection, and immunoglobulin levels. In women with breast cancer, seven months of moderate exercise increased resting NK cell activity levels [11]. In addition to NK cells, lymphocytes have been used as a measure of immune response. Lymphocyte apoptosis, which would cause a decrease in immune function, was measured in healthy individuals after two treadmill tests. One test was to simulate moderate exercise (60% of maximal oxygen uptake) and the other was to simulate exhaustive exercise (80% of maximal oxygen uptake). Lymphocyte apoptosis was significantly increased directly after exercising at 80% of the maximal oxygen uptake, but no change was seen after exercising at 60% of the maximal oxygen uptake [38]. In parallel with the mouse study previously described, the effect of exercise on the immune function of aging
people was investigated. Specifically, anti-influenza antibodies were analyzed to determine the immune response to vaccination against the flu. Participants exercised three days per week for ten months at 65-75% heart rate reserve as a measure of moderate exercise. Individuals who exercised had a significantly higher antibody reaction to the flu vaccine. This suggests that older individuals who exercise have a more responsive immune system compared to their sedentary counterparts [39]. In general, there is an increase in NK cell activity with moderate exercise, an increase in lymphocyte apoptosis with intense exercise, and an increase of antibody production in response to influenza with moderate exercise. Moderate and exhaustive exercise affects the immune system differently.

The degree of perceived intensity of an exercise program can vary from person to person depending on their health status and prior fitness level. A significant amount of research has been done to determine the effect of intense, exhaustive exercise on immune function due to the notion that it is detrimental to the immune function of all individuals. Due to the complexity of the human immune system, researchers have looked at several different immune components, but the most common immune components investigated are immunoglobulins. A few studies have shown IgA levels to immediately decrease after an intense training session, but not be affected with moderate exercise [4, 40]. Studies have shown that intense exercise can increase the risk of developing an upper respiratory tract infection (URTI) due to a decrease in immune function. The decrease in immune function is quantified by a decrease in B and T cell function and suppressed NK cell function. An increase in cytokines and a decrease in mucosal IgA are also reported. The suppression due to intense exercise can last up to two weeks post-exercise [5]. A
nineteen-week study of military parachuters did not show any difference in IgA concentration or types of lymphocytes present compared to their non-active counterparts. The evidence was inconclusive as to the prevalence of URTI due to intense activity [41].

Over a seven-month training season, elite swimmers were tested for salivary IgA levels and risk to infection. IgA levels correlated inversely with infection rates in the elite swimmers and moderately exercising controls. As the concentration of salivary IgA decreased more infections were diagnosed [7, 42]. The data support a decrease in IgA as a possible key to the mechanism of protection against an URTI. After eight weeks of strenuous interval exercise, male participants showed a decrease in IgA and IgM levels immediately post-exercise, but there was no difference in immunoglobulin levels due to training. IgG levels did not change [43]. Salivary IgA levels also decreased significantly in male runners after exhaustive exercise testing and returned to normal levels within the hour. The runners trained for ten weeks and did a maximal treadmill test at week zero and ten. Conclusions from the study suggest that moderate exercise does not affect IgA levels, but maximal effort does significantly decrease IgA levels [44]. A second study supports the finding that salivary IgA levels decrease immediately post-exercise and return to normal levels within the hour [7, 45]. The ability of salivary immunoglobulin levels to return to resting levels in a short time period may be indicative of the ability of the immune system to cope with the stress of exercise [7]. The immune system responds differently to varied intensities of exercise. Moderately exercising runners did not show a change in salivary IgA levels, but elite runners training three consecutive days did show a decrease in IgA levels on days two and three of training [46]. Generally, IgA and IgM levels decrease after intense exercise, IgG levels stay constant, B, T, and NK cell function
decreases, and cytokines increase, which indicated a decrease in immune function that could lead to a greater susceptibility to infection.

IgA is the most common measure of the mucosal immune system with IgG and IgM being repeated less frequently in the literature [40]. Immunoglobulin levels may vary with the total amount of protein in the saliva and the saliva flow rate. The verdict is still out as to which measure of immunoglobulin levels is appropriate. Some investigators suggest the immunoglobulin secretion rate (μg/min) is the best measure due to varying saliva flow rates. Others still insist that the concentration is the best measure of immunoglobulin levels [14]. Moderate exercise does not seem to affect the salivary flow rate, while intense exercise may decrease saliva flow [7]. In studies using moderate exercise, actually immunoglobulin concentration is an appropriate measure of immunoglobulin levels. Many cancer survivors and patients show signs of immunosuppression [34] with one indicator being a decrease in salivary IgA as well as other immunoglobulins [4, 16, 29].

Moderate exercise has been shown to increase immune function indicated by an increase in immunoglobulin levels. One study involving women showed an increase in serum IgA, IgG, and IgM after engaging in moderate exercise [47]. Moderate exercise, five forty-five minute sessions per week of walking at 60% of heart rate reserve, for fifteen weeks significantly increased serum immunoglobulin (IgA, IgG, IgM) levels at six weeks of training and at fifteen weeks compared to the non-active control group. The women in the study had no previous physical activity and were slightly obese [48]. Other studies have shown that low to moderate intensity exercise increases salivary IgA levels suggesting an improvement in immune function in the elderly population studied. This
improvement was seen whether the program was long-term (12 months) or short-term (3 months) [49, 50]. A decrease in illness, specifically influenza, was seen in individuals that were moderately exercising for twelve weeks. The decrease in illness was also associated with an increase in salivary IgA [51]. Moderate exercise has been shown to increase immune function and possibly decrease the risk of infection. It is thought that a moderate exercise program will improve the immune function of cancer survivors [16] because moderate exercise has increased immunoglobulin levels in other populations indicating an enhancement of the immune system.

Significance of the Project

While there are many immune molecules to target, immunoglobulins are currently the most commonly investigated immune parameter. There has been little research investigating the immune function and response of cancer patients on a moderate exercise regimen. It is thought that cancer patients who undergo a moderate exercise program will show immune enhancement while non-cancer participants will show little to no change. To date no study has compared exercise-induced immunoglobulin alterations of cancer survivors and non-cancer participants as a measure of immune function. This is important to distinguish the molecular changes that occur throughout the body because of malignant cells and cancer treatment. It would be unproductive and dangerous to the health of the patients to assume cancer patients would respond to exercise the same as healthy individuals. Immune enhancement due to moderate exercise would be shown by an increase in immunoglobulin levels (IgA, IgG, IgM) in the cancer patients as opposed to the non-cancer participants.
The goal of this project was to identify exercise-induced changes in the mucosal immune system in cancer survivors compared to non-cancer participants. By identifying the effect exercise has on the immune system of cancer survivors compared to non-cancer participants, recommendations can be made to improve the survivors’ quality of life. The goal is to use these recommendations to positively influence the exercise habits of cancer survivors to benefit their overall health. Findings from this research could aide in identifying and preventing the events that lead to debilitating long-term fatigue in cancer survivors. The goal is to positively influence the exercise habits of cancer survivors to increase immune system function, decrease fatigue, and increase overall quality of life.
Materials and Methods

Acquisition of Samples

The saliva of five cancer and six non-cancer exercise-trained participants were analyzed for changes in levels of immunoglobulin isotypes (IgA, IgG, and IgM) to assess the effect of exercise on the immune response. The patients participated in a moderate aerobic exercise program supervised by trainers at the Rocky Mountain Cancer Rehabilitation Institute (RMCRI) at the University of Northern Colorado (UNCO) in Greeley, CO. The moderate exercise program consisted of training 40 minutes three times per week in a one-on-one setting. Each participant walked slowly for five minutes as a warm-up then continued with 30 minutes of walking at 55-77% of the participants’ heart rate maximum. Each session was concluded with a five minute cool down walk. The subjects performed an incremental peak treadmill test to exhaustion at the start of the program and after 8 weeks of training. Timed saliva samples were collected at each peak exercise test prior to testing, immediately post-exercise (IPE), and 30 minutes post-test [52]. All samples were analyzed for IgA, IgG, and IgM isotypes in quadruplicate and compared to human immunoglobulin controls in order to quantify immunoglobulin levels. Samples were not able to be analyzed at UNCO, but have been stored by Dr. Carole Schneider, director of the RMCRI.
Cancer patients included presented with a variety of malignancies and were six weeks or less out of treatment (surgery, radiation, and/or chemotherapy). It has been assumed that the burden of all tumors has been removed through treatment. Subjects were matched for age and gender and all participants were sedentary prior to the study. All participants involved in the study were given the same specifications for food intake, sleep, and when the last meal prior to the peak exercise test was to be consumed. Caffeine intake was restricted the day of testing for all participants. All participants involved in the study were not taking any mood enhancing medication or herbal supplements and did not smoke or consume alcohol. All study participants were cleared for exercise by their physician and signed an informed consent. The informed consent was approved by the University of Northern Colorado Institutional Review Board (IRB). This review board also approved the use of human subjects for all procedures. All research was conducted in accordance with all Federal and University guidelines and in compliance with Conflict of Interest Guidelines [52].

**Enzyme-Linked ImmunoSorbent Assay (ELISA)**

A sandwich ELISA was used to quantify IgA, IgG, and IgM levels in saliva samples to investigate potential differences in the immune response to exercise in cancer patients and non-cancer participants. All ELISAs were run using a human IgA, IgG, or IgM ELISA quantitation kit from Bethyl Laboratories (catalog no. E80-102) along with the Bethyl Laboratories ELISA Starter Accessory Package (catalog no. E101-127). The ELISA protocol consisted of six basic steps. The 96-well ELISA plate was coated with capture antibody that was either an anti-human IgA, IgG, or IgM unconjugated antibody (1 mg/mL), which was added to the plate at a dilution of 1:100 in 0.05M NaCO₃ (pH 9.6)
coating buffer (100 μL antibody in 10 mL coating buffer). The plates were incubated at 4°C overnight. Blocking/post-coat buffer (100 μL/well of 0.05mM Tris buffered saline + 1% BSA), for non-specific binding, was added to the plate following application of wash buffer (50mM Tris buffered saline, pH 8.0 + 0.05% Tween-20). Covered plates were incubated at room temperature for 2 hours then washed. Prior to the addition of samples to the ELISA plate, each sample was diluted in sample/conjugate diluent (0.5mL 10% Tween-20 in 100 mL blocking/post-coat buffer). Samples were diluted in different ratios depending on the isotype targeted for the assay and run in quadruplicate. Saliva contains different amounts of each isotype [6, 14, 40] and to optimize the results, different starting concentrations were used for each isotype. Samples to be analyzed for IgA were serially diluted 1:500, 1:2500, and 1:12500 with sample/conjugate diluent. Saliva for IgG was serially diluted 1:50, 1:250, and 1:1250. Saliva analyzed for IgM was serially diluted 1:10, 1:50, and 1:250. The purpose of the dilutions was to find the optimal working dilution for the sample in relation to the human reference serum that was used as the standard. The reference serum was run in the first two rows of each plate and serially diluted 1:2. IgA plates started with 1000 ng/mL (2 μL in 2.4 mL diluent), IgG 500 ng/mL (1 μL in 2.4 mL diluent), and IgM 2000 ng/mL (4 μL in 2.4 mL diluent). Plates were incubated 1-2 hours and washed before the addition of the detection antibody. Anti-human IgA, IgG, or IgM HRP-conjugated antibody (diluted 1:1000 in sample/conjugated diluent) was added to the plate (100 μL/well). The plates were washed prior to addition of the substrate for enzymatic color reaction. Equal parts of TMB peroxidase substrate and peroxidase solution B were mixed to form the substrate that was added 100 μL/well.
Plates were allowed to react 20-35 minutes until the enzymatic reaction was blue. Plates were then read using a Biorad plate reader with Microplate Manager Software to determine the immunoglobulin concentration of each sample compared to the standard run on each plate. Due to the use of human IgA, IgG, and IgM standards with the ELISA, concentrations of the human immunoglobulins in the saliva samples were able to be obtained.

**Statistical Analysis**

The study design consisted of two groups, cancer patients and non-cancer participants. The two groups were matched in all other variables except the presence or absence of cancer. Each participant partook in a moderate exercise program for eight weeks monitored by a cancer exercise specialist at RMCRI to an intensity of 55-77% of each individual’s heart rate maximum. Each person participated in an incremental peak treadmill test to exhaustion at the start of the program and after 8 weeks of training. Timed saliva samples were collected at each peak exercise test prior to testing, immediately post-exercise (IPE), and 30 minutes post-test.

To determine the effect of moderate exercise on the mucosal immune system, assessed by immunoglobulin (IgA, IgG, IgM) concentration determined by ELISA, on cancer patients and non-cancer participants, means and standard deviation for each group (cancer and non-cancer) at each timed sampling point were calculated in Microsoft Excel. Mean values were then compared with its matched value between baseline (week 0) immunoglobulin concentration and the eight week concentration for each immunoglobulin (IgA, IgG, IgM) at all timed sampling points. Comparisons were measured with a paired, two-tailed students’ t-test in Microsoft Excel. All values for
each immunoglobulin isotype were averaged and compared using Sigma Stat. Averages for IgA, IgG, and IgM were compared using a one-way ANOVA and multiple comparisons were run using the Student-Newman-Keuls Method. P-values of <0.05 were considered significant.
Results

IgA, IgG, and IgM are three of five classes of immunoglobulins. Concentrations of these three were specifically analyzed because IgA is the immunoglobulin class that is present in large quantities in secretions, particularly saliva [3, 4, 6]. IgG is the antibody primarily responsible for the memory immune response and is found in large amounts in blood serum. IgM is the antibody predominantly responsible for the primary immune response [3, 6]. Few studies have been done on the presence of IgG and IgM in human saliva, but it is possible that these isotypes could give us information regarding exercise-induced immune alterations. These immunoglobulins can be detected and quantified by an Enzyme-Linked ImmunoSorbent Assay (ELISA), which is relatively quick to produce results.

The saliva of five cancer patients and six non-cancer participants were analyzed for alterations in levels of immunoglobulin isotypes (IgA, IgG, and IgM) to assess the effect of moderate exercise on immune function. The participants participated in an eight week moderate aerobic exercise program supervised at the RMCRI. The subjects performed an incremental peak treadmill test to exhaustion at the start of the program (week 0) and after 8 weeks of training. Timed saliva samples were collected at each peak exercise test prior to testing, immediately post-exercise (IPE), and 30 minutes post-test [52]. All samples were analyzed for IgA, IgG, and IgM isotypes and compared to human immunoglobulin controls in order to quantify immunoglobulin levels.

To examine exercise-induced immunoglobulin alterations an ELISA was used to assess any changes in IgA, IgG, and IgM concentrations between cancer patients and
non-cancer participants (Figure 1). IgA concentrations were not different between cancer patients and non-cancer participants at any of the testing points: baseline (week 0) pre-exercise test (1), immediately post-test (2), and 30 minutes post-test (3); week 8 pre-exercise test (4), immediately post-test (5), and 30 minutes post-test (6) (Figure 1A). At each time point respectively, cancer patients IgA concentrations (ng/mL) (± standard deviation) were 1.2 (± 0.5), 1.3 (± 0.1), 0.9 (± 0.2), 1.2 (± 0.2), 1.1 (± 0.4), and 1.3 (± 0.2) (Figure 1A). Non-cancer patient IgA concentrations (ng/mL) (± standard deviation) were 1.2 (± 0.3), 1.0 (± 0.3), 0.9 (± 0.5), 1.1 (± 0.2), 1.1 (± 0.3), and 1.2 (± 0.4) (Figure 1A). IgG concentrations were not different between cancer patients and non-cancer participants at any of the testing points: baseline (week 0) pre-exercise test (1), immediately post-test (2), and 30 minutes post-test (3); week 8 pre-exercise test (4), immediately post-test (5), and 30 minutes post-test (6) (Figure 1B). At each time point respectively, cancer patients IgG concentrations (ng/mL) (± standard deviation) were 6.2 (± 1.6), 6.2 (± 2.0), 6.8 (± 2.4), 7.4 (± 1.8), 6.8 (± 1.9), and 8.5 (± 3.5) (Figure 1B). Non-cancer patient IgG concentrations (ng/mL) (± standard deviation) were 6.3 (± 1.0), 4.6 (± 1.4), 7.0 (± 0.4), 6.8 (± 1.3), 4.6 (± 1.2), and 6.5 (± 0.9) (Figure 1B). IgM concentrations were not different between cancer patients and non-cancer participants at any of the testing points: baseline (week 0) pre-exercise test (1), immediately post-test (2), and 30 minutes post-test (3); week 8 pre-exercise test (4), immediately post-test (5), and 30 minutes post-test (6) (Figure 1C). At each time point respectively, cancer patient IgM concentrations (ng/mL) (± standard deviation) were 143.8 (± 64.1), 131.8 (± 55.5), 107.2 (± 65.2), 140.1 (± 47.4), 152.0 (± 21.5), and 141.2 (± 59.5) (Figure 1C). Non-cancer patient IgM concentrations (ng/mL) (± standard deviation) were 123.6 (± 51.2), 85.1 (±
61.0), 76.9 (+ 65.6), 103.4 (+ 66.8), 110.0 (+ 27.5), and 86.3 (+ 48.5) (Figure 1C). Results show that there were no exercise-induced differences between cancer patients’ and non-cancer participants’ immunoglobulin concentrations at each individual time point. Since there were no significant differences between cancer patients and non-cancer participants, immunoglobulin changes were analyzed within individual patient groups (cancer or non-cancer) between baseline (week 0) and week 8 for each isotype (IgA, IgG, IgM) at each time point.

Exercise-induced IgA alterations in non-cancer participants were analyzed to determine if there was a training effect after 8 weeks of moderate exercise on immune function (Figure 2). The average IgA concentration of non-cancer participants detected by ELISA did not change after 8 weeks of moderate exercise prior to the treadmill test (Figure 2A), immediately post-test (Figure 2B), and 30 minutes after the test (Figure 2C). Prior to the treadmill test the average IgA value at week 0 was 1.2 ng/mL and week 8 was 1.1 ng/mL (Figure 2A). Immediately following the treadmill test the average IgA value at week 0 was 1.0 ng/mL and week 8 was 1.1 ng/mL (Figure 2B). Thirty minutes after the treadmill test the average IgA value at week 0 was 0.9 ng/mL and week 8 was 1.2 ng/mL (Figure 2C). Non-cancer participants’ salivary IgA alterations were not significant in response to 8 weeks of moderate exercise at any of the time points.

To determine if a training effect after 8 weeks of moderate exercise was present in cancer patients, IgA concentrations were measured (Figure 3). The average IgA concentration of cancer patients assessed by ELISA did not change after 8 weeks of moderate exercise prior to the treadmill test (Figure 3A) and immediately post-test (Figure 3B), but did exhibit a significant increase 30 minutes after the test (Figure 3C,
p<0.05). In cancer patients, prior to the treadmill test the average IgA value at week 0 and week 8 was 1.2 ng/mL (Figure 3A). Immediately following the treadmill test the average IgA value at week 0 was 1.3 ng/mL and week 8 was 1.1 ng/mL (Figure 3B). Thirty minutes after the treadmill test the average IgA value at week 0 was 0.9 ng/mL and week 8 was 1.3 ng/mL (p<0.05) (Figure 3C). Increases in cancer patients’ salivary IgA were significant in response to 8 weeks of moderate exercise at the 30 minutes post-test time point.

To examine IgG alterations in non-cancer participants after 8 weeks of moderate exercise IgG concentrations were analyzed (Figure 4). The average IgG concentration of non-cancer participants detected by ELISA showed a significant decrease following 8 weeks of moderate exercise prior to the treadmill test (Figure 4A) and an increase 30 minutes after the test (Figure 4C) (p<0.05). IgG concentrations did not change immediately post-test (Figure 4B). In non-cancer participants, prior to the treadmill test the average IgG value at week 0 was 6.3 ng/mL and week 8 was 4.6 ng/mL (p<0.05) (Figure 4A). Immediately following the treadmill test the average IgG value at week 0 was 6.9 ng/mL and week 8 was 6.8 ng/mL (Figure 4B). Thirty minutes after the treadmill test the average IgG value at week 0 was 4.6 ng/mL and week 8 was 6.5 ng/mL (p<0.05) (Figure 4C). Non-cancer participants had decreased concentrations of salivary IgG that were significant in response to 8 weeks of moderate exercise at rest, and IgG concentrations significantly increased at the 30 minutes post-test time point.

The IgG concentrations of cancer patients were analyzed to determine if 8 weeks of moderate exercise enhanced immune function (Figure 5). The average IgG concentration of cancer patients detected by ELISA did not change after 8 weeks of
moderate exercise prior to the treadmill test (Figure 5A), immediately post-test (Figure 5B), and 30 minutes after the test (Figure 5C). In cancer patients, prior to the treadmill test, the average IgG value at week 0 was 6.2 ng/mL and week 8 was 6.2 ng/mL (Figure 5A). Immediately following the treadmill test, the average IgG value at week 0 was 6.8 ng/mL and week 8 was 7.4 ng/mL (Figure 5B). Thirty minutes after the treadmill test the average IgG value at week 0 was 6.8 ng/mL and week 8 was 8.5 ng/mL (Figure 5C). Cancer patients’ salivary IgG alterations were not significant in response to 8 weeks of moderate exercise in cancer patients at any of the time points tested.

To examine IgM alterations due to 8 weeks of moderate exercise ELISA was used to quantify IgM concentrations (Figure 6). The average IgM concentration of non-cancer participants did not change after 8 weeks of moderate exercise prior to the treadmill test (Figure 6A), immediately post-test (Figure 6B), and 30 minutes after the test (Figure 6C). In non-cancer participants, prior to the treadmill test the average IgM value at week 0 was 123.6 ng/mL and week 8 was 85.1 ng/mL (Figure 6A). Immediately following the treadmill test the average IgM value at week 0 was 76.9 ng/mL and week 8 was 103.4 ng/mL (Figure 6B). Thirty minutes after the treadmill test the average IgM value at week 0 was 110.0 ng/mL and week 8 was 86.3 ng/mL (Figure 6C). Non-cancer participants’ salivary IgM alterations were not significant in response to 8 weeks of moderate exercise at any of the time points.

To examine immune alterations in cancer patients after 8 weeks of moderate exercise IgM concentrations were analyzed by ELISA (Figure 7). The average IgM concentration of cancer patients did not change after 8 weeks of moderate exercise prior to the treadmill test (Figure 7A), immediately post-test (Figure 7B), and 30 minutes after
the test (Figure 7C). In cancer patients, prior to the treadmill test the average IgM value at week 0 was 143.8 ng/mL and week 8 was 131.8 ng/mL (Figure 7A). Immediately following the treadmill test the average IgM value at week 0 was 107.2 ng/mL and week 8 was 140.1 ng/mL (Figure 7B). Thirty minutes after the treadmill test the average IgM value at week 0 was 152.0 ng/mL and week 8 was 141.2 ng/mL (Figure 7C). Cancer patients’ salivary IgM alterations were not significant in response to 8 weeks of moderate exercise at any of the time points tested.

To specifically examine the relative concentrations of IgA, IgG, and IgM in the saliva samples analyzed by ELISA, the concentrations of each antibody in the human saliva for all time points were averaged to get an average concentration for each isotype (Figure 8). IgA is supposed to be the most concentrated antibody in secretions such as saliva. However, the data demonstrate that IgA was not the most abundant or concentrated antibody. Analysis of immunoglobulin concentrations show there was a significantly higher concentration of IgM (115 ng/mL) in the saliva of non-cancer participants and cancer patients compared to IgA (1.1 ng/mL) and IgG (6.4 ng/mL). IgG concentrations were significantly higher than IgA concentrations in the saliva (p<0.05) (Figure 8). The finding that IgM concentrations were significantly higher than IgA and IgG concentrations in human saliva was unexpected and contradictory to published findings.

In conclusion, the IgA concentration of cancer patients increased from baseline (week 0) to week 8 at the 30 minute post-test time point due to moderate exercise. The IgG concentration of non-cancer participants decreased pre-exercise test and increased 30 minutes post-test in response to moderate exercise. IgA and IgG concentrations were
able to be altered with moderate exercise in cancer patients and non-cancer participants depending on the sample time.
Figure 1. Levels of immunoglobulins (IgA, IgG, and IgM) in human saliva were not different between cancer patients and non-cancer participants in response to moderate exercise. Saliva samples were obtained from patients at designated times during 8 weeks of a moderate exercise program—Week zero: pre-exercise test (1), immediately post-test (2), 30 minutes after the test (3), and after 8 weeks of moderate exercise: pre-exercise test (4), immediately post-test (5), and 30 minutes after the test (6). Samples were analyzed by ELISA for immunoglobulin (IgA, IgG, IgM) levels. Analysis of salivary IgA (Figure 1A), IgG (Figure 1B), and IgM (Figure 1C) show there was no difference in the amounts of salivary antibodies between cancer and non-cancer patients who moderately exercise.
**Figure 2.** Non-cancer participants’ salivary IgA alterations were not significant in response to 8 weeks of moderate exercise at any of the time points tested.

The average IgA concentration of non-cancer participants detected by ELISA did not change after 8 weeks of moderate exercise prior to the treadmill test (Figure 2A), immediately post-test (Figure 2B), and 30 minutes after the test (Figure 2C).

**Figure 3.** Cancer patients’ salivary IgA alterations were significant in response to 8 weeks of moderate exercise only at the 30 minutes post-test time point.

The average IgA concentration of cancer patients detected by ELISA did not change after 8 weeks of moderate exercise prior to the treadmill test (Figure 3A) and immediately post-test (Figure 3B), but did exhibit a significant increase 30 minutes after the test (Figure 3C, p<0.05).
**Figure 4.** Non-cancer participants’ salivary IgG alterations were significant in response to 8 weeks of moderate exercise at rest and 30 minutes post-test. The average IgG concentration of non-cancer participants detected by ELISA showed significant changes following 8 weeks of moderate exercise prior to the treadmill test (Figure 4A) and 30 minutes after the test (Figure 4C) (p<0.05). IgG levels did not change immediately post-test (Figure 4B).

**Figure 5.** Cancer patients’ salivary IgG alterations were not significant in response to 8 weeks of moderate exercise in cancer patients at any of the time points tested. The average IgG concentration of cancer patients detected by ELISA did not change after 8 weeks of moderate exercise prior to the treadmill test (Figure 5A), immediately post-test (Figure 5B), and 30 minutes after the test (Figure 5C).
Figure 6. Non-cancer participants' salivary IgM alterations were not significant in response to 8 weeks of moderate exercise at any of the time points tested. The average IgM concentration of non-cancer participants detected by ELISA did not change after 8 weeks of moderate exercise prior to the treadmill test (Figure 6A), immediately post-test (Figure 6B), and 30 minutes after the test (Figure 6C).

Figure 7. Cancer patients’ salivary IgM alterations were not significant in response to 8 weeks of moderate exercise at any of the time points tested. The average IgM concentration of cancer patients detected by ELISA did not change after 8 weeks of moderate exercise prior to the treadmill test (Figure 7A), immediately post-test (Figure 7B), and 30 minutes after the test (Figure 7C).
**Figure 8.** IgM levels were significantly higher than IgA and IgG levels in human saliva.

Immunoglobulin concentrations in human saliva were determined using ELISA. Analysis of immunoglobulin levels show there was a significantly higher concentration of IgM (*) in the saliva of non-cancer participants and cancer patients compared to IgA and IgG. IgG (+) levels were significantly higher than IgA levels in the saliva (p<0.05).
Discussion

The goal of this project was to identify exercise-induced changes in the mucosal immune system in cancer survivors compared to non-cancer participants and to use these recommendations to positively influence the exercise habits of cancer survivors to benefit their overall health. Findings from this research could aide in identifying and preventing the events that lead to debilitating long-term fatigue in cancer survivors. The project goals were facilitated through investigation of immune alterations in immunoglobulin (IgA, IgG, IgM) levels between cancer patients and non-cancer participants due to moderate exercise.

The main finding in this study was that in cancer patients the IgA concentration increased after 30 minutes post-exercise testing between baseline (week 0) and week 8 of a moderate exercise program (Figure 3C). This result suggests that the cancer patients’ immune responses were boosted due to a moderate exercise program for eight weeks. It is possible to use IgA as a marker for susceptibility to infection in cancer patients as research supports a relationship between decreased IgA levels and an increase in infection [42]. This may help identify patients who are immunocompromised marked by decreased levels of IgA in their body. IgA is important because the dimeric form of IgA found in saliva allows it to bind and sequester pathogens in mucosal tissues and secretions, preventing the invasion and subsequent infection by pathogens [13]. By
increasing IgA levels through exercise, the immune system of cancer patients has greater defense mechanisms against infection.

Levels of IgA varied depending on the time point the sample was taken and if the group included cancer patients or non-cancer participants. In non-cancer participants, there were no significant alterations in IgA at any of the time points (Figure 2). IgA levels have shown variable responses to moderate exercise as seen here. Salivary IgA has been shown to increase [49, 51] or stay the same with moderate exercise [43, 46]. In cancer patients, IgA levels did not change at the pre-testing (Figure 3A) and immediately post-test (Figure 3B) time points. IgA levels were significantly increased from baseline (week 0) to week 8 of the moderate exercise program at 30 minutes post-test (Figure 3C). Cancer patients’ immune function was enhanced by moderate exercise seen through a significant increase in salivary IgA levels 30 minutes post-exercise test. Results suggest that cancer patients’ immune function was enhanced by the 8 week moderate exercise program due to the increase of IgA 30 minutes post-testing.

Salivary IgG levels showed different responses in non-cancer participants depending on the sampling time (Figure 4) and showed no significant changes in cancer patients (Figure 5). Non-cancer participants’ IgG levels significantly decreased from baseline (week 0) to week 8 of the moderate exercise program at the pre-test time point (Figure 4A). This result suggests a negative effect on IgG from moderate exercise, but more likely, some other unknown extenuating circumstance is having an effect on IgG levels before the exercise test was administered. No change was seen in IgG levels immediately post-exercise test (Figure 4B). Salivary IgG levels have been previously known to stay at the same levels immediately post-exercise and no training effect has
been seen [43]. In non-cancer participants only, a significant increase was seen in IgG levels at the 30 minute post-test time point (Figure 4C). This result suggests an enhancement of immune function in non-cancer participants. While this may be a notable finding, it is also important to view it in context. IgG is the main immunoglobulin in serum and is generally more crucial for clearing infection, rather than preventing infection like IgA in saliva [10]. The IgA and IgM findings together, demonstrate an immune benefit for individuals that moderately exercise, but the specific immune enhancement may vary between individuals due to differences in their immune function because of existing disease. Thus, the increased salivary IgG in non-cancer participants and the increased salivary IgA in cancer patients may demonstrate the components of an immune response that are most sensitive to the influence of moderate exercise.

There was no difference between cancer patients and non-cancer participants at any of the time points in IgA, IgG, and IgM levels (Figure 1). Since the study was only eight weeks long, it is possible that the exercise program was not implemented long enough. Studies that show significant immunoglobulin exercise-induced alterations ranged from twelve weeks to forty-eight weeks [48-51]. One study did see significant serum IgA, IgG, and IgM increases after six weeks of moderate exercise compared to non-exercising controls [48], but as mentioned previously, the humoral immune system responds differently to exercise because it is regulated by different mechanisms [3, 6]. Immunoglobulin levels may have shown different responses if measured further out from the exercise test. It could be beneficial to collect samples at different time points such as one hour, six hours, twelve hours, and twenty-four hours post-exercise test to determine how efficient the immune system responds to stress, specifically exercise.
Immunoglobulins are known to return to baseline pre-exercise values one hour [7, 45], at the least, to twenty-four hours [42, 48, 49] after the session. It is also possible that no difference was seen due to the small sample size. While it was large enough to be statistically relevant, other studies have larger sample sizes to better represent the population of interest. In studies that show significant improvement in immunoglobulin levels, the sample size ranged from nineteen to forty-five participants [48-51]. Although significant differences were not seen between cancer patients and non-cancer participants, differences were seen in the overall average IgM level for cancer patients and non-cancer participants compared to IgG and IgA.

Interestingly, our analysis of these samples demonstrated that the overall level of IgM in the human saliva samples was significantly higher than either IgG or IgA. The average concentration of IgM (115.0 ng/mL) in the non-cancer and cancer saliva samples was significantly higher than the average concentration of IgG (6.4 ng/mL) and IgA (1.1 ng/mL) (Figure 8). Interestingly, the concentrations of IgA and IgG in our study were lower than other researchers have reported, while IgM concentrations were high, which is contrary to published findings [7, 14, 41-43, 45, 51]. High levels of IgM in blood samples are known to indicate a variety of health issues such as viral hepatitis, arthritis, impairment of kidney function, parasitic infection, or a new infection. The participants involved in this study could have the start of a new infection due to the high IgM levels, but most likely, IgM levels can increase in response to low levels of IgA in the host [7, 40, 53, 54] because IgM can function similarly to IgA in some individuals [10]. Low levels of IgA in serum are generally seen in people with leukemia and kidney problems. It has also been reported that individuals can be born with low levels of IgA [31]. One
study reports low levels of IgA in saliva to be less than 10 ng/mL [54]. In this study, the average IgA concentration was 1.1 ng/mL and therefore falls into the category of low to no IgA in the saliva samples, which is supported by high levels of IgM. The low IgA and high IgM can be explained in the cancer patients (at least six weeks out of treatment) by immunosuppression through the effects of treatment and disease up to six months after treatment [16], but the reason for low IgA and high IgM levels in non-cancer participants remains unknown.

In conclusion, our results demonstrate that immune function in cancer patients is enhanced (as demonstrated by increased salivary IgA levels) by 8 weeks of moderate exercise. Although these findings are important, it is necessary to view the results in context as there was not a group of non-exercising cancer patients to control for recovery post-treatment not related to the moderate exercise. Comparing the non-exercise cancer patients to the exercising cancer patients would allow for conclusions to be made about the effect of exercise alone on the cancer patients’ immune function. This is an important factor that was not taken into consideration.

The increased immune function may protect cancer patients against infection, enhancing their quality of life. Findings from this research may be used to support the study of exercise methods that further increase immune function in an effort to help cancer patients achieve a healthy state while recovering from treatments. This research supports the implementation of the moderate exercise programs prescribed at the Rocky Mountain Cancer Rehabilitation Institute in Greely, CO.
Future Direction

Future directions for this project would be to continue the exercise program for a longer length of time since other investigations had better success in tracking immunoglobulin changes over a longer time frame such as six months to a year. In the future, it would be advantageous to track immune changes further out from the exercise test at time points six hours, twelve hours, and twenty-four hours post-test to see if immune components continued to increase or decrease and then plateau or return to resting values. It would also be beneficial to increase the number of participants (non-cancer and cancer) to more accurately represent the populations under investigation. Also, it may be important to separate male and female participants when analyzing data due to differences in their immune response and baseline immune levels. Patients should be stratified by the type of cancer and treatment received because certain types of cancer may affect the immune system differently and treatment can be immunosuppressive up to six months after completion affecting different immune components depending on the treatment type. For publication, an additional study group of non-exercising cancer patients needs to be added to control for general recovery from the disease and treatment. Only then can conclusions be made about the direct effect of exercise on the cancer patients’ immune function.
References


