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Effects of Exercise Training on Neutrophil Proliferation and Function In Cancer Patients

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EFFECTS OF EXERCISE TRAINING ON NEUTROPHIL PROLIFERATION AND FUNCTION IN CANCER PATIENTS

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ABSTRACT

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Cancer and its treatments, namely chemotherapy and radiation, lead to negative physiological consequences. Neutrophils are the most abundant leukocytes in circulation with several studies pointing to the impact of cancer and its treatments lowering the count and function of this cell line. Exercise has been shown to positively impact to neutrophil count and function in the generally healthy population. However, this effect is less understood in the cancer population.

The purpose of this study was to quantify and characterize neutrophil populations in cancer patients and examine the effects of a prescribed exercise intervention on neutrophil count and neutrophil function. It was hypothesized that cancer patients completing a 12-week individualized and prescribed exercise intervention would have an increased absolute neutrophil count. It was also hypothesized that neutrophils from cancer patients completing an individualized and prescribed exercise intervention would demonstrate enhanced oxidative burst activity.

Participants (N = 24) were recruited by physician referral. Participants were grouped based on treatment status (in-treatment [IT] or post-treatment [PT]) and performed low to moderate intensity exercise over a 12-week period. Blood was collected from each participant after the initial visit and intervention. Neutrophils were isolated from blood by magnetic

separation. Isolated cells were characterized and quantified via flow cytometry to quantify absolute neutrophil count (ANC). Neutrophils were induced to activate via N-formylmethionine-leucyl-phenylalanine (fMLP). After 45 minutes, cell membrane expression of rhodamine-123 was measured via flow cytometry to assess the rate of oxidation from rest. Neutrophils were measured for functional capacity over a 48-hour period. After 24 and 48 hours, neutrophils were assessed via flow cytometry for CD16 brightness as a surrogate for functional capacity.

Absolute neutrophil count (was significantly improved in both IT (0.71 ± 0.38 to 0.96 ± 0.52 K cells/µl; $p = .05$) and PT (0.52 ± 0.32 to 0.90 ± 0.51 K cells/µl; $p < .05$). Neutrophil function was not significantly changed in IT, but PT saw significant improvement to neutrophil function (80.35 ± 65.54% to 167.16 ± 121.28%; $p = .05$). There was no change to neutrophil functional capacity in the IT group. There was no change to neutrophil functional capacity in either IT or PT after 24 and 48 hours. Neutrophil functional capacity significantly decreased from baseline 48 hours after the intervention period in the PT group ($p < .05$).

A 12-week individualized and prescribed exercise intervention could significantly increase ANC of cancer patients both during treatment and after treatment. Exercise also increased the function of neutrophils in a post-treatment population of cancer patients while maintaining function of neutrophils in an in-treatment population.

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CHAPTER I

INTRODUCTION

Cancer and related treatments carry side effects and toxicities detrimental to physiological systems within the human body. Immunosuppression, immune-evasion, and immune dysfunction are particularly problematic in cancer progression and treatment (Biswas, 2015; Hurt et al., 2017; Spiegel et al., 2016). As a result, cancer survivors have a compromised ability to fight off acute and chronic infection in a timely and efficient manner (Biswas, 2015; Dinan et al., 2015; Sweeney et al., 2015).

Innate immunity is a crucial wing involved with the response to pathogenic attack. Cells of the innate immune response include leukocytes and their subsequent proliferation and response to infection (Murphy & Weaver, 2017). Of the leukocytes found throughout the human body, granulocytes such as neutrophils, eosinophils, mast cells, and basophils perform critical processes such as phagocytosis and propagation of an inflammatory response to signal further immune activity (Murphy & Weaver, 2017). Neutrophils in particular carry a high phagocytic load as they are the most abundant leukocytes in the human body (Murphy & Weaver, 2017). Neutrophils respond to initial signals of infection and infiltrate the perturbed tissue to continue the attack against the responsible pathogen. Furthermore, neutrophils propagate further inflammation, which facilitates an increased immune response and allows for the adaptive arm of the immune system to locate and assist in fighting the infection (Murphy & Weaver, 2017).

When the neutrophil response is impaired, as is the case with cancer and many cancer treatments, an individual will have an impaired innate response to infection (Netterberg et al.,

2017; Zhao et al., 2017). This might hinder downstream activity of other immune cell types. Treatment for cancer commonly leads to suppression of neutrophils and to a state of low neutrophil count or neutropenia (Hurt et al., 2017; Mouchemore et al., 2018; Netterberg et al., 2017). Neutropenia is a common condition associated with chemotherapy treatment (Amundsen et al., 2012; Fung et al., 2014; Netterberg et al., 2017). Individuals with neutropenia have abnormally low neutrophil counts and thus a poor response to infection, not just the cancer itself. Lack of adequate response to infection will lead to poor prognosis and an inability to fight off conditions that would typically be alleviated in the generally healthy individual.

Cancer itself manipulates neutrophil function, further impairing the innate response associated with this cell type. Through immune-editing of the tumor and thus selective proliferation of poorly immunogenic tumor cells, the cancer can essentially mask itself from immune surveillance (Veglia et al., 2018). Furthermore, the tumor propagates mechanisms that allow for phenotypical changes in the neutrophil, thus making it more pro-tumorigenic. Termed tumor-associated neutrophils (TAN), this phenotype is characterized by its immature morphology, low density, and expression of plasma membrane receptors not typically found in mature neutrophils (Fridlender et al., 2009; Hurt et al., 2017; Veglia et al., 2018). Among the TAN proliferation in the tumor, a subtype of myeloid derived suppressor cell (MDSC) is also implicated for its pro-tumorigenic properties (Veglia et al., 2018; Zhou et al., 2018). The polymorphonuclear MDSC (PMN-MDSC) phenotype expresses similar properties to the TAN and thus functions in a similar manner (Veglia et al., 2018; Zhou et al., 2018).

Exercise has been shown to be an effective method for alleviating common side effects of treatment in the cancer population (Betof et al., 2013; Brown et al., 2019; Pedersen et al., 2016; Schneider et al., 2007a). However, its role in the maintenance and preservation of immune

function is less understood. When assessing immune function in a generally healthy population, intensity of exercise is the primary variable in preservation and enhancement of immune function (Keast et al., 1988; Nieman, 1994; Simpson et al., 2012, 2015). Moderate exercise has been shown to enhance immune function over time in a generally healthy population (Nieman, 1994; Sellami et al., 2018; Simpson et al., 2015). This concept of optimal intensity of exercise and its relation to enhancing immune function in the cancer survivor has no consensus for programming and should be investigated with regard to survivors who are in-treatment, post-treatment, and across varying diagnoses.

Therefore, the purpose of this study was to quantify and characterize neutrophil populations in cancer survivors and examine the effects of a prescribed exercise intervention on neutrophil count and neutrophil function. The role of intensity of exercise and treatment status factors into the analysis of innate immune function. It was hypothesized that a prescribed exercise intervention in cancer survivors would increase total neutrophil count and enhance the functionality of neutrophils whether in-treatment or post-treatment regardless of intensity.

CHAPTER II

REVIEW OF LITERATURE

Exercise has been shown to benefit generally healthy individuals by promoting proper immune function when performed at a moderate intensity (Bartlett et al., 2016; Nieman, 1994, 2012; Simpson et al., 2020). The role of exercise in this capacity is less understood in the cancer population who are at increased risk for immune dysfunction both from the disease itself and from specific treatments (Sellami et al., 2018; Shephard & Shek, 1995; Turbitt et al., 2019; Zhang et al., 2019). As a result, those diagnosed with cancer might be at an increased risk of acute and chronic infection by micro-organisms (Biswas, 2015; Dinan et al., 2015; Park et al., 2016). By establishing a role of exercise in promoting proper immune function in the cancer population, clinical exercise physiologists and those associated with exercise-based rehabilitation programs would be better equipped to design rehabilitation programs with this specific goal in mind.

The immune system is comprised of various barriers and cells that provide protection against and resolution of substances that would otherwise disrupt homeostasis (Murphy & Weaver, 2017). These conditions might be environmental, owed to natural conditions such as air quality or lifestyle factors (Nieman, 2020; Shephard & Shek, 1995; Vivier et al., 2018). Others might be due to opportunistic infection, whether bacterial, viral, fungal, or parasitic (Murphy & Weaver, 2017). Depending upon the nature of the harm incurred, barriers might provide enough protection to prevent the body from harm (Murphy & Weaver, 2017). Barriers to infection include skin, mucous membranes, and stomach acid. In general, these barriers prevent

microorganisms and environmental conditions from altering the body; however, opportunistic infections that directly enter the cellular environment require a cellular response (Murphy & Weaver, 2017).

The cellular response of the immune system is comprised of two overarching arms: innate immunity and adaptive immunity (Dominguez-Andres & Netea, 2019; Murphy & Weaver, 2017; Vivier et al., 2018). Both arms function to guard the body from pathogenic invasion and "non-self" antigens. Innate immunity functions as the initial responder to a foreign substance, acting to promote a state of inflammation, works to actively destroy the pathogen, and, if necessary, establishes communication with the adaptive arm (Dominguez-Andres & Netea, 2019; Vivier et al., 2018). Adaptive immunity acts in a manner that not only removes the pathogen from the body but also establishes preventative measures should the individual become exposed to this agent in the future (Murphy & Weaver, 2017; Vivier et al., 2018).

Neutrophils are the most abundant cells of innate immunity, continuously circulating throughout the body (Fridlender et al., 2009; Mollinedo, 2019). These cells are one of the first to respond to a pathogen by generating an inflammatory response and performing phagocytosis in an attempt to destroy the pathogen (Lin & Loré, 2017; Mollinedo, 2019; Odobasic et al., 2016). The process of degranulation assists in this response as neutrophils release proteins that lead to inflammation and aid in combating the infection (Mollinedo, 2019; Odobasic et al., 2016).

Immunity

Innate Versus Adaptive Immunity

The immune system is broken into two arms: innate immunity (or nonspecific) and adaptive (or specific) immunity (Murphy & Weaver, 2017). These two arms represent various aspects of the physiological characteristics of immunity such as cellular response, mechanism of

action, time course of response, and primary and secondary immune tissue (Murphy & Weaver, 2017). Innate immunity is primitive in nature as it has carried across the entirety of human existence. This arm of immunity is considered nonspecific as it works in a preventive manner against all substances that would aim to cause harm to the host. Protective mechanisms include natural barriers (e.g., mucus lining, skin, bodily secretions) and cells (e.g., natural killer cells, dendritic cells, monocytes, and granulocytes; Murphy & Weaver, 2017). Natural barriers provide an ever-present layer of protection that work to prevent entry of foreign substances into the internal environment of the body. Cells of innate immunity constantly patrol the body yet respond to pathogens in distinct manners. Natural killer cells, for example, inspect cells for expression of pathogenic markers and initiate apoptosis of those indicating dysfunction or invasion (Crinier et al., 2020). On the other hand, neutrophils patrol the body for injury or pathogen in an inflammation-dependent manner (Lin & Loré, 2017; Odobasic et al., 2016). Commonly referred to as damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), these upregulate in response to injury or infection and promote neutrophil chemotaxis to the site so they might enact their response (Graham et al., 2018; Petri & Sanz, 2018).

Adaptive immunity is mediated by cells that interact with cells of innate immunity to propagate an immune response deemed too excessive for an innate response alone (Nieman, 2020; Vivier et al., 2018). Cells of adaptive immunity include T and B cells among other secondary cells (Dogra et al., 2020; Hawke et al., 2020; Vivier et al., 2018). B cells function to produce antibodies that bind to the plasma membrane of infected cells or foreign bodies to allow for recognition by other immune cells by tagging them for destruction (Murphy & Weaver, 2017). T cells are further divided into cytotoxic T cells, helper T cells, regulatory T cells, and

memory T cells (Anthony et al., 2012; Murphy & Weaver, 2017). Cytotoxic T cells respond to antibody presentation and initiate apoptosis of infected cells (Anthony et al., 2012). Helper T cells regulate and guide the specific response of ill immune cells by upregulating specific factors to attack such agents as bacterial infection, viral infection, and fungal infection (Graham et al., 2018; Spitzer et al., 2017). Regulatory T cells, a subset of helpers, act in a manner to dampen an immune response by avoiding an overblown response to a pathogen and allowing for the body to cease the immune response (Renner et al., 2017; Zhou et al., 2016). Memory T cells are naïve throughout an immune response and mature into effector T cells at the end of an immune response (Anthony et al., 2012; Murphy & Weaver, 2017). These cells linger and retain the antigen recognition generated during the immune response so any future exposure will initiate a rapid response to the pathogen (Anthony et al., 2012; Murphy & Weaver, 2017).

Myeloid Versus Lymphoid Lineage

Immune cells are derived from a common progenitor (Curran et al., 2020; Gedye et al., 2014; Zhu et al., 2018). However, the area of maturation dictates classification as myeloid or lymphoid in lineage. Cells of myeloid lineage undergo maturation within the bone marrow of an individual and then migrate into systemic circulation (Veglia et al., 2018; Zhao et al., 2017). While cells of lymphoid lineage do undergo production within the bone marrow, the area of maturation is what distinguishes these cells from those of myeloid lineage. Lymphoid cells migrate in their immature state to lymphatic tissues for maturation such as the spleen or thymus (Dogra et al., 2020; Hawke et al., 2020; Vivier et al., 2018).

Time-Course of Immune Response

Response to a pathogen requires coordination and transition between innate and adaptive immunity (Petri & Sanz, 2018; Summers et al., 2010; Zhou et al., 2016). Dependent upon the

degree of infection, innate immunity might be all that is required to excise the pathogen and return the body to a homeostatic state (Dominguez-Andres & Netea, 2019; Petri & Sanz, 2018). This response is rapid and occurs and recedes within minutes to hours (Petri & Sanz, 2018; Summers et al., 2010). Should the pathogen require a greater response, cells of innate immunity present the antigen to adaptive immune cells (namely dendritic cells) and initiate a transition to an adaptive immune response (Odobasic et al., 2016; Vivier et al., 2018). During this transitionary phase, innate immune cells still work to control the infection while adaptive immune cells work to recognize and propagate the response (Algood, 2020; Cronin et al., 2016; Odobasic et al., 2016). Due to the nature of this transition, the full response of the adaptive arm might not come into effect for days to weeks (Campbell & Turner, 2018; Spitzer et al., 2017). As pathogenic activity is managed and alleviated, signal transduction subsides and regulatory cells act to temper the immune response and return the body to its homeostatic immune set point (Anthony et al., 2012; Dominguez-Andres & Netea, 2019; Summers et al., 2010).

Neutrophils

The Role of Neutrophils in the Immune Response

Neutrophils are the most abundant leukocytes in the body. As such, they are commonly the first immune cell to respond to areas of infection or inflammation and are typically recruited within minutes of a trauma. The relatively short lifespan of most neutrophils and their ability to respond to threats quickly leads to a high rate of turnover in this cell population (Pillay et al., 2010). Original thought was neutrophils were short-lived and only remained in circulation for no more than a few hours (Pillay et al., 2010). This idea has been refuted and it is now believed neutrophils can remain in circulation for days before either extravasating or undergoing apoptosis (Pillay et al., 2010; Van Raam et al., 2008).

When infection or inflammation occurs, a neutrophil migrates to the site of distress and initiates processes of degranulation (Ekpenyong et al., 2015; Lood et al., 2017). This process involves the release of antimicrobial agents from the neutrophil (Nussbaum et al., 2013). These in turn mediate the affliction and promote further immune response through increased inflammation (Hann et al., 2020; Nussbaum et al., 2013). Once the process of degranulation is underway, the neutrophil continues to promote these antimicrobial and inflammatory factors until the condition has ceased (Ekpenyong et al., 2015; Zhang et al., 2010). Through opsonization, neutrophils are also capable of phagocytosing pathogens (Bartlett et al., 2017; Chen et al., 2018). This process involves phagosome formation and internalization of the microbe at which time the neutrophil undergoes a "respiratory burst" and forms reactive oxygen species and hydrolytic enzymes to ingest the microbe (Bartlett et al., 2017; Chen et al., 2018). Upon completion of its immune response, the neutrophil is removed by macrophages and cleared from the area of infection (Chung et al., 2016; Odobasic et al., 2016).

Mechanisms of Action

Neutrophils operate through a variety of actions to establish and promote an immune response and to clear the site of pathogens (Kenny et al., 2017; Lin & Loré, 2017; Odobasic et al., 2016). Degranulation, phagocytosis, and the formation of neutrophil extracellular traps (NETs) are all common processes in which the neutrophil promotes an inflammatory response and rids the body of pathogens (Boeltz et al., 2019; Kenny et al., 2017; Mollinedo, 2019).

Degranulation occurs by neutrophil release of intracellular, antimicrobial products in response to a pathogen (Mollinedo, 2019). Three distinct granules are packaged and contents released during this process. The primary, secondary, and tertiary granules each contain specific

antimicrobial proteins to initiate the immune response, propagate the immune response, and directly attack the pathogen (Borregaard & Cowland, 1997).

Neutrophil phagocytosis generally occurs prior to degranulation but can also occur as a result of degranulation processes (Dahlgren et al., 1995; Leliefeld et al., 2018). Internalization of a pathogen into the inner environment of the neutrophil occurs through encapsulation into a phagosome (Leliefeld et al., 2018). Whether this occurs as the primary step of immune response of as a result of degranulation, the neutrophil ultimately eliminates the pathogen and induces release antimicrobial factors, inflammatory factors, and NETs to properly stimulate necessary follow-up responses by neutrophils and other immune cells to the site of infection (Lin & Loré, 2017; Odobasic et al., 2016).

The formation of NETs allows the neutrophil to ensnare a pathogen for elimination by the neutrophil (Boeltz et al., 2019). The process by which neutrophils produce NETs is termed NETosis (Kenny et al., 2017). As a result, neutrophils undergo a unique method of apoptosis when initiating this process (Kenny et al., 2017). This product is particularly efficient as it minimizes the damage to surrounding cells and tissue (Boeltz et al., 2019). However, NETs have been implicated in aiding the metastasis of certain cancer cells by allowing them to travel more efficiently in circulation and extravasate at new tissue beds (Demers et al., 2016; Lee et al., 2019; Yang et al., 2020).

It is worth noting that studies pointed to sex influencing the functional and pathogenic properties of neutrophils. Pokhrel et al. (2020) found significantly higher reactive oxygen species (ROS) levels ($p < .0001$) and production ($p < .01$) from neutrophils of female participants versus male participants, pointing toward enhanced phagocytic properties of neutrophils in the female sex. These data suggested potential sex influence on the functional capabilities of neutrophils.

Characterization

Characterization of neutrophils has been an area of debate and lack of consensus (Bronte et al., 2016; Fridlender et al., 2009; Veglia et al., 2018). Due to the volatile nature of neutrophils and their low capacity for mechanical stress, isolation and handling methods have been given particular scrutiny when establishing methods of characterization (Veglia et al., 2018). Furthermore, the phenotype for the mature neutrophil varied from study to study (Condamine et al., 2016; Veglia et al., 2018). Even within agreed phenotyping of a neutrophil, there has been debate as to whether or not the cells are truly neutrophils or the similar in profile, yet immunosuppressive, myeloid-derived suppressor cell (MDSC; Condamine et al., 2016; Veglia et al., 2018; Zhou et al., 2018).

While there are areas of debate of how to isolate and classify neutrophils, some plasma membrane markers were commonly reported across research studies (Condamine et al., 2016; Eruslanov et al., 2017; Veglia et al., 2018). The most commonly used markers for distinguishing a neutrophil from various other immune cells included the CD11b and CD66b surface markers (Veglia et al., 2018; Wikberg et al., 2017). CD11b is expressed on the vast majority of innate immune cells and as such is a common marker for filtering out non-innate cells (Du et al., 2008; Fridlender et al., 2009). CD66b is a cell adhesion and migration molecule that is exclusive to granulocytes (Wikberg et al., 2017; Zhou et al., 2016).

Through the use of CD11b and CD66b, researchers attempted to narrow down neutrophil-specific markers (Condamine et al., 2016; Eruslanov et al., 2017; Veglia et al., 2018). As a result, other markers were shown to be indicative of the mature neutrophil phenotype. CD14 and CD15 emerged as two additional markers to determine whether or not mature neutrophils were present (Droeser et al., 2013; Wagner et al., 2003). CD14 was specific to

macrophages and as such allowed researchers to filter out cells positive for this marker (Wagner et al., 2003). CD15 was shown to be a marker of cells from the myeloid lineage and therefore allowed filtering out of lymphoid lineage cells (Droeser et al., 2013; Vivier et al., 2018). More recently, the surface marker LOX-1 was shown to distinguish a mature neutrophil from the MDSC population with cells positive for LOX-1 indicative of the MDSC population (Condamine et al., 2016; Veglia et al., 2018). Taken together, these five surface markers provided a phenotypic profile that narrowed down a cell population sample to what was believed to be an accurate phenotype of the mature neutrophil.

Cancer and Its Effect on Immune Function

Cancer is a condition of cellular mutation (Marin-Valencia et al., 2012; San-Millán & Brooks, 2017). As such, the immune system response might be altered due to the nature of cancer's progression, the tissues impacted, and the upregulation of various factors by the tumor cells (Berry et al., 2017; Biswas, 2015; Hurt et al., 2017; Mishalian et al., 2013). Furthermore, treatment for cancer carries side-effects that might impact the production of immune cells and inhibit the efficacy of those cells in circulation (Balaji et al., 2019; Bower, 2014; Bower et al., 2011; Wu et al., 2020). While some cancer treatment might affect the body in this manner, it is important to recognize the ever-evolving impact of immunotherapy (Chang & Pearce, 2016; Chen & Mellman, 2017; Cloughesy et al., 2019). Immunotherapy plays upon the body's own cellular immune defenses to attack cancerous cells (McKean et al., 2020; Spitzer et al., 2017). This area of therapeutics has consistently showcased promising results in the treatment of cancer but more research and strengthening evidence of the long-term impact are necessary.

Cancer Influence on Innate and Adaptive Immunity

Cancer carries profound influence on both the innate and adaptive arms of immunity by working to promote factors that would effectively mask the tumor from immunosurveillance Chen & Mellman, 2017; Souto et al., 2011). This impairs proper immune function systemically and allows for a state of chronic inflammation to foster within the tumor microenvironment (TME; Andrejeva & Rathmell, 2017; Lacroix et al., 2018). In addition to inflammation within the TME, several immune cells undergo a phenotypic shift by changing from an anti-tumor to pro-tumor phenotype (Corzo et al., 2010; Fridlender et al., 2009). The two cell types most influenced by this shift include the macrophage (shifting to the tumor-associated macrophage, TAM) and the neutrophil (shifting to the TAN; Bohn et al., 2018; Shaul & Fridlender, 2019). These cells can exhibit both anti-tumor and pro-tumor properties, indicating a need for future research to establish the exact role of these cells within the tumor (Fridlender et al., 2009; Hoves et al., 2018).

Along with phenotypic changes to immune cells, upregulation of immune cells to aid in tumor proliferation occurs (Granot & Fridlender, 2015). Such cells as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) are upregulated to suppress the immune response that initially propagates in the TME (Veglia et al., 2018; Zhou et al., 2016). This dampening might be achieved via tumor upregulation of hematopoietic factors or via the hijacked, tumor-associated cells mentioned (Eruslanov et al., 2014; Hurt et al., 2017). Zhou et al. (2016) sought to establish the role of Treg influence on the TME and found a TAN-dependent infiltration of Tregs into the TME. Injection of TANs into hepatic tumors showed significant infiltration of Tregs into the TME and proliferation of hepatic cancer cells compared to hepatic tumors that were not injected with TANs ($p < .01$; Zhou et al., 2016). This infiltration of immunosuppressive

factors and cells resulted in increased immunoevasion by the tumor, further promoting its growth and development in the tissue bed.

Tumor-Associated Neutrophils

Tumor-associated neutrophils represent a distinct phenotype of the neutrophil that is pro-tumorigenic (Berry et al., 2017; Shaul & Fridlender, 2019) and might be referred to as N2 neutrophils in the literature (Shaul & Fridlender, 2017). These cells shifted from the canonical N1 phenotype (anti-tumorigenic) to the N2 phenotype (Shaul & Fridlender, 2017). The driving force behind this shift was not well defined but the factor most often implicated in supporting this shift was transforming growth factor-beta (TGF-β; Fridlender et al., 2009). Studies aimed at defining this shift implicated TGF-β as a key player affecting the mature neutrophil to become more tumor promoting and potentially immune-suppressive (Fridlender et al., 2009; Shaul & Fridlender, 2017). Fridlender et al. (2009) found this shift was highly dependent on TGF-B, showcasing greater infiltration of TANs of Balb/C mice into the tumor by 45% relative to a control when TGF-B was inhibited ($p < .02$; Fridlender et al., 2009). In this study, TGF-β blockade produced TANs with significant down-regulation of messenger RNA expression for the immunosuppressive protein arginase by nearly 5-fold (control = 1; treatment = 0.19, $p < .001$) and significant upregulation of messenger RNA expression for the anti-tumor protein tumor-necrosis factor-alpha (TNF-α) by more than 7-fold (control = 1; treatment = 7.5, $p < .001$; Fridlender et al., 2009). This is a different characteristic from immature neutrophil production seen with cancer. These cells are considered to be a type of MDSC, specifically polymorphonuclear (PMN) MDSCs (Veglia et al., 2018; Zhou et al., 2018).

The TAN has been an area of interest in the treatment and delay in the progression of cancer (Mishalian et al., 2013; Shaul & Fridlender, 2019). Distinguishing between anti-tumor

and pro-tumor neutrophils is critically important to establishing the prognosis and degree of impact these cells have on cancer and its ability to progress and metastasize (Berry et al., 2017; Shaul & Fridlender, 2019). Some groups highlight the differences in expression of certain membrane markers and the production, or lack thereof, of antimicrobial and antitumor properties as a method to distinguish between the N1 neutrophil and the TAN (N2). Common differences in membrane-bound properties include higher VEGF and MMP-9 expression in N2 neutrophils relative to their N1 counterparts, promoting a pro-tumorigenic microenvironment (Jablonska et al., 2005; Zhou et al., 2016). Furthermore, the N2 neutrophil is characterized by a lower capability to produce and release hydrogen peroxide and tumor necrosis factor-α, factors that are readily produced by N1 neutrophils, dampening the overall pathogenic response in the TME (Mishalian et al., 2013).

Even with a proper absolute neutrophil count (ANC) performed, it is unclear whether individuals have higher circulating numbers of N2 or N1. The answer to this question would provide a wealth of information about the prognosis of one diagnosed with cancer. The factors these two phenotypes of a neutrophil release would determine whether the TME would be conducive to the cancer progression or if the neutrophils were working to prevent its progression. Current literature was conflicting in this area. While the general thought was TANs were pro-tumorigenic, there was some evidence to conclude they might have a positive prognostic value (Berry et al., 2017). These factors included increased infiltration of TANs that express high levels of myeloperoxidase (MPO; Shaul & Fridlender, 2019). However, studies implicating factors driving the phenotypic switch and subsequent release of pro-tumorigenic factors by N2 neutrophils outweighed the little emerging evidence indicating otherwise (Mishalian et al., 2013; Shaul & Fridlender, 2019). Increased expression of programmed death-ligand 1 (PD-L1), ROS

release, and interleukin-8 (IL-8) production have all been implicated in aiding the progression of cancer development directly or through inhibition of proper immune responses (Shaul & Fridlender, 2019). These factors contributed to the wide variability of TAN characteristics in the TME as a result of specific cancer types. For example, in hepatocellular carcinoma, increased TAN expression of PD-L1 enhanced survivability of TANs in the intratumoral space, decreasing overall survival of these patients (Shaul & Fridlender, 2019). Additionally, in colorectal cancer, an IL-8-dependent increase in ROS production by TANs in the intratumoral space lowered the proliferation of T cells and might have lowered overall survival of these patients (Shaul & Fridlender, 2019).

There is a need for a better system to distinguish the N1 from the N2 phenotype and the use of the neutrophil-to-lymphocyte ratio (NLR) for prognosis might address that need (Ferrucci et al., 2016; Shaul & Fridlender, 2019). The NLR is indicative of infiltrating neutrophils and lymphocytes into the TME with a higher NLR indicative of a poor prognosis (Ferrucci et al., 2016; Jiang et al., 2019). Ferrucci et al. (2016) reported a high NLR resulted in a significant difference in median overall survival between those with a NLR < 3 (9.2 months) compared to those with a NLR ≥ 3 (2.7 months; $p < 0.01$). If these neutrophils were in fact anti-tumor and the cancer was diagnosed at an early stage of progression, perhaps NLR could then guide clinicians to target these cells for therapeutic purposes (Ferrucci et al., 2016). The amount of N1 neutrophils in this ratio would need to be determined to truly say whether the neutrophils in the NLR were beneficial or not; future research should aim to distinguish between the N1 and N2 phenotype in the NLR. Furthermore, whether use of this ratio to guide therapeutics could delay or prevent the conversion of anti-tumor neutrophils to the pro-tumor phenotype has yet to be

determined but would need to be answered when attempting to target neutrophils that have infiltrated the TME.

Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) are an area of interest in cancer development that has gained substantial interest and understanding in the last decade (Bronte et al., 2016; Sica et al., 2017; Veglia et al., 2018). Myeloid-derived suppressor cells are generally classified into two subsets: granulocyte or polymorphonuclear MDSCs (PMN-MDSC) and monocyte MDSCs (M-MDSCs; Bronte et al., 2016; Sica et al., 2017; Veglia et al., 2018). Both are characterized by immunosuppressive properties in a multitude of clinical conditions, namely pregnancy, autoimmunity, and cancer (Bronte et al., 2016; Sica et al., 2017; Veglia et al., 2018).

These cells act in a manner that suppresses immune response to pathogens. These cells work in a manner to dampen the response by $CD8^+$ and $CD4^+$ T cells while promoting regulatory T cell response, effectively canceling the adaptive immune response in a tissue environment (Nagaraj et al., 2010; Wu et al., 2014). Due to their phenotypic similarity to common innate immune cells, namely monocytes and neutrophils, MDSCs are upregulated in cancer to lower the concentration of these immune cells and to promote immune-evasion through suppression/promotion of T cell populations (Nagaraj et al., 2010; Wu et al., 2014). Literature describing the exact mechanism of action in diseased populations and a true role of these cells in general was elusive. Research is currently aimed at characterizing these cells to truly distinguish them from similar canonical cell types (Veglia et al., 2018). Until this characterization is solidified, research is sporadic in surface markers used to characterize these cells (Bronte et al., 2016; Dumitru et al., 2012; Veglia et al., 2018). Furthermore, quantification of associated factors

that might or might not act to promote MDSC proliferation and even phenotypic shift of other immune cells was not well defined (Herrero et al., 2015; Oh et al., 2013; Veglia et al., 2019).

Common markers associated with both MDSCs and mature innate immune cells include CD11b, CD14, CD15, and CD16 (Veglia et al., 2018). Studies that include a large panel of defining markers will only aid in eliminating cells, which should not be considered MDSCs, and help to hone a definitive phenotype that truly characterizes these cells. The ability to distinguish between a healthy, mature immune cell and MDSCs is pivotal due to the immunosuppressive properties of MDSCs. Under pathological expansion of MDSC populations, particularly in those with cancer, other immune functions are hindered (Corzo et al., 2010; Nagaraj et al., 2010; Ostrand-Rosenberg, 2018). This leads to a lessened immune response by healthy cells and might effectively eliminate the surveillance of healthy immune cells at the tumor (Nagaraj et al., 2010; Oh et al., 2013; Veglia et al., 2019).

Treatment Impact/Toxicities

The diagnosis of cancer itself carries its own influence on the immune system and its various functions. Unfortunately, this influence is not only impacted by the disease itself but might also be deterred due to treatment. Several immune-related side effects from cancer could be attributed to treatment rather than to the disease itself, namely as a result of chemotherapy and/or radiotherapy (Bower, 2014; Bower et al., 2011; Miller et al., 2016). The most prevalent of these conditions includes neutropenia and myelosuppression, both of which impact cellular function of immunity directly or indirectly (Chen et al., 2015; Hurt et al., 2017; Netterberg et al., 2017; Sweeney et al., 2015).

While treatment does garner unfortunate side-effects toward immunity, interventions might be implemented to preserve immune function if the side-effects are severe enough to

warrant their inclusion (Ashcraft et al., 2019; Lacroix et al., 2018). These immune-boosting agents and interventions work to mobilize factors to promote immune cell production and function (Ashcraft et al., 2019; Boland et al., 2017; Steele et al., 2016). Common methods of inducing an immune response include pharmacological interventions and lifestyle modifications, namely exercise (Ashcraft et al., 2019; Byun et al., 2017; Idorn & Per Thor Straten, 2017). These strategies might not directly impact the immune response to the cancer itself but they could aid in response to other pathogens the body might need to protect against.

Finally, utilization of the immune system itself to combat the cancer is a promising and growing avenue of treatment. Immunotherapy utilizes the host's own immune cells to combat the cancer via *ex vivo* manipulation and reinjection into the body (Chen & Mellman, 2017; Cloughesy et al., 2019; Spitzer et al., 2017). In effect, these therapies work around the methods utilized by the cancer to mask itself from immune surveillance or diminish a proper immune response (Byun et al., 2017; Cloughesy et al., 2019). In turn, the immune system gains the capacity to recognize tumor cells and works to mitigate their influence with the end goal of eradicating the cancer via a natural cellular response (Byun et al., 2017; McKean et al., 2020).

Neutropenia

Neutropenia is defined as a state of low cellular concentration of neutrophils in circulation, predisposing one to a heightened risk of opportunistic infection (Amundsen et al., 2012; Hurt et al., 2017). Cancer treatment, in particular chemotherapy and radiotherapy, impacts the production and maturation of neutrophils and other granulocytes by depleting the overall number and negatively affecting the function of mature cells in this population (Hurt et al., 2017). Furthermore, treatment might lead to unintended destruction of these cells, perpetuating the low number and promoting risk of infection (Dinan et al., 2015; Hurt et al., 2017).

Without any intervention to control and enhance production of these cells, the rate of mortality from common infection increases (Lyman et al., 2014; Netterberg et al., 2017). The most prominent of these conditions is a state of febrile neutropenia. This condition manifests in a similar fashion to the common cold (Dinan et al., 2015; Lyman et al., 2014). However, because of impaired mechanisms that would normally eradicate the infection, it is allowed to flourish and therefore imposes a fatal risk on the host (Dinan et al., 2015).

This impact on the health of a cancer patient has led to research into enhancing neutrophil count both pharmacologically and physiologically (Choi et al., 2014; Mouchemore et al., 2018; Van Raam et al., 2008). Agents aimed to enhance such factors to promote myelopoiesis, such as granulocytic colony stimulating factor (G-CSF), have emerged as standard options for improving neutrophil count in neutropenic individuals (Jurcevic et al., 2015; Van Raam et al., 2008). This intervention promotes the production of neutrophils by bone marrow for use in general circulation and prevents such states as febrile neutropenia from occurring (Jurcevic et al., 2015). Jurcevic et al. (2015) found a significant increase in G-CSF after the administration of a CXCR2 antagonist relative to a placebo ($p < .05$), indicating a capacity for neutrophil production after pharmacological intervention. Van Raam et al. (2008) also found G-CSF administration delayed the apoptosis of neutrophils compared to a control ($p < .05$) through inhibition of calpain activation upstream of caspase-3, suggesting the pharmacological administration of G-CSF might prolong the functional capacity of mature neutrophils in circulation. Other interventions have aimed at increasing production of neutrophils through natural physiological mechanisms.

Exercise has been implicated as a mechanism that provides immune benefit and stimulates production of immune cells (Keast et al., 1988; Simpson et al., 2015). Through physiological stress induced by exercise, the body initiates a local immune response at the

impacted muscle and thus increases production of immune cells (Hwang et al., 2020; Idorn & Per Thor Straten, 2017; Simpson et al., 2015). Chronic exercise stress has been shown to enhance immune surveillance of the body on a global scale, laying claim that exercise might induce adaptations to the immune system that extend out of the local musculature (Campbell & Turner, 2018; Hwang et al., 2020). Further research into the immunologic benefits of exercise is needed, especially in clinical populations.

Myelosuppression

Myelosuppression is another common side effect of cancer treatment that impairs the function of various immune cells and associated cell types (Chen et al., 2015; Netterberg et al., 2017). This condition is defined as a state of low myelopoiesis or cellular production within the bone marrow (Chen et al., 2015; Netterberg et al., 2017). This state impacts not only the production of various white blood cells but might also impair production of erythrocytes and clotting factors such as platelets (Elter et al., 2009; Netterberg et al., 2017). This condition carries with it impaired immune function as well as potential for decreased tissue oxygenation and damage repair (Chen et al., 2015).

Reversal of myelosuppression might occur at the cessation of treatment as one is more capable of stimulating bone marrow activity through higher loading (Netterberg et al., 2017). If the condition is severe enough, a bone marrow transplant might be recommended (Du et al., 2008; Netterberg et al., 2017). This intervention carries prolonged risk of infection as the body attempts to accept the transplanted marrow; thus, it would not be conducive for one who is already in an immunosuppressed state. As such, bone loading interventions such as exercise rehabilitation and physical therapy might be recommended prior to the operation (Elter et al., 2009; Emmelot-Vonk et al., 2008). These interventions allow for an environment where bone

loading can be monitored to ensure safety of the patient until they reach a safe set point to undergo a transplant (Lyman et al., 2014; Tenório et al., 2018). More research is necessary to understand the risks and benefits associated with implementing a preoperative intervention in the cancer population that presents with myelosuppression requiring a bone marrow transplant.

Immunotherapy

Immunotherapy is steadily becoming a common treatment option for cancer as it utilizes the patient's own immune system to directly target cancer cells (Byun et al., 2017; Cloughesy et al., 2019; Glitza et al., 2020; Renner et al., 2017). While this therapy is ever-growing in its efficacy, knowledge gaps must still be filled to fully appreciate the use of this treatment option for any and all cancer diagnoses. Risks associated with immunotherapy include autoimmunity, unresponsiveness by the individual, and death due to the time course necessary to properly culture and prime the cells (Balaji et al., 2019; Delanoy et al., 2019). These carry additional side-effects such as financial strain, increased risk of opportunistic infection, and dysfunction in other cell lines associated with immunity (Balaji et al., 2019). Research is now being conducted to not only improve the efficacy of immunotherapy but also to establish methods to reduce risks associated with this treatment and provide a clearer strategy for immunotherapy options that would be most effective for the patient (Balaji et al., 2019; Delanoy et al., 2019; Spitzer et al., 2017).

Common immunotherapies include the use of checkpoint inhibitors and T cell receptors (Chang & Pearce, 2016; Cloughesy et al., 2019; Steele et al., 2016). These options act to improve the responsiveness of already circulating immune cells by enacting restraints on the cancer cells (checkpoint inhibitors) or to enhance the function of immune cells *ex vivo* to target the cancer

cells when administered *in vivo* (e.g., CAR-T therapy; Byun et al., 2017; Chang & Pearce, 2016; Steele et al., 2016).

Recent studies implicated the NLR as a prognostic marker for overall survival and progression-free survival in response to immunotherapy, namely PDL-1 inhibitors (Jiang et al., 2019; Raphael et al., 2020; Topalian et al., 2015). High NLR was reported to be indicative of lower overall survival and less time of progression-free survival in patients receiving immunotherapy (Jiang et al., 2019). Jiang et al. (2019) reported a significant correlation between elevated NLR and overall survival ($r = 0.585$; $p = .036$) when examining those treated with PD-L1 inhibitors; with those exhibiting a high NLR, having a median overall survival of 2.7 months compared to 16 months by those with low NLR ($p < .001$; Jiang et al., 2019). Furthermore, the same group found a significant difference in progression-free survival between those with a high NLR (1.9 months) versus those with a low NLR (4.7 months; $p < .001$) when administered a PD-L1 inhibitor (Jiang et al., 2019). As previously discussed, TANs might increase this NLR at the TME, resulting in poorer outcomes (Jiang et al., 2019; Raphael et al., 2020). Future research to assess the impact of interventions on lowering this value at the TME might allow for enhanced efficacy of treatment, namely PD-1 inhibition therapy.

Clinical Significance of Immune Health in Cancer Patients

Complete Blood Counts

Complete blood counts (CBCs) are routinely ordered for cancer patients to assess various cells and markers of overall health within systemic circulation (Aarts et al., 2017; Al-Gwaiz & Babay, 2007; Ferrucci et al., 2016). One variable measured as part of a standard marker for general immune health includes quantification of the ANC (Al-Gwaiz & Babay, 2007; Ferrucci et al., 2016). This value allows physicians to determine the risk of opportunistic infection in their

patients as a result (Al-Gwaiz & Babay, 2007; Ferrucci et al., 2016). An ANC might be deemed high, normal, safe, or low, depending on the number quantified in cells per microliter. High ANC values (neutrophilia) are indicative of a mounting immune response and, therefore, are commonly seen in those exposed to opportunistic infection (Al-Gwaiz & Babay, 2007; Souto et al., 2011). Low values (neutropenia) are commonly found as a result of chemotherapy and/or radiation in the cancer population and dampen the ability of the patient to defend against opportunistic infection from foreign bodies (Al-Gwaiz & Babay, 2007; Netterberg et al., 2017). Other situations in which a neutropenic state might occur include viral infections such as hepatitis and human immunodeficiency virus (HIV; Al-Gwaiz & Babay, 2007; Amundsen et al., 2012; Lyman et al., 2014). These conditions provide the greatest use of the ANC values as physicians gain valuable insight into the changes in innate immunity over time and allow prescription of necessary immune boosting agents, should the need arise (Al-Gwaiz & Babay, 2007).

Febrile Neutropenia

As a result of low CBCs, especially with regard to the ANC, the risk of acute illness could become a major concern when determining the ongoing course of treatment (Al-Gwaiz & Babay, 2007; Lyman et al., 2014; Netterberg et al., 2017). Chemotherapy has been shown to have myelosuppressive properties and, as such, predisposes patients to increased risk of infection (Chen et al., 2015; Netterberg et al., 2017). The most common condition described as a result of this treatment, neutropenia with fever (or febrile neutropenia [FN]), occurs when patients have neutrophil counts so low a common fever becomes severe in nature (Dinan et al., 2015; Lyman et al., 2014). As such, physicians elect to lower the dosage of treatment to prevent the onset of such conditions (Chang, 2000; Lyman et al., 2014). Once this occurs, the treatment course is

elongated and efficacy of treatment is reduced, leading to increased risk for further toxicity of treatment, lowered response toward the cancer itself, and increased financial burden (Chang, 2000; Crawford et al., 2008; Lyman et al., 2014). A study by Crawford et al. (2008) found the likelihood of FN to be highest during the first cycle of chemotherapy, resulting in a significantly higher incidence of reduction in chemotherapy dosage by the second cycle compared to those who were not diagnosed with FN (11.1% for FN versus 5.9% without FN; $p = .0033$). The same group also found individuals with FN who were diagnosed during their first cycle also had a significantly higher likelihood of delaying the initiation of their second cycle of treatment compared to those not diagnosed with FN (22.2% versus 13.5%; $p = .001$; Crawford et al., 2008).

Several risk factors increase the chance one might develop FN through treatment (Lyman et al., 2014; O'Brien et al., 2014). Underlying conditions might prove to be an underlying cause of this condition as inflammation is heightened throughout the body (Lyman et al., 2014). This condition might result in increased susceptibility for infection due to a dampened response at sites of infection through low supply and ineffective activity (Lyman et al., 2014; O'Brien et al., 2014). Furthermore, environmental factors might play a role in the development of FN. The hospital environment naturally exposes one to new risks and opportunistic pathogens (O'Brien et al., 2014). When receiving treatment, it is especially important that conditions are as sanitary as possible to decrease this risk (Lyman et al., 2014; O'Brien et al., 2014). Outside of the hospital setting, there is an even greater likelihood of exposure to factors that could result in infection. Lack of control in these situations should be counteracted by proper patient education to limit the risk of infection through daily environmental interactions.

Development and diagnosis of FN carries unusual risk relative to generally healthy individuals in fighting off common conditions such as the common cold (Lyman et al., 2014; O'Brien et al., 2014). Therefore, it is important to take treatment and environmental considerations into account when educating patients on their treatment plan during the time period when onset of FN is particularly high. Future research should aim to establish safe and effective interventions, pharmacological or not, to minimize this risk and allow for the most optimal treatment plan to be followed. One method of improving immune response to illness is exercise (Chen et al., 2018; Simpson et al., 2015). Literature suggested chronic, moderate exercise is enough to promote greater cytotoxic and phagocytic capabilities in circulating immune cells and lower the overall inflammatory response to infection (Pedersen & Bruunsgaard, 2003; Phillips et al., 2010; Woods et al., 1999; Yan et al., 2001).

Prognostic Value of Dihydrorhodamine-123-Positive (+) Neutrophils

The functional capability of a neutrophil in response to a pathogen can be measured via degranulation and respiratory burst processes as well as through the various factors released (Chen & Junger, 2012; Pyne et al., 1995; Schmidt et al., 2014). During the process of degranulation, a neutrophil releases factors in primary, secondary, and tertiary granules (Dahlgren et al., 1995; Droeser et al., 2013; Pflieger et al., 2018). These are packaged intracellularly and migrate to the plasma membrane for release into the cytoplasm (Dahlgren et al., 1995; Pflieger et al., 2018). The respiratory burst results in the release of reactive oxygen species (ROS), namely the superoxide anion and hydrogen peroxide (Dahlgren & Karlsson, 1999; Van Pelt et al., 1996). Release of these factors carries a multitude of effects from propagation of inflammation driving further immune signaling to degradation of internalized pathogens (Dahlgren & Karlsson, 1999; Pflieger et al., 2018).

One factor of particular interest in measuring the respiratory burst process of neutrophils is dihydrorhodamine 123 (DHR123; Chen & Junger, 2012; Henderson & Chappell, 1993; Van Pelt et al., 1996). The DHR123 fluorescent marker ultimately is converted to rhodamine 123 (Rho123) during neutrophil respiratory burst in a myeloperoxidase (MPO) and NADPH-dependent manner of oxidation (Henderson & Chappell, 1993; Van Pelt et al., 1996). This process allows for measurement of activity but is of greater importance in its direct indication of neutrophil phagocytosis (Chen & Junger, 2012; Henderson & Chappell, 1993). Rho123 indicates the activity of phagocytic processes by the neutrophil as it is directly involved in the upregulation of ROS, namely superoxide (Chen & Junger, 2012; Henderson & Chappell, 1993).

Due to the high degree of ROS release by the neutrophil, the value of DHR123 and Rho123 used for quantifying response and function could reflect the amount of mature neutrophils undergoing respiratory burst in response to a pathogen (Dahlgren & Karlsson, 1999; Kenny et al., 2017; Suzuki, 2019). A study by Hashiguchi et al. (2005) found the measurement of DHR123 to be the most suitable method of evaluating oxidative burst compared to cytochrome C, homovanillic acid, or Amplex Red. The results from this study indicated a change in DHR123 response of nearly threefold when neutrophils were stimulated with fMLP from the blood of healthy donors and a near fivefold response in trauma patients (Hashiguchi et al., 2005). By measuring this enhanced neutrophil expression, one could quantify the degree to which an individual's neutrophil cell population was able to mount a proper response to a pathogen (Chen & Junger, 2012; Pyne et al., 1995). Utilizing DHR123 in this manner allows researchers and clinicians to better understand the health of patients' neutrophil population (Chen & Junger, 2012; Dahlgren & Karlsson, 1999).

The Immune Response to Exercise

Exercise has been shown to improve both arms of immunity (Keast et al., 1988; Malm, 2006; Nieman, 1994; Peake et al., 2017). The response by innate and adaptive immunity have similarities in how the cell types, as well as primary and secondary immune organs, adapt in their proliferation, responsiveness, and overall function due to exercise (Davison, 2011; Nieman, 1994; Sellami et al., 2018). The time course of response differs between both wings of immunity, with the innate wing experiencing a more immediate response that may persist for days (Bessa et al., 2016; Estruel-Amades et al., 2020; Peake et al., 2017). The adaptive wing will experience an uptick in activity later into the time course of recovery from exercise (Bessa et al., 2016; Peake et al., 2017). This may manifest due to the stress of exercise itself, or in response to the indirect outcomes of exercise, such as dampening the overall immune response to tissue damage or prevention in risk of opportunistic infection post-exercise (Malm, 2006; Simpson et al., 2020).

Two lines of thought have dominated the study of exercise immunology. Both counterarguments are rooted in the inflammatory response associated with exercise but differ in how acute and chronic adaptations present. One side argued that exercise produced an immunosuppressive effect, generally in an acute manner (Simpson et al., 2020). The other proposed that exercise enhanced immunosurveillance and thus improved overall immune function, generally in response to the long-term effect of exercise (Campbell & Turner, 2018; Hwang et al., 2020). Current literature differed in this effect, in particular due to the varying populations recruited in each study. This was particularly evident when comparing studies that differed in the age, exercise history, and exercise intensity of the population sampled or mode of exercise utilized (Campbell & Turner, 2018; Malm, 2006; Simpson et al., 2012). Furthermore,

the response when one initiates a structured exercise program versus the response seen in general physical activity might differ (Bartlett et al., 2016, 2017; Davison, 2011; Turner, 2016).

Overall, exercise will lead to an immune response, acutely or chronically, but it might not be reflective of the response in a general population. However, this might be altered in a clinical population (Ashcraft et al., 2019; Evans et al., 2009; Idorn & Hojman, 2016; Messaggi-Sartor et al., 2019). There is a lack of research explaining the physiological response and adaptation to the immune system in the clinical population. The available literature was skewed with more attention given to common and easily assessed populations over those that varied in presentation or frequency of diagnosis (Alghadir et al., 2016; Carpio-Rivera et al., 2016; Pal et al., 2013). For example, more was known about the immune response in individuals with cardiovascular and metabolic diseases than conditions such as cancer and other hematological disorders (Alghadir et al., 2016; Cornelissen et al., 2011; Hoekstra et al., 2020). Alghadir et al. (2016) found type 2 diabetics who practiced moderate or high intensity physical activity, compared to physically inactive type 2 diabetics, showcased significantly lower fasting blood glucose (156.4±12.2 mg/dL and 140.1±14.1 mg/dL versus 198±29.3 mg/dL ; $p < .05$ and $p < .01$, respectively) and glycosylated hemoglobin (HbA1c; 6.95±0.74% and 6.46±0.69% versus 8.9±1.1%; $p < .05$ and $p < .01$, respectively), resulting in improved antioxidant concentration, less oxidative stress, and a reduction in symptoms reported by participants. Furthermore, Tenório et al. (2018) supported this finding in obese adolescents, showcasing a direct impact of exercise on enhancing neutrophil proliferation after low intensity exercise training (4.5±1.7 μL^{-1} x 10^3 – 5.2±3.3 μL^{-1} x 10^3; $p = .01$).

Upper respiratory infection rate and risk is the most common marker of immune function in response to exercise (Campbell & Turner, 2018; Malm, 2006; Nieman, 1994; Simpson et al.,

2020). Studies assessed this risk in response to long endurance competition (generally events greater than 10 kilometers in distance) with participants showing a significant decrease in immune cell phagocytic capacity by as much as -0.3±0.05 particles/cell ($p \leq .05$) and increased susceptibility to infection 24 hours post high intensity exercise compared to the same amount of time after moderate intensity exercise (Hack et al., 1994; Malm, 2006; Nieman, 2000). Furthermore, microtrauma in musculature was also assessed in response to these events, which in turn gave insight into the immune infiltration to highly active muscle groups through the increase in concentration of exercise-induced cytokines (Bessa et al., 2016; Hwang et al., 2020). Bessa et al. (2016) found a significant increase in creatine kinase, a common marker for muscle-related damage, post-exercise by twofold from rest ($p < .01$). This resulted in a 40% increase in concentration of the pro-inflammatory cytokine, MCP-1, along with a significant increase in neutrophil (70% from baseline; $p < .05$) and lymphocyte (+55% from baseline; $p < .05$) infiltration into the working skeletal muscle up to three hours post-exercise, indicating a damage-induced, inflammation-driven increase in immune response to local muscle groups (Bessa et al., 2016).

While important, the focus on aerobic exercise left a wide array of questions concerning immune system response, adaptation, and function after participation in nonaerobic exercise. Resistance training led one to think there was greater infiltration into the musculature by immune cells after training but it did not provide a global level view. While the musculoskeletal system has a wide range of physiological and physical functions, the lack of understanding about how the immune system responds to exercise needed to be established. Current literature stated the benefit of resistance training might lead to improved bone mineral density and overall bone health (Bartholomeu-Neto et al., 2015; dos Santos et al., 2016; Nilsen et al., 2016). Furthermore,

some research suggested enhanced phagocytic capacity of immune cells after resistance exercise (Bartholomeu-Neto et al., 2015). Bartholomeu-Neto et al. (2015) found resistance training resulted in a significantly higher phagocytic index in the neutrophils of elderly women compared to sedentary counterparts (221.2±105.8 vs 140.4±69.5, p <.001). Future research should aim to confirm this finding. With these known benefits, it could be inferred that diseased populations in particular might benefit from increased bone loading and resistance exercise to stimulate the production of healthy bone tissue and subsequent production of healthy immune cells, healing factors, and other factors related to defense mechanisms
(Bartholomeu-Neto et al., 2015; Nilsen et al., 2016). A consensus on these hypotheses has yet to be achieved and requires further analysis to establish the biological adaptations that occur to improve health and performance under these conditions of exercise.

Other forms of training have begged similar questions to the global effect of immune system adaptation to other forms of exercise, namely flexibility training and high intensity interval training (Bartlett et al., 2018; Reis et al., 2018). Greater focus has been given to mental health-related exercise (e.g., yoga) as an avenue of structured exercise that provides benefit to physiological health as well as mental health (Hung et al., 2018; Schulz et al., 2017). High intensity interval training provides a quick form of exercise that has begun to show similar benefits to traditional forms of exercise (aerobic and resistance; Bartlett et al., 2018; Schulz et al., 2018). This has proved to be a popular option for individuals who are entering into structured exercise as it allows them to plan a period of time throughout the day that is seen as manageable and efficient (Schulz et al., 2018). However, the benefit from this form of exercise for the immune system has been elusive and given the nature of this style of training, it must be thoroughly investigated and compared to the aforementioned research into high intensity and

high duration forms of exercise that have dominated the study of exercise immunology (Devin et al., 2019; Schlüter et al., 2019). As the popularity of these forms of exercise continues to grow, the immune adaptation should be further explored. Furthermore, the growing body of literature exposing the benefits of aerobic and resistance training on the immune system should consider inclusion of these forms of exercise in the study design.

Neutrophil Response to Exercise

The response to exercise in the neutrophil population varies depending on the length of the intervention—either immediately post exercise (acute adaptation) or after a lengthy exercise program (chronic adaptation; Bartlett et al., 2016, 2018; Borges et al., 2018; Davison, 2011). Current literature showed an elevated concentration in neutrophil count post-acute exercise (Barry et al., 2017; Davison, 2011), which might have been attributed to the inflammatory aspect of exercise (Suzuki, 2019). This response might have also been due to the general immune response to the microtrauma induced by exercise (Bessa et al., 2016; Peake et al., 2017). Neutrophils are signaled to begin the clearance of debris caused by exercise and to stimulate other immune cells to circulate to the affected area (Bessa et al., 2016; Borges et al., 2018; Peake et al., 2017). Borges et al. (2018) noted a significant increase in neutrophil production of IL-8 (+59.75% from baseline, $p < .01$) and TNF-α (+49.23% from baseline, $p < .01$) in dancers after an acute bout of high-intensity street dancing exercise. The elevation in response generally subsided within hours following an acute bout of exercise, returning to baseline levels (Borges et al., 2018; Davison, 2011; Quindry et al., 2003).

Chronic adaptation to exercise in the neutrophil population is less understood and varying in results were found in the literature (Bartlett et al., 2017, 2018; Leicht et al., 2017). Some studies showed a change to the neutrophil count in the general healthy population while others

saw no significant change to the absolute value of neutrophils in circulation (Barry et al., 2017; Bartlett et al., 2018). In assessing this phenomenon, Bartlett et al. (2018) found no significant change in total white blood cell count or neutrophil count in a sedentary, but generally healthy, adult population after a 10-week exercise intervention (Total: 5.6 ± 1.1 μL^{-1} x 10^3 to 5.5 ± 1.2 μL^{-1} x 10^3, $p = .322$; Neutrophil: 2.8 ± 0.8 μL^{-1} x 10^3 to 2.9 ± 0.9 μL^{-1} x 10^3, $p = .217$). Furthermore, Chen et al. (2018) found a significant increase in neutrophil count from rest (3.03 ± 0.36 to 4.14 ± 0.25, $p < .05$) in sedentary men in response to hypoxic exercise training. However, the same group found no significant difference in neutrophil count, pre to post, after a four-week intervention period (Chen et al., 2018). Due to this lack of chronic effect in generally healthy individuals, more research has focused on the change in the functional capacity of neutrophils after an exercise program. Current literature suggested aspects of neutrophil response to pathogens and infiltration into impacted tissue were enhanced with exercise. Such characteristics as improved chemotaxis were seen, indicating an improved sensitivity by the neutrophil to migrate toward a pathogen (Barry et al., 2017; Chen et al., 2018). Barry et al. (2017) found a significant improvement in CCR5-mediated neutrophil infiltration to tissue of obese adults in response to a short-term exercise intervention (65.12 ± 9.04 %-CCR5 positive to 70.53 ± 6.43 %-CCR5 positive neutrophils, $p < .05$). The results seen across multiple studies were rather inconclusive as some studies varied in their assessment of chemotaxis by the neutrophil after exercise.

In addition to enhanced chemotaxis and pathogen sensitivity, the phagocytic and degranulation properties of neutrophils were optimized as a result of exercise training (Bartlett et al., 2017, 2018; Chen et al., 2018). Research was indicative of an increased bactericidal capacity and oxidative activity of neutrophils in response to structured exercise (Bartlett et al., 2017; Chen

et al., 2018). Bartlett et al. (2017) found improvement to both phagocytic capacity and oxidative burst activity in neutrophils of sedentary adults in response to both high intensity (phagocytosis: 130.66 ± 16.6 to 152 ± 17.2 median fluorescence intensity (MFI), $p < .05$; oxidative burst: 69 ± 24.5 to 74.6 ± 22.1 MFI, $p < .05$) and moderate intensity exercise interventions (phagocytosis: 126.2 ± 12.5 to 145.5 ± 14.1 MFI, $p < .05$; oxidative burst: 77.6 ± 18.2 to 104.8 ± 16.5 MFI, $p < .01$). Furthermore, Chen et al. (2018) confirmed these findings in sedentary men having undergone hypoxic exercise training exhibiting a significant increase in phagocytosis ($p < .05$) and oxidative burst ($p < .05$) in neutrophils. These results were shown in various levels of exercise intensity and in differing training statuses of individuals (Leicht et al., 2017; Pyne et al., 1995). Furthermore, some studies suggested a significant change in neutrophil function in response to exercise by the elderly, potentially alluding to a preservation or enhancement of neutrophil activity, regardless of age (Bartlett et al., 2016, 2018).

Within various diseased and special populations, a lack evidence supports the exercise benefit in the neutrophil population. Conditions in which the impact of neutrophil dysfunction are common and detrimental should receive focus. These populations include those diagnosed with cancer, autoimmune disorders, and chronic inflammatory conditions. The role of neutrophil function in these populations has the potential to alter prognosis and treatment plans. As such, establishing non-pharmacological means to improve function and proliferation of neutrophils would aid directly to the choices made by patients and physicians for treatment dosage, financial planning, and length of hospital stay.

Exercise in Cancer Patients

The field of exercise oncology has grown dramatically over the past decade. As such, various sites to conduct exercise rehabilitation for the cancer population have been introduced

into communities in both in-patient and out-patient settings. The initial justification for this field of rehabilitation stemmed from the toxicities associated with cancer treatment. For example, the cardiotoxicity of chemotherapeutic agents, such as anthracyclines, resulted in increased risk of cardiovascular disease upon completion of treatment (Dent et al., 2020; Mortimer et al., 2017). This complication led to examination of a role for exercise to combat this toxicity due to the already known benefit for reducing the risk of cardiovascular disease (Schneider et al., 2007a, 2007b). Establishment of this benefit led to increased research in other physiological systems impacted by treatment. As such, the field of exercise oncology has grown from a traditional sense of physical therapy, orthopedic devices, and other mechanical regimes into one of the primary modes of rehabilitation for the cancer population.

The growth of this field has resulted in many governing bodies for exercise science and oncology re-evaluating their positions on exercise in the cancer population and the guidelines for prescribing exercise to this population. The American College of Sports Medicine et al. (2018) recently revised the guidelines for exercise prescription in the cancer population as new research had been completed (Patel et al., 2019). These guidelines resembled similar guidelines for the generally healthy population but had guidelines for management of cancer diagnosis-specific complications. The American Society of Clinical Oncology (Dent et al., 2020) issued statements guiding physicians to educate patients on the benefits of exercise during and post-treatment. Finally, the American Cancer Society continuously released guidelines and recommendations for cancer survivors seeking positive lifestyle changes via exercise and other avenues (Picon-Ruiz et al., 2017; Siegel et al., 2017).

Due to the high degree of variability between patients, programming exercise for the cancer population has remained elusive of a unified stance (Idorn & Per Thor Straten, 2017;

Rajarajeswaran & Vishnupriya, 2009). Several institutions are working resolve this and establish formal and unified guidelines for exercise programming and prescription. Highlighted by the American College of Sports Medicine, the University of Northern Colorado Cancer Rehabilitation Institute (UNCCRI) is one example of a formal exercise rehabilitation program that carries into account the cancer history and present complications from treatment into designing programs that adhere to specific guideline for exercise frequency, intensity, and duration while still maintaining the unique individuality of each case (Brown et al., 2019; Shackelford et al., 2017). Research from the UNCCRI has shown significant improvement to several fitness-related aspects of the health of cancer patients actively undergoing chemotherapy and/or radiotherapy performing 12-weeks of structured exercise such as fatigue (5.1 ± 2.4 to 3.8 ± 2, $p < .001$), VO_{2peak} (20.9 ± 7.4 ml/kg/min to 23.5 ± 7.9 ml/kg/min, $p < .001$), and estimated one repetition maximum on the leg press (81 ± 33 kg to 91 ± 42 kg, $p < .05$) and chest press (27 ± 16 kg to 32 ± 19 kg, $p < .001$; Brown et al., 2019). This and other programs nationwide provide a structured and safe environment for cancer patients to interact and receive proper exercise.

Research into this field continues to progress—evolving from performance-based studies to now include studies aimed at assessing the role of exercise in reshaping the internal environment in such a way as to mobilize factors for bodily defenses to combat the cancer, slow its progression, and prevent reoccurrence (Hamada et al., 2019; Jones et al., 2007; Juvet et al., 2017; Nilsen et al., 2016). Furthermore, the role of exercise and its direct impact on the TME is being evaluated (Brown & Gilmore, 2020). As this field continues to grow, the role of exercise rehabilitation for the cancer population will continue to receive recognition and acceptance from clinical societies and insurance companies.

Exercise as a Complement to Traditional Treatment Options

The inclusion of exercise as a complement to combat the complications of treatment while ongoing, to alleviate lingering side effects post-treatment, and to prepare a patient before treatment have all gained increasing evidence and support from researchers and clinicians (Ashcraft et al., 2019; Idorn & Per Thor Straten, 2017; Miura et al., 2019). As such, exercise should now be a standard for inclusion in the treatment plan for the cancer patient. However, this is not universally the case. Lack of education for patients and healthcare providers is a key barrier to the incorporation of exercise into the treatment plan with prominent organizations such as the American Society of Clinical Oncology acknowledging the disconnect in how they might guide practitioners to best manage treatment-related side effect via exercise and lifestyle modification (Dent et al., 2020; Idorn & Per Thor Straten, 2017). Environmental and behavioral barriers also provide a significant inhibition for the initiation of an exercise program (Liu et al., 2009; Miura et al., 2019). Therefore, the body of evidence to support exercise oncology should be bolstered and access should be made readily available for the treatment team and the patient.

The treatment-related impact at all points during the time course of treatment highlighted the need for inclusion of exercise for the patient (Cešeiko et al., 2020; Guinan et al., 2017; Idorn & Per Thor Straten, 2017; Liu et al., 2009). Due to the mechanism of action from common cancer treatments, namely radiotherapy and chemotherapy, many systems are negatively impacted (Ashcraft et al., 2019; Hiraoui et al., 2019). Recent studies showcased the benefit of exercise during treatment in reduction of treatment related side-effects and in preserving the function of a system during treatment (Dent et al., 2020; Mohamady et al., 2017). Mohamady et al. (2017) found exercise to significantly alleviate the effects of chemotherapy-induced anemia compared to a sedentary group ($p < .05$) and actually improved those patients' oxygen carrying

capacity (hemoglobin pre-treatment: 11.52 ± 0.62 g/dL, post-treatment: 12.10 ± 0.59 g/dL, $p < .001$) and red blood cell count (RBC; pre-treatment: 4.24 ± 0.37 10^6/µl, post-treatment: 4.49 ± 0.42 10^6/µl, $p < .001$) over the course of treatment. The cardiovascular and musculoskeletal systems were the most apparent in toxicity from treatment. As such, several exercise intervention studies aimed to assess the role of exercise in preserving and enhancing these systems during and after treatment (Cešeiko et al., 2020; Clauss et al., 2017; Hiraoui et al., 2019; Messaggi-Sartor et al., 2019). Results have shown maintained or improved function and activity of cardiomyocytes, protein synthesis, oxygen carrying capacity, and motor unit recruitment among other adaptations (Cešeiko et al., 2020; Clauss et al., 2017; Hiraoui et al., 2019; Messaggi-Sartor et al., 2019). Cešeiko et al. (2020) found significant improvement in quadricep muscle mass of breast cancer survivors compared to controls (1.01 kg vs 0.79 kg, $p < .05$) as a result of a 12-week exercise intervention, indicating greater protein synthesis in response to exercise. Furthermore, depending on the form of treatment, impact on the pulmonary system and on bone marrow activity were also present with reductions in pulmonary capacity and respiratory rate, and impaired production of immune and healing factors from the bone marrow (Bobbio et al., 2008; Liu et al., 2009; Schneider et al., 2007b). Current studies reported improvement to this system and tissue in response to exercise through enhanced activity of intercostal musculature, improved ventilation capacity, and increased production of platelets, hemoglobin, and healing factors from the bone marrow (Bobbio et al., 2008; Liu et al., 2009; Messaggi-Sartor et al., 2019; Schneider et al., 2007a). Messaggi-Sartor et al. (2019) found combined aerobic training and respiratory muscle training resulted in significant improvement to the generation of inspiratory and expiratory pressures as a result of eight weeks of exercise in non-small cell lung cancer survivors, indicating greater improvement to intercostal muscle strength compared to a control group (inspiratory

pressure between group difference: 13.42 ± 5.11 cmH_2O, p = .016; expiratory pressure between group difference: 18.76 ± 7.59 cmH_2O, p = .023).

Cancer-related fatigue (CRF) has been the most reported side effect throughout treatment and at the onset of an exercise intervention (Giacalone et al., 2010; Stone et al., 2000). Cancer-related fatigue is associated with detrimental consequences in one's ability to perform activities of daily living and maintaining energy and alertness throughout the day (Giacalone et al., 2010; Stone et al., 2000). Exercise has been shown to reverse this side effect with studies showing a significant reduction in fatigue as a result of a formal exercise intervention (Brown et al., 2019; Dhillon et al., 2012). Brown et al. (2019 reported as much as a 25% reduction in fatigue of cancer patients actively undergoing chemotherapy and/or radiation in response to a 12-week exercise intervention ($p < .001$).

Exercise itself also has a positive impact on treatment administration itself. Several studies have shown increased efficacy of treatment in those who participated in exercise or physical activity leading up to and during treatment (Ashcraft et al., 2019; Dent et al., 2020; Jurcevic et al., 2015; Miller & Thyfault, 2020). Jurcevic et al. (2015) found healthy volunteers receiving a CXCR2 antagonist were able to increase ANC at a rate similar to a placebo group (approximately 40% change from baseline, p = NS) in as little as 10 minutes of exercise, indicating a retained ability of neutrophils to mobilize despite receiving a treatment that could impair their production. Results such as these could translate to the oncologic realm, allowing physicians to confidently prescribe dosages of harmful treatments such as chemotherapy with the knowledge that their patients could retain immune function. Ultimately, more results such as these could lead to lower dosages of treatment, faster recovery time from surgery, and less social and financial burden on the patient (Ashcraft et al., 2019; Dhillon et al., 2012). These results also

lend support for enhanced efficacy of novel treatment in response to exercise, namely toward immunotherapy (Ashcraft et al., 2019; Idorn & Per Thor Straten, 2017).

Immune Response to Exercise in Cancer Patients

The immune response to exercise has been well documented, albeit at times conflicting in interpretation, in the generally healthy population (Campbell & Turner, 2018; Simpson et al., 2020). Generally healthy individuals showed a highly responsive immune system that responded to exercise in a manner that improved phagocytic and cytotoxic properties of immune cells and lowered the inflammatory response to a pathogen (Phillips et al., 2010; Simpson et al., 2020). This interaction has also been explored in the aging population due to the immunosenescence that occurs during this phase of life (Bartlett et al., 2016; Simpson et al., 2012). Immune response to exercise in the aging population is typically correlated with habitual physical activity throughout the lifetime with those having practiced greater amounts of consistent physical activity having a lesser reduction in immunosenescence compared to their counterparts who were less consistent (Bartlett et al., 2016). Furthermore, immune response to exercise has been shown in clinical populations, namely in metabolic disorders and chronic inflammatory diseases such as rheumatoid arthritis and fibromyalgia (Nieman, 2020; Suzuki, 2019). Individuals in these populations have a reduced inflammatory profile in response to exercise, providing evidence for alleviating chronic inflammation as a result of exercise (Simpson et al., 2020; Suzuki, 2019). While the impact of cancer on the immune system and strategies to combat this impact are becoming clearer, the specific impact of an individualized exercise program for those diagnosed with cancer and its impact on the immune health of these individuals is not well understood.

Current literature supported the inclusion of exercise in the treatment plan of cancer patients but the impact of exercise on immune health and function might prove to be a barrier for

some to participate (Campbell & Turner, 2018; Sellami et al., 2018; Shephard & Shek, 1995; Zhang et al., 2019). Low counts of immune cells might hinder referral to an exercise program by the physician or through perceived fatigue experienced by the patient (Ashcraft et al., 2019; Idorn & Per Thor Straten, 2017; Shephard & Shek, 1995). This might lead to apprehension toward environmental factors associated with an exercise facility. Furthermore, time of year when disease risk is highest might prohibit participation from severely immunocompromised patients. Despite the apprehension, literature supported improved immune response in cancer patients after completing a prescribed exercise intervention. A study by Abdalla et al. (2014) found a significant difference in interferon gamma (approximately +5 pg/mL difference, p = .0112) and TGF-B (approximately –100 pg/mL difference, p = .0453) between tumor-bearing BALB/c mice after eight weeks of exercise relative to an untrained control, indicating improvement in innate immune function as a result of exercise.

Furthermore, Glass et al. (2015) found significant improvement in cytokine (-3.5% from exercise versus +2.6% from control in IL-4, p = 0.012) and angiogenic factors (-23% from exercise versus +1.2% from control in VEGF, p = .043) in cancer survivors after 12 weeks of aerobic exercise relative to a control, indicating a role for exercise in modulating immune-effectors in cancer patients. The aforementioned mental barriers could be overcome with education but more research is necessary to understand the most effective manner of education.

The importance of establishing a role for exercise in the immune health of cancer survivors extends beyond the potential physiological adaptations (Idorn & Per Thor Straten, 2017; Zhang et al., 2019). By improving this aspect of one's health, the inherent fear of opportunistic infection throughout the seasons will decrease, thus increasing the outlook of participants in social and recreational activity (Hyatt et al., 2020; Idorn & Per Thor Straten,

2017). Financial security would improve due to lowered frequency and duration of hospital visits from elected or admitted procedures and conditions (Hyatt et al., 2020). Psychological health could also improve from less time spent in a state of anxiety and/or fear of environmental influence of the immune system of this population that is told of their immunocompromised state from the diagnosis of their cancer (Campbell & Turner, 2018; Idorn & Per Thor Straten, 2017).

In conclusion, there are several factors to consider when determining a proper plan of action for initiating an exercise intervention for a cancer patient. While a multitude of research is available highlighting the benefits of exercise in this population, the knowledge of this benefit for the immune system is less understood. Current research provided the blueprint for anticipated adaptations to the immune system of a cancer patient whether they are actively undergoing treatment or removed from treatment. However, more research is needed to clearly demonstrate with empirical evidence the effects of exercise on immune function in the cancer population. This evidence is critical in order to provide a sound theoretical basis for incorporating exercise into the treatment plan and to effectively monitor patient progress during and after cancer treatment.

CHAPTER III

METHODOLOGY

Site of Study

The purpose of this study was to quantify and characterize neutrophil populations in cancer survivors and examine the effects of a prescribed exercise intervention on neutrophil count and neutrophil function. This study was conducted at the University of Northern Colorado Cancer Rehabilitation Institute (UNCCRI) located at the Ben Nighthorse Campbell Center and Ross Hall in Greeley, Colorado.

Institutional Review Board Approval and Informed Consent

Performance of this study by participants adhered to all ethical guidelines for human research and was approved by the Institutional Review Board of the University of Northern Colorado (see Appendix A). All participants received verbal and written detail about the guidelines of the study and requirements of participation. All participants gave oral and written informed consent prior to participation (see Appendix B).

Participant Population

A total of 24 participants were recruited for this study and were actively undergoing chemotherapy and/or radiotherapy treatment (n = 12) or completed chemotherapy and/or radiotherapy treatment within two years of recruitment (n = 12). These individuals were referred to UNCCRI by a physician who cleared their participation in an exercise-based rehabilitation program. Those who consented to participate underwent a comprehensive exercise-based

assessment that evaluated current fitness status and degree of physiological detriment because of cancer diagnosis, treatment, and/or lifestyle factors. After completing the initial physical assessment, participants completed an individualized, prescribed, supervised 12-week exercise intervention that included both aerobic and resistance training. At the end of the exercise intervention, another comprehensive physical assessment was conducted to determine pre to post changes (see Appendix C for study participation timeline).

Inclusion and Exclusion Criteria

Participants had to be currently enrolled in the UNCCRI Phase Program to be eligible for inclusion. A minimum of 10 weeks of participation was required for a participant to have received enough stimulus in frequency and intensity of exercise to induce a chronic adaptation in the immune system (Bartlett et al., 2018; Baslund et al., 1993; Mejías-Peña et al., 2017; Thijssen et al., 2006). Participants who did not complete at least 10 weeks of exercise, were removed from the study. Participants must have been actively undergoing chemotherapy and/or radiotherapy or had completed treatment within two years prior to beginning the study. Individuals who were actively receiving treatment that included an immune boosting agent (e.g., Neulasta) were excluded from participation. During the course of the intervention period, any changes to the treatment regimen that would artificially induce the production of neutrophils resulted in the participant being dropped from the study. Those with autoimmune disorders or chronic inflammatory conditions were excluded from participation. Any history of acute infection less than two weeks prior to beginning the study excluded the individual from participation.

Potential Risk

Potential risks involved in participation in this study included associated risks of blood draw and exercise. When performing a blood draw, potential risks included bruising and

irritation at the site as well as light-headedness in the immediate period after the draw. Common risks associated with exercise included elevated heart rate, respiratory rate, body temperature, and blood pressure. Individuals might have also experienced a decreased oxygen saturation and dehydration. Less common risks associated with exercise included mild angina, arrhythmia, or, in very rare instances, death.

Incentive(s) for Participation

Participation in the study provided each participant with individualized exercise designed to combat the toxicities associated with their cancer and its treatment. Each participant also received a physiological assessment of various systems within the body at the initial visit and follow-up visit. Finally, each participant also received a free analysis of immune function and an explanation of how chronic exercise enhanced this physiological system.

Phase Training Protocol

Upon completion of the physical assessment, participants were grouped into phases as outlined by the UNCCRI Phase Training protocol (Brown et al., 2019). Based on their cancer-related health history, an incoming participant entered the program as Phase 1 or Phase 2. The phase at intake governed the intensity of exercise for the participant throughout the 12-week intervention. Cardiovascular exercise intensities were based on a percentage of heart rate reserve (HRR), which was calculated using the Karvonen formula. Once a phase was completed, each participant completed a follow-up assessment of all physiological parameters.

Phase 1

Phase 1 was defined as any participant entering rehabilitation while actively undergoing chemotherapy and/or radiotherapy. Phase 1 clientele trained in an intensity range developed to preserve health status and combat toxicities of treatment. This range was classified as low to

moderate in intensity or 30-45% of the individual's HRR and estimated one-repetition maximum.

Phase 2

Phase 2 was defined as any participant entering rehabilitation post-treatment or not actively undergoing chemotherapy and/or radiotherapy. Phase 2 clients trained in an intensity range designed to elicit chronic adaptations of exercise as well as built a foundation for proper technique and corrective movement. This range was classified as moderate intensity or 40-60% of their HRR and estimated one-repetition maximum.

Exercise-Based Assessment

The exercise-based assessment was performed as outlined by the UNCCRI Phase Training protocol (Brown et al., 2019). In brief, participants underwent an assessment of physiological parameters under the supervision of a staff physiologist and two clinical cancer exercise specialists (CCES). Parameters assessed included body composition, balance, pulmonary function, cardiovascular endurance, muscular strength and endurance, flexibility, and quality of life surveys. Upon completion of the assessment, the staff physiologist and CCES team worked together to analyze the assessment data and formulate an exercise prescription to govern the exercise intervention (see Appendix D for Exercise Assessment Protocol).

Exercise Intervention

Exercise Session

Each participant performed exercise at a frequency of three days per week for 12 weeks. The duration of each session was 60 minutes. Each session was composed of 20 minutes of aerobic exercise, 30 minutes of resistance and balance training, and 10 minutes of flexibility training. The intensity of exercise was governed by each participant's phase and determined from

the analysis of the assessment data by the researcher and CCES assigned to the participant. Each participant progressed through their rehabilitation in a manner deemed appropriate by the assigned CCES and the researcher.

Exercise Programming

The exercises chosen for each training session were dictated by the goal of each phase. Participants in Phase 1 had exercise programmed to primarily alleviate the side-effects of treatment. Those participants assigned to Phase 2 had exercises programmed to primarily increase the technique and functional deviations and asymmetries found with performance of movement. Each phase's participants had exercise programmed to enhance physiological systems, activities of daily living, and independence.

Blood Sample Collection and Analysis

Blood Draw Timeline

Blood sample collection was performed by a registered nurse or a certified phlebotomist. Procurement of blood samples was performed within one week of the initial assessment or reassessment performed at UNCCRI. As such, data were presented as PRE and POST intervention utilizing a paired sample experimental design. All samples were collected prior to the participant's exercise session UNCCRI with each participant coming to the facility having fasted and prior to initiation of physical activity. During each visit, one vial of blood totaling no more than 10 milliliters (mL) was collected to account for various forms of analysis.

Protocol

Blood Sample Collection

Prior to sample collection, a phlebotomist was scheduled to perform a blood draw on the specific participant(s) and a timeslot for use of the Attune NxT flow cytometer in the Ross Hall

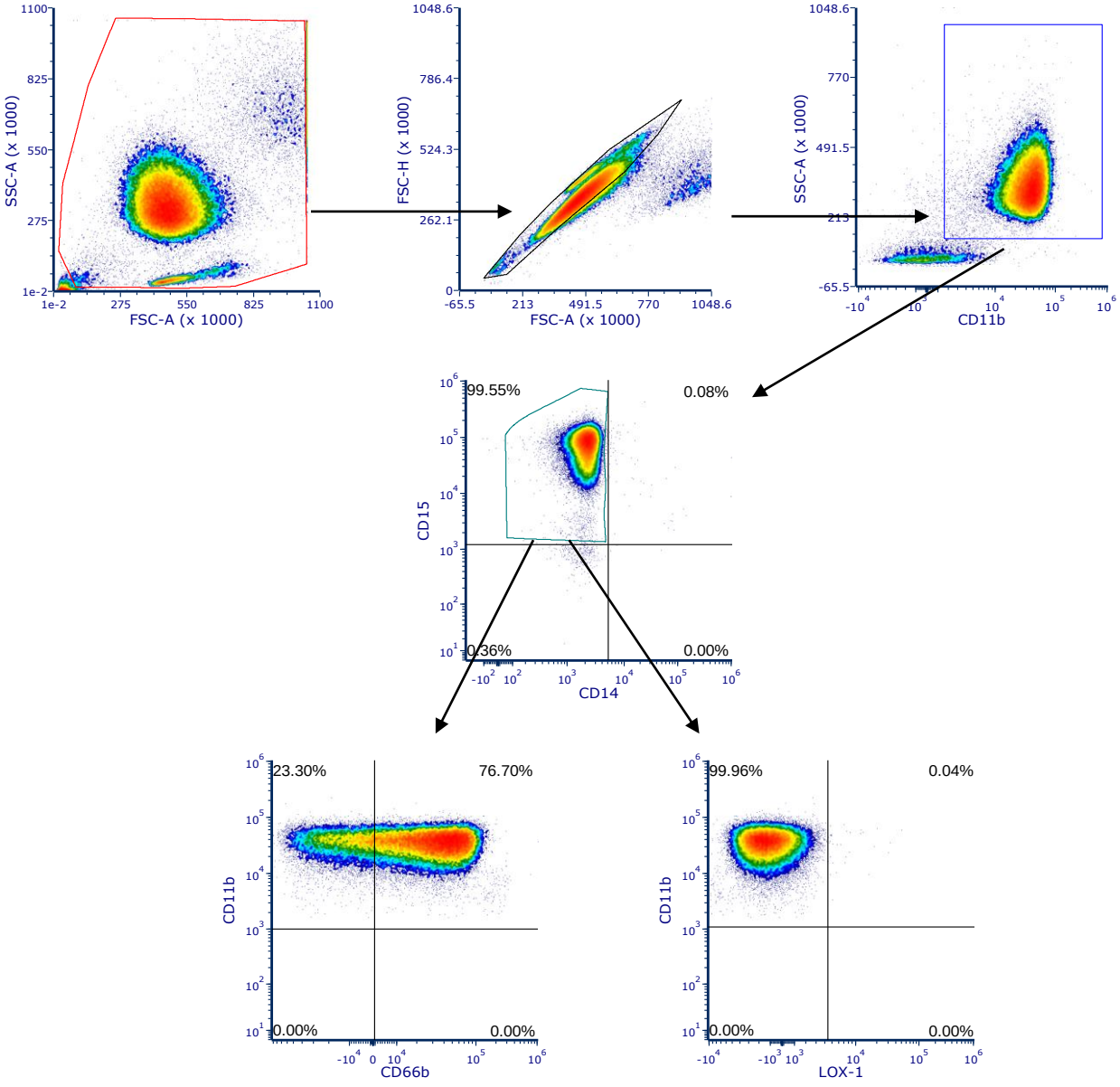
Imaging Suite was obtained. Supplies was available at UNCCRI to perform all blood draws. Supplies included, but were not limited to, EDTA vacutainer collection tubes (10 mL), an ice bucket with crushed ice, needles, cotton balls, Band Aids, alcohol wipes, rubber tourniquet bands, vacutainer rack, and black Sharpies.

Neutrophil Isolation and Phenotyping

Once a sample was collected, it was transported to a secondary laboratory for processing and preparation for flow cytometry analysis. Neutrophil isolation was performed with 3 mL of whole blood via negative selection as described by the *EasySep Direct Human Neutrophil Isolation Kit* by STEMCELL Technologies. Briefly, this kit utilized a combination of magnetic particles, monoclonal antibodies, and Fc block to bind with all non-neutrophil cell populations, leaving isolated neutrophils within the supernatant. Once isolated, samples were stained and incubated with fluorescent-labeled antibodies for LOX-1 (anti-human LOX-1-Briliant Violet 421, BioLegend, Catalog #358609), CD14 (anti-human CD14-APC, BioLegend, Catalog #301808), CD15 (anti-human CD15-PE-Cy7, BioLegend, Catalog #323030), CD11b (anti-human CD11b-FITC, BioLegend, Catalog #301330), and CD66b (anti-human CD66b-Pacific Blue, BioLegend, Catalog #305111). Samples were run on an Attune NxT flow cytometer (Thermo Fisher Scientific, Waltham, MA). Gates were set to analyze neutrophils in each sample. Cell counts were provided for the total neutrophil, mature neutrophil, and immature neutrophil populations. Distinguishing between the various sub-types of neutrophils allowed for further examination of the extent that circulating neutrophils were truly canonical or representative of another population of cells similar in character to neutrophils (i.e., MDSCs).

Figure 1

Neutrophil Characterization Strategy



Gating of neutrophils after isolation from whole blood. Characterization and count based on CD11b+CD14-CD15+CD66b+LOX1- phenotype. All samples were gated against an unstained control sample to ensure accuracy.

Neutrophil Functional Measure

Neutrophil functional analysis was conducted following the protocol for the Abcam Respiratory Burst Assay Kit. Stimulation via N-formylmethionine-leucyl-phenylalanine (fMLP) at a 10X concentration induced a NADPH oxidative-dependent respiratory burst response. Measurement of respiratory burst utilized fluorescence of rhodamine 123 after its conversion from dihydrorhodamine 123 (DHR 123) during sample preparation. DHR 123 is freely permeable across the cell membrane and upon stimulation by fMLP was oxidized into rhodamine 123 for fluorescent measurement. After 45 minutes of stimulation, the sample was immediately assessed on the Attune NxT flow cytometer for positive expression of the rhodamine 123 relative to an unstimulated sample and unstained control.

Additional analysis of neutrophil functional capacity was assessed in a time-dependent manner. Isolated neutrophils were incubated at 37ºC with PBS and assessed for loss of CD16 expression at three separate time points: 0, 24, and 48 hours, following established methodology (Robinson et al., 1997). A CD16 expression was used as a surrogate marker of neutrophil functional capacity over time.

Independent and Dependent Variables

Independent Variables

The independent variables for this study included intensity of exercise (low or moderate), fMLP concentration, and incubation time.

Dependent Variables

The dependent variables for this study included ANC, neutrophil rhodamine 123 expression, and CD16 brightness.

Primary Outcome Measure(s)

Primary outcome measures for this study included change to ANC and change to neutrophil functional capacity. Reference ranges utilized for determining ANC of each participant included high (neutrophilia): >8.0 K cells/µl; normal: 1.5 – 8.0 K cells/µl; safe: 0.5 – 1.5 K cells/µl; and low (neutropenia): <0.5 K cells/µl. High values were indicative of infection or stress. Normal and safe ranges indicated no restriction in activities of daily living. Low values indicated a severe risk for chronic infection. Neutrophil functional capacity was quantified via the change in expression of rhodamine 123 protein on the plasma membrane of purified neutrophils in response to fMLP stimulation. Currently, no literature exists to present normative data for fMLP-induced functional responses in neutrophils. However, literature supported an increase in neutrophil respiratory activity and ROS production in response to stimulation under homeostatic conditions and as a result of exercise in the generally healthy and rheumatoid arthritis populations (Bartlett et al., 2018; Davison, 2011; Kenny et al., 2017). These data were used to support inclusion of this assessment as an outcome measure, giving researchers evidence to expect a changed response in neutrophil respiratory activity because of fMLP presentation coupled with an exercise intervention.

Statistical Analysis

Statistical analyses were performed via IBM SPSS software (IBM, Armonk, New York) and FCS Express software (De Novo Software, Glendale, California). Tests performed included paired sample *t*-tests and two-sample *t*-tests to assess primary outcome variables within and between groups, respectively. A priori power analysis indicated a minimum of seven participants in each group to achieve 80% power for detecting effect at an alpha level of .05.

CHAPTER IV

RESULTS

Participant Demographics

A total of 24 participants enrolled in the study (female, *n* = 16) with 12 participants allocated to each group based on treatment status: in-treatment (IT) or post-treatment (PT). No adverse events were reported for either group during the study. Total adherence to exercise programming across the participant population was 87 ± 13%. In-treatment patients had an adherence rate of 88 ± 13 while PT patients had an adherence rate of 87 ± 14%. Adherence to the intervention was calculated as the number of sessions attended divided by the total number of sessions programmed over the 12-week intervention: (adherence = [attended sessions]/36]). No statistically significant differences were found between groups for adherence. Post-treatment patients were, on average, 7.5 months removed from treatment at the time of enrollment. The total population was 64 ± 10 years of age and comprised primarily of Caucasian individuals (*n* = 21) with the only other ethnic group represented being Latinx (*n* = 3). No statistically significant differences were observed between groups for any of the participant demographics. A summary of participant demographics is presented in Table 1.

Table 1

Summary of Participant Demographic Information

	IT (n = 12)	PT (n = 12)	Average (N = 24)
Age (years)	60 ± 9	67 ± 12	64 ± 10
Resting Heart Rate (bpm)	84 ± 15	80 ± 14	84 ± 17
Height (m)	1.65 ± 0.09	1.64 ± 0.14	1.64 ± 0.12
Weight (kg)	74.9 ± 18.0	82.8 ± 18.1	79.2 ± 18.5
Body Mass Index (kg/m^2)	27.5 ± 0.2	30.6 ± 0.3	29.1 ± 0.3
Blood Pressure (mmHg)			
Systolic BP	122 ± 16	134 ± 17	130 ± 17
Diastolic BP	81 ± 15	84 ± 7	83 ± 11
Cancer Diagnoses			
Breast, *n* (%)	4 (34)	5 (42)	9 (38)
Colorectal, *n* (%)	1 (8)	3 (25)	4 (17)
Lung, *n* (%)	2 (16)	0 (0)	2 (8)
Prostate, *n* (%)	2 (16)	0 (0)	2 (8)
Kidney, *n* (%)	1 (8)	0 (0)	1 (4)
Tongue, *n* (%)	0 (0)	1 (8)	1 (4)
Esophageal, *n* (%)	1 (8)	0 (0)	1 (4)
Testicular, *n* (%)	1 (8)	0 (0)	1 (4)
Ovarian, *n* (%)	0 (0)	1 (8)	1 (4)
Hodgkin's Lymphoma, *n* (%)	0 (0)	1 (8)	1 (4)
Myelodysplastic Syndrome, *n* (%)	0 (0)	1 (8)	1 (4)
Cancer Stage			
Stage 0, *n* (%)	0 (0)	1 (8)	1 (4)
Stage I, *n* (%)	2 (16)	6 (50)	8 (33)
Stage II, *n* (%)	2 (16)	1 (8)	2 (8)
Stage III, *n* (%)	4 (34)	1 (8)	5 (21)
Stage IV, *n* (%)	2 (16)	1 (8)	3 (12.5)
Gleason, *n* (%)	2 (16)	0 (0)	2 (8)
Unknown/Not Staged, *n* (%)	0 (0)	3 (25)	3 (12.5)
Treatment History			
Surgery, *n* (%)	11 (92)	11 (92)	22 (92)
Radiation, *n* (%)	9 (75)	10 (83)	19 (79)
Chemotherapy, *n* (%)	8 (67)	8 (67)	16 (67)
Hormone Therapy, *n* (%)	3 (25)	5 (42)	8 (33)
Immunotherapy, *n* (%)	4 (33)	3 (25)	7 (29)
Time Since Treatment (months)	0 ± 0	7.5 ± 4	

Values represent sample mean (± standard deviation) of the given population.

Sixteen participants indicated they were currently physically active at the time of enrollment, whether due to occupation or recreation. These participants reported that they performed approximately 14 minutes of physical activity per week, prior to enrolling in the current study. Those who reported engaging in occupational physical activity indicated the intensity of this activity as low (*n* = 4) or moderate (*n* = 12). Those who reported engaging in recreational physical activity indicated the intensity of this activity as low (*n* = 10). Every participant who engaged in recreational physical activity also reported engaging in occupational physical activity. Every participant indicated they experienced a change to their physical activity within the last year prior to enrollment and/or directly due to their cancer diagnosis regardless of physical activity habits reported at enrollment. A summary of physical activity history can be found in Table 2.

Table 2

Self-Reported Physical Activity History

	IT (*n* = 12)	PT (*n* = 12)	Total (*N* = 24)
Physically Active Participants			
Occupational, *n* (%)	7 (58)	9 (75)	16 (67)
Recreational, *n* (%)	4 (33)	6 (50)	10 (42)
Physical Activity per Week (minutes)	12 ± 8	15 ± 9	14 ± 9
Intensity of Occupational Physical Activity			
Low, *n* (%)	4 (33)	0 (0)	4 (17)
Moderate, *n* (%)	3 (25)	9 (75)	12 (50)
Intensity of Recreational Physical Activity			
Low, *n* (%)	4 (33)	3 (25)	7 (29)
Moderate, *n* (%)	0 (0)	3 (25)	3 (12.5)

Values represent number (%) or sample mean (± standard deviation) of the given population.

Exercise-Based Assessment

Prior to and after the intervention, participants underwent exercise-based assessment for evaluation of body composition, balance, pulmonary function, cardiovascular endurance, muscular strength and endurance, flexibility, and quality of life. After 12 weeks of exercise, the total population saw a significant increase in upper body strength-to-weight ratio (0.41 ± 0.17 to 0.51 ± 0.13; $p < .05$). After 12 weeks of exercise, the IT group saw a significant increase in cardiovascular endurance (18.8 ± 6.2 to 22.8 ± 6.2 mL/kg/min, $p < .05$) and lower body strength-to-weight ratio (1.05 ± 0.39 to 1.26 ± 0.37; $p < .05$). After 12 weeks of exercise, the PT group saw a significant increase in upper body strength-to-weight ratio (0.38 ± 0.15 to 0.54 ± 0.14; $p < .05$), lower body strength-to-weight ratio (1.11 ± 0.35 to 1.39 ± 0.48; $p < .05$), and muscular endurance measured by sit-to-stand repetitions in 30 seconds (9 ± 3 to 12 ± 2 repetitions, $p < .05$). A comparison between groups found no differences between IT and PT patients at baseline. After 12 weeks, the IT patients were significantly different from PT patients in muscular endurance ($p < .05$). A summary of various parameters measured can be found in Table 3.

Table 3

Exercise Assessment Results

	IT (n = 12)	PT (n = 12)	Total (N = 24)
Body Composition (percent body fat)			
Pre	31.2 ± 12.8	38.9 ± 9.7	35.6 ± 11.9
Post	37.9 ± 4.9	41.8 ± 8.2	39.4 ± 6.7
Balance (LOS)			
Pre	78 ± 18	86 ± 4	84 ± 11
Post	83 ± 5	85 ± 7	84 ± 6
Pulmonary Function (percent of predicted)			
FVC Pre	103 ± 25	80 ± 26	91 ± 25
FVC Post	95 ± 22	98 ± 13	96 ± 18
FEV_1 Pre	95 ± 25	74 ± 20	84 ± 23
FEV_1 Post	96 ± 20	92 ± 10	94 ± 15
Cardiovascular Endurance (mL/kg/min)			
Pre	18.8 ± 6.2	18.3 ± 8.2	18.2 ± 7.4
Post	22.8 ± 6.2*	21.6 ± 7.3	22.2 ± 6.8
Muscular Strength (strength-to-weight ratio)			
Upper Pre	0.45 ± 0.18	0.38 ± 0.15	0.41 ± 0.17
Upper Post	0.48 ± 0.12	0.54 ± 0.14*	0.51 ± 0.13*
Lower Pre	1.05 ± 0.39	1.11 ± 0.35	1.08 ± 0.38
Lower Pre	1.26 ± 0.37*	1.39 ± 0.48*	1.31 ± 0.42
Muscular Endurance (sit-to-stand reps)			
Pre	14 ± 5	9 ± 3	12 ± 4
Post	16 ± 4	12 ± 2*[a]	14 ± 3
Flexibility (sit-and-reach inches)			
Pre	9.53 ± 3.83	11.67 ± 3.91	10.03 ± 4.39
Post	11.16 ± 4.51	14.25 ± 1.25	10.54 ± 5.54
Quality of Life (FACT Total)			
Pre	108 ± 20	117 ± 23	111 ± 25
Post	103 ± 19	95 ± 25	99 ± 22

Values represent sample mean (± standard deviation) of the given population. * Denotes significance within group from baseline ($p < .05$). [a] Denotes significant difference between groups; $p < .05$.

Absolute Neutrophil Count

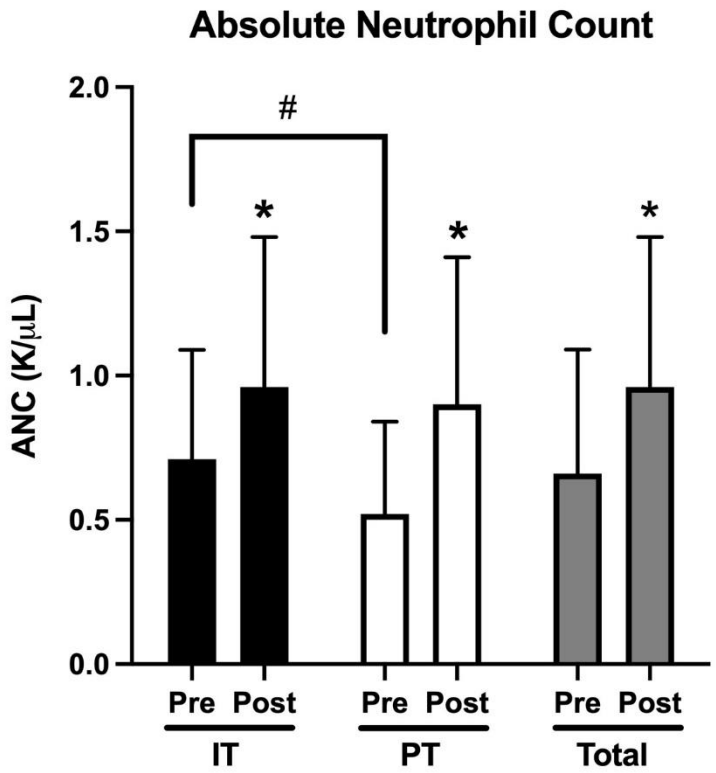
Upon entry, the total population had an ANC of 0.66 ± 0.43 K/µL with IT patients at an ANC of 0.71 ± 0.38 K/µL and PT patients at an ANC of 0.52 ± 0.32 K/µL. Based on normative data for ANC in the generally healthy population, both groups were classified as “safe” in their

initial ANC values. The IT group's ANC upon entry was comparable to cancer patients while in treatment as the instance of low neutrophil counts was well document during treatment (Chen et al., 2015; Van Waart et al., 2015). Interestingly, the PT group's ANC upon entry was significantly lower than that for the IT group. In fact, the PT group was on the cusp of severe neutropenia. It was reported in the literature that recovery from neutropenia could be expected within three months after each treatment cycle with some authors reporting no clear pattern of recovery (Grazziutti et al., 2006; Hughes et al., 2002; Moore, 2016). It should be noted that this recovery was hastened with pharmacological intervention (e.g., G-CSF agonists), which none of the participants in this study was prescribed (Alvarado Ibarra et al., 1999; Choi et al., 2014). The PT group was on average 7.5 months post-treatment, which could influence ANC if some participants enrolled closer to their end of treatment date.

After completing the exercise intervention, within a sample of 1 mL of blood, the total population saw a significant increase in ANC from pre-intervention (i.e., baseline) values (0.66 ± 0.43 to 0.96 ± 0.52 K/µL; $p < .05$; see Figure 2). The IT group showed a significant increase in ANC from baseline (0.71 ± 0.38 to 0.96 ± 0.52 K/µL; $p = .05$; see Figure 2), and the PT group also showed a significant increase in ANC from baseline (0.52 ± 0.32 to 0.90 ± 0.51 K/µL; $p < .05$; see Figure 2). Between groups comparison found a significant difference in ANC between groups at baseline. After the exercise intervention, between groups comparison found no significant difference in ANC.

Figure 2

Absolute Neutrophil Count ± Standard Deviations Before (Pre) and After (Post) 12 Weeks of Exercise Training



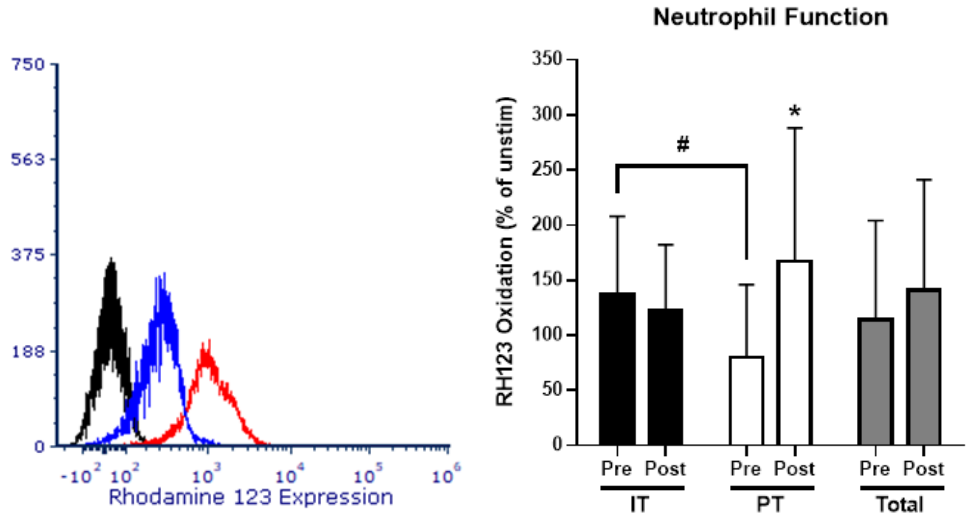
Note. Count is based on $CD11b^{+}CD14^{-}CD15^{+}CD66b^{+}LOX1^{-}$ phenotype. # denotes a difference in ANC between IT-Pre and PT-Pre; $p < .05$. *$p < .05$ compared to pre-intervention values.

Neutrophil Function

After the exercise intervention, the total population saw no change in the neutrophil-induced rate of rhodamine oxidation from baseline (114 ± 90% to 141 ± 100%; no significance [NS]). The IT group saw no change from baseline (137 ± 71% to 122 ± 60%; NS), while the PT group saw a significant increase in the rate of rhodamine oxidation after the exercise intervention (80 ± 66% to 167 ± 121%; $p = .05$). Between groups comparison found a significant difference in rate of oxidation at baseline ($p = .02$). After the exercise intervention, between groups comparison found no significant difference in rate of activation (see Figure 3).

Figure 3

Neutrophil Function Rate of Response to Formylmethionine-Leucyl-Phenylalanine Stimulation from Rest Before and After 12 Weeks of Exercise



Note: Left: Representative results of fluorescence from neutrophil rhodamine oxidation via flow cytometry. Black = unstained control, blue = unstimulated sample, red = 45-minute fMLP-stimulated sample. Right: Neutrophil rate of rhodamine oxidation relative to an unstimulated sample. # denotes difference in rate of oxidation between IT-Pre and PT-Pre; $p = .02$. *$p < .05$ compared to PT Pre value.

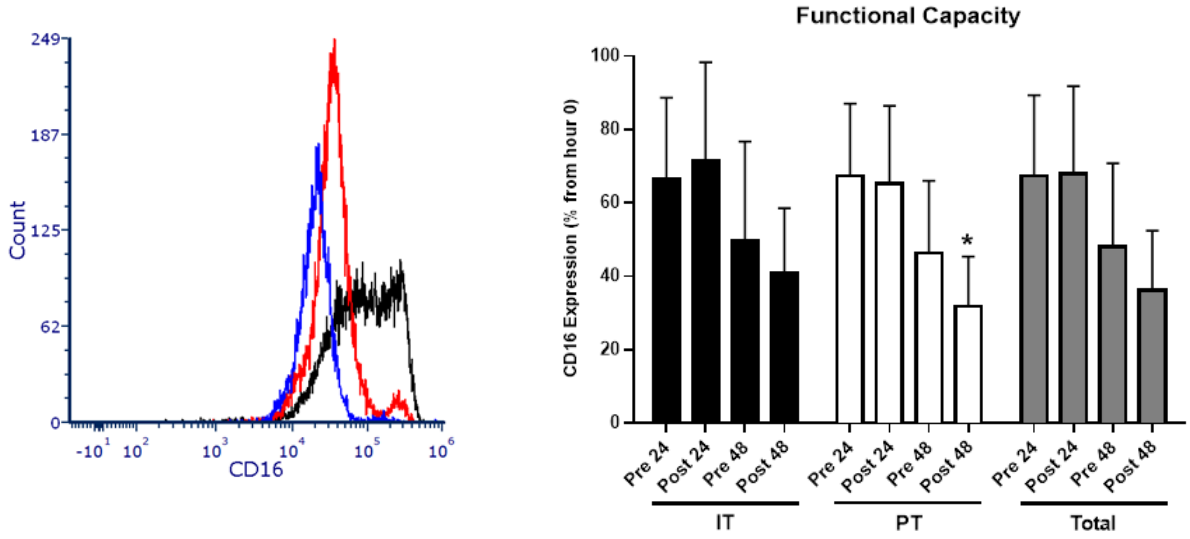
Neutrophil Functional Capacity

After the exercise intervention, the total population saw no change in neutrophil functional capacity, defined as the rate of diminished CD16 fluorescence relative to hour 0, at any time point over a 48-hour period compared to baseline. Neither group saw a change in functional capacity over a 24-hour period compared to baseline. The PT group saw a significant decrease in functional capacity after 48 hours from hour zero compared to baseline (46 ± 21% to 32 ± 14%; $p < .05$). Between groups comparison found no significant difference in functional capacity at 24 or 48 hours at baseline. After the exercise intervention, between groups

comparison found no significant difference in functional capacity at 24 or 48 hours (see Figure 4).

Figure 4

Neutrophil Functional Capacity as Determined by CD16 Expression Over a 48-Hour Period Before and After 12 Weeks of Exercise



Note. Left: Representation of CD16 brightness, measured via flow cytometry, relative to hour 0 (black) measured at 24 (red) and 48 (blue) hours. Right: Percent of CD16 expression at each time point relative to hour 0 for pre- and post-intervention measurements, respectively. *$p < .05$ compared to baseline.

CHAPTER V

DISCUSSION AND CONCLUSION

Discussion

This study aimed to assess the impact of exercise on the absolute neutrophil count and neutrophil function in cancer patients participating in a 12-week exercise intervention who were actively receiving chemotherapy and/or radiation or who had completed these forms of treatment within two years of enrollment. Based on the results of this study, exercise significantly increased ANC after 12 weeks of exercise (as seen in Figure 2) in patients both in-treatment and post-treatment. Furthermore, after cancer treatments had been completed, exercise improved neutrophil function after 12 weeks of exercise (as seen in Figure 3).

It has been well established that acute exercise increases circulating neutrophil count and activity for restorative processes (e.g., clearance of debris, driving inflammation to recruit lymphocytes to recovering muscle) and returns to baseline values within six hours after completing the exercise (Bessa et al., 2016; Sellami et al., 2018). This acute effect is seen in both generally healthy individuals and in the cancer population (Schauer et al., 2020). The impact of chronic exercise on expanding neutrophil proliferation in the cancer population has not been well established. However, the results of the current study indicated chronic exercise in the cancer population was capable of stimulating a significant increase in neutrophil count. Current literature did not support chronic exercise to directly increase the number of neutrophils in circulation; however, it did implicate upregulation of precursors for neutrophil differentiation such as granulocyte colony stimulating factor (G-CSF) and increased hematopoietic progenitor

cell concentration in the bone marrow, which would allow for an increase in circulating neutrophils (De Lisio & Parise, 2012; Emmons et al., 2019). The increase in ANC seen in the current study might also have been attributed to these mechanisms.

Granulocyte colony stimulating factor induces a cascade of events during infection to expand neutrophil count by mobilizing neutrophils in the bone marrow and inducing neutrophil differentiation. This mechanism is stimulated pharmacologically in the cancer population to induce the differentiation of neutrophils from progenitor cells during chemotherapy. This pathway acts to promote expansion through stimulation by inflammatory cytokines, namely IL-1β. This inflammatory-dependent action promotes G-CSF upregulation in the bone marrow and initiates neutrophil differentiation from progenitor cells. This has proven to be a valuable therapeutic by aiding individuals who are suffering from neutropenia. However, exercise has been shown to reduce the dosage of G-CSF necessary for expansion (Courneya et al., 2007). Courneya et al. (2007) found that individuals with breast cancer who participated in exercise programming had a significantly lower rate of G-CSF administration compared to usual care controls and that exercise trained patients who were administered G-CSF had a lower dosage, indicating exercise served a role in reducing the need for pharmaceutical intervention of G-CSF. The current study supported the notion that exercise could enhance neutrophil proliferation even in the absence of any G-CSF intervention.

The increase in ANC might also be influenced by increased hematopoietic progenitor cell expansion, allowing for a greater potential for neutrophil differentiation. It is proposed that this mechanism acts through catecholamine activity to promote expansion of the pool of progenitor cells in response to acute exercise training (Maryanovich et al., 2018; Tops et al., 2016). Beta-adrenergic signaling via noradrenaline and adrenaline act on β_3 pathways to stimulate bone

marrow rejuvenation and β_2 pathways to mobilize stem cells into the blood stream, respectively (Agha et al., 2018; Maryanovich et al., 2018). Bone marrow turnover and increased mobilization of stem cells into the blood stream would allow for an increased bank of precursor cells for neutrophil differentiation. Unfortunately, this effect has yet to be replicated through chronic exercise in the human population. However, animal models suggest that oxidative stress induces an upregulation in antioxidant properties in the bone marrow, providing a mechanism for long-term protection of progenitor cells (De Lisio & Parise, 2012). If these effects were confirmed in humans after chronic training, it would indicate that exercise could be a modality to produce a higher supply of progenitor cells that might differentiate into neutrophils when activated and therefore provide a protective mechanism to ensure their survival until called upon. While measurement of catecholamine proliferation and function was beyond the scope of the current study, it did provide a potential mechanism for chronic exercise effects on the upregulation of neutrophil precursors.

Increased ANC in the current study further supported the incorporation of structured exercise as close as possible to the initiation and cessation of treatment. Results of this study provided evidence that low and moderate intensity exercise was adequate stimuli to promote changes to ANC. This result also implicated exercise as a mode for prevention of severe neutropenia development in the exercising cancer population as no group in the current study fell into the “low” category of ANC as defined by the American Society of Hematology (Boxer, 2012). This result is especially important for in-treatment patients when considering the instance of neutropenia development and the increased risk of mortality. Many prior studies have explored the impact of treatment status on ANC and it is well known that active treatment diminishes ANC in cancer patients (Chen et al., 2015; Schauer et al., 2020). Chen et al. (2015)

found 79% of gastric cancer patients experienced neutropenia during their treatment regimen with lower baseline neutrophil counts indicating an increased risk of mortality. Furthermore, several studies have shown exercise to be beneficial in reducing the risk of neutropenia and hospitalization during chemotherapy in the cancer population compared to usual care controls (Dimeo et al., 1997; Mijwel et al., 2020; Van Waart et al., 2015). In fact, Van Waart et al. (2015) found a lower rate of neutropenia development during chemotherapy in breast cancer patients who performed exercise when compared to a usual care control. Dimeo et al. (1997) found a significant difference in the duration of neutropenia in cancer patients who exercised during chemotherapy compared to a usual care control. Furthermore, Mijwel et al. (2020) found a lower rate of hospitalization due to neutropenic events (3% versus 13%) in breast cancer patients who exercised compared to usual care controls. The results of this study supported exercise as a mode of therapy to prevent severe neutropenia. Additionally, the similar response to exercise between groups could also be a relevant finding for referring oncologists as it increases confidence for referral of all patients to an exercise program regardless of treatment status.

While results from the current study provided evidence suggesting the benefit of exercise for protection against severe neutropenia, other studies have failed to demonstrate a benefit of exercise on neutropenia in cancer patients (Chamorro-Vina et al., 2010; Jarden et al., 2009; Shim et al., 2019; Zimmer et al., 2013). There were, however, some notable methodological differences in these studies. Several were conducted on patients with leukemia and lymphoma who were receiving high-dose chemotherapy or stem cell transplantation or they implemented a home-based exercise intervention. These conditions in particular could have predisposed participants to depressed immune function, inhibiting their ability to adequately resupply neutrophils into circulation despite the exercise intervention or simply did not provide an

adequate exercise stimulus to elicit a change. In particular, Shim et al. (2019) found colorectal cancer patients who completed a home-based exercise program during chemotherapy saw no difference in the instance of neutropenia when compared to a sedentary control. The results of this study might indicate the necessity of supervised and structured exercise in the cancer population to protect against the instance of neutropenia as we found improvement to these values when implementing an individualized and prescribed form of exercise that required one-on-one supervision from a clinical cancer exercise specialist.

The change in functional activity via rhodamine oxidation by neutrophils seen in this study was promising for both IT and PT groups. The IT group might not have shown a significant change but more importantly, there was no significant decrement to function as a result of the exercise intervention. This is important to consider in the context of the treatment regimen. As a cancer patient undergoes treatment, maintenance of immune function is critical in order to combat potential infections that might occur during treatment without adjusting the dosage of treatment. Prior studies have shown chemotherapy could reduce neutrophil function for up to three weeks between cycles (Mendonça et al., 2006). The recovery of function to neutrophils after treatment has ended in adult humans is not well understood but pediatric leukemia patients and animal models indicated a loss of function could persist in the weeks to months following the end of treatment (Ravanbakhsh et al., 2021; Tanaka et al., 2009).While exercise is thought to be a prophylactic option for combatting the treatment-related impact on neutrophil function during treatment, little evidence explains neutrophil function in exercising cancer patients after treatment is complete. Despite the lack of evidence on the long-term impact of exercise on neutrophil function in cancer patients after treatment, studies in other populations have shown beneficial functional changes to neutrophils via enhanced phagocytosis, increased

reactive oxygen species production, and improved oxidative burst activity, which could also provide an explanation for the results of the current study (Bartlett et al., 2017; Castellani et al., 2019; Syu et al., 2012). For example, Syu et al. (2012) found chronic moderate intensity exercise over a two-month period was enough to cause improved phagocytic activity of neutrophils in healthy individuals compared to sedentary controls, 36% versus 20% phagocytic neutrophils. Bartlett et al. (2017) found patients with stable rheumatoid arthritis who participated in a high intensity walking program for 10 weeks saw a significant increase in neutrophil ROS production after PMA stimulation. Furthermore, the Bartlett group also reported enhanced ROS production and oxidative burst of neutrophils in sedentary adults in response to exercise (Bartlett et al., 2017). Castellani et al. (2019) found cystic fibrosis patients who received G-CSF therapy showed enhanced ROS production after fMLP stimulation. Granulocytic colony stimulating factor in particular acts on neutrophil function by recruiting neutrophils in an inflammatory-dependent manner to sites of infection through signaling via IL-17 and TNFα; it is also shown to prime neutrophils for NET formation by stimulation via complement protein, namely C5a (Laan et al., 2003; Yousefi et al., 2009). Should an upregulation of G-CSF have occurred in participants in the current study, that could explain the increased oxidation of DHR123, a ROS, to rhodamine after fMLP exposure via priming of the neutrophils to better respond to activating stimuli.

Functional changes to neutrophils could be due to improved sensitivity to inflammatory cytokines and expression of migratory factors. Inflammatory cytokines such as IL-8 and IL-17 signal for chemotaxis of neutrophils to a site of inflammation (Bartlett et al., 2016; Laan et al., 2003). Bartlett et al. (2016) reported significantly higher neutrophil chemotaxis toward IL-8 in physically active older adults when compared to sedentary counterparts ($p = .044$) with a significant positive correlation between physical activity and chemotactic index in these

individuals (r = 0.689, p = .001). Chemo-attractants such as CXCR2 and CCR5 present possible mechanisms by neutrophils are capable to migrate toward a stimulus. Bartlett et al. (2016) also found that improved sensitivity to inflammatory cytokines was seen without any change to CXCR2 expression, suggesting the migratory improvement in physically active individuals could occur independent of improved chemo-attractant expression. This might explain the lack of change in neutrophil function for the IT group in the present study. Perhaps circulating neutrophils for patients who are undergoing treatment exhibited neither of these changes, thus inhibiting any real functional change when stimulated. Currently, no studies have shown that active cancer treatment directly impacts these factors to the point of inhibiting an effect from exercise. Additionally, Barry et al. (2017) found a significant change in neutrophil expression of CCR5, a chemokine receptor that promotes neutrophil infiltration into tissue, in obese individuals who participated in high-intensity interval training (pre-training 65 ± 9% vs. post-training 71 ± 6% neutrophils expressing CCR5, $p < .05$) after 10 sessions of exercise. Overall, improved expression of chemotactic markers and sensitivity to inflammatory cytokines might mobilize neutrophils at a faster rate, promoting greater oxidation such as was seen in the PT group. These data from prior studies could explain the improvement to DHR123 oxidation in the PT group and provide a possible indication as to why the IT group saw no improvement.

To date, no studies have provided such an extensive neutrophil characterization in a broad range of cancer survivors following a prescribed, individualized, supervised exercise intervention. Furthermore, despite the lack of evidence for the impact of exercise on chemotactic factors and inflammatory cytokine sensitivity in the cancer population, the current study described a structured exercise intervention that might garner beneficial outcomes. Unlike prior studies mentioned, the current study utilized an evidence-based approach to prescribing exercise

by going further than simply home-based or unmonitored forms of exercise (Brown et al., 2019; Shim et al., 2019). Additionally, the intensities prescribed were based on individual performance, providing a method to tailor the effects of exercise to the individual rather than utilizing a generalized approach.

The current study utilized CD16 expression as a marker of functional capacity since it is a common surface marker on a variety of immune cells that respond to stimuli in a non-discriminant manner to induce functional responses from the immune cell (i.e., degranulation of a neutrophil). It is expected that as neutrophils age, their fluorescence of CD16 will diminish (Robinson et al., 1997). The aim of this measure used in the present study was to determine if there was a reduction in the rate at which CD16 fluorescence diminished over a 48-hour period on neutrophils from participants after exercise training compared to samples obtained at baseline. The general lack of change to CD16 was not surprising as current literature supported no change in CD16 expression on neutrophils in response to exercise (Bartlett et al., 2017). While no significant reduction in CD16 fluorescence was seen in the IT group, the PT group showed significant reduction in CD16 fluorescence over a 48-hour time period after completing the exercise intervention, an effect that was not observed prior to initiating the exercise intervention. This effect might be explained as an alteration in neutrophil turnover after 48 hours. Current evidence is conflicting with some studies showed delayed-onset apoptosis while others reported a rapid increase in neutrophil apoptosis in response to acute exercise (Chen et al., 2018; Mooren et al., 2012; Syu et al., 2011). Chen et al. (2018) demonstrated increased rates of neutrophil apoptosis in exercising individuals who participate in hypoxic exercise training. While this showed the potential for exercise to increase apoptosis of neutrophils, thus providing an explanation of increased turnover leading to diminished CD16 fluorescence in PT, hypoxic

exercise training is likely not a practical intervention for the cancer population. Syu et al. (2011) showed that chronic moderate intensity exercise increased mitochondrial membrane potential in neutrophils, providing a reduction in apoptotic signaling to these cells and reducing the instance of spontaneous apoptosis. Furthermore, Mooren et al. (2012) implicated G-CSF as a survival signaling compound for neutrophils in response to a bout of acute exercise, delaying neutrophil apoptosis via upregulation of the anti-apoptotic protein MCL-1 and allowing for longer neutrophil survival during the post-exercise recovery period. So rather than increased turnover as the reason for increased rate of diminished CD16 fluorescence, it might be that neutrophils were upregulating pathways to delay apoptosis. This would allow for a greater quantity of neutrophils to survive the 48-hour time period after the exercise intervention. However, while a higher number of neutrophils might have survived, they might not have been capable of upregulating CD16 expression. Currently, no studies explain upregulation of CD16 in the aged neutrophil.

Limitations

The current study was not without limitations. A potential limitation of this study was population heterogeneity. Individuals referred to UNCCRI entered the program with a wide range of characteristics. As a result, these factors might have affected outcomes based on this variability. Such characteristics included solid versus liquid cancers, different treatment regimens (namely chemotherapy, radiotherapy, and immunotherapy), variations in each of the treatment regimen types, time out of treatment for the PT group, cancer stage, and varying fitness levels between participants at the time of entry into the study. Lack of a control group posed another limitation to the current study. This limitation has been acknowledged and participants are being actively recruiting for a control group prior to moving ahead with publication of these data (preliminary control data shown in Appendix E).

Variability in participant characteristics the day of their scheduled blood draw posed potential limitations. Hydration and feeding status could potentially have affected neutrophil counts since data were normalized to a volume of blood. This was controlled by the simultaneous quantification of hematocrit when drawing blood (not reported) and a requirement of overnight fasting prior to a blood draw. The process for assessment of neutrophil function following fMLP exposure had the potential to over-activate neutrophils during the respiratory burst process and, as such, posed a risk of a reduction in the total amount of observable events via flow cytometry. To address these limitations, all instruction for participation was standardized, emphasized, and clearly stated to all study participants to ensure all samples were collected under the exact same conditions. Furthermore, blood collection and handling were standardized and closely scrutinized to safeguard the integrity of all samples. To avoid overstimulation of cells with fMLP, aliquots of 10X stock solution were made to ensure equal concentrations of fMLP for every sample.

Future Directions

Results of the current study highlighted the need for a better understanding of the biological mechanisms by which neutrophils expand and alter function in response to exercise, particularly in the cancer population. Various factors might be beneficial for exploration but G-CSF seems to be a likely first candidate as this molecule influenced both proliferation and function of neutrophils. Exercise itself should also be examined to determine the proper dose, mode, and frequency to institute optimal changes to neutrophil expansion and function. The variety of interventions used in the literature to determine an effect of exercise on neutrophils presented a great deal of difficulty in generalizing findings.

Conclusion

In conclusion, this study demonstrated the benefit of exercise in promoting increases in neutrophil proliferation and function in exercising cancer patients following a 12-week exercise intervention. Furthermore, improving neutrophil count by exercise could occur while in treatment and once treatment has ended. As well, exercise could induce an increase in neutrophil function in response to stimulation in cancer patients who begin an exercise program after their treatment has ended. This effect was not observed in cancer patients who initiated exercise while in treatment but no decrease in functional response was observed. This study showcased the safety and efficacy of initiating an exercise program without risk of negative impact on the neutrophil population in cancer patients. Furthermore, these data highlighted the benefit of exercise for general immune health and maintenance in the cancer population. These data supported the inclusion of exercise into the treatment plan of cancer patients at any time during the treatment continuum.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL

UNIVERSITY OF
NORTHERN COLORADO

Institutional Review Board

Date:	09/16/2021
Principal Investigator:	Reid Hayward
Committee Action:	**APPROVED – Renewal**
Action Date:	09/16/2021
Protocol Number:	2004000320R002
Protocol Title:	Exercise Interventions to Attenuate the Negative Side-Effects of Cancer Treatments
Expiration Date:	08/16/2022

Based on the information submitted, your study is currently: Active - Open to Enrollment. The University of Northern Colorado Institutional Review Board (IRB) for the protection of human subjects has reviewed and approved your renewal application.

As a reminder, all research must be conducted in accordance with the procedures outlined in your approved protocol.

This protocol is approved until the expiration date listed above and must be reviewed for renewal on an annual basis for as long as the research remains active. The investigator will need to submit a request for Continuing Review at least 30 days prior to the expiration date. If the study's approval expires, investigators must stop all research activities immediately (including data analysis) and contact the Office of Research and Sponsored Programs for guidance.

As principal investigator of this research project, you are responsible to:

- Conduct the research in a manner consistent with the requirements of the IRB and federal regulations 45 CFR 46.
- Obtain informed consent and research privacy authorizations using the currently approved forms and retain all original, signed forms, if applicable.
- Request approval from the IRB prior to implementing any/all modifications.
- Promptly report to the IRB any unanticipated problems involving risks to subjects or others and serious and unexpected adverse events.

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Institutional Review Board

- Maintain accurate and complete study records.
- Report all Non-Compliance issues or complaints regarding the project promptly to the IRB.

Please note that all research records must be retained for a minimum of three (3) years after the conclusion of the project. Once your project is complete, please submit the Closing Report Form.

If you have any questions, please contact Nicole Morse, Research Compliance Manager, at 970-351-1910 or nicole.morse@unco.edu. Please include your Protocol Number in all future correspondence. Best of luck with your research!

Sincerely,

Michael Aldridge
IRB Co-Chair, University of Northern Colorado: FWA00000784

Silvia Correa-Torres
IRB Co-Chair, University of Northern Colorado: FWA00000784

APPENDIX B

INFORMED CONSENT

INFORMED CONSENT

[FOR PARTICIPATION IN RESEARCH AT UNCCRI]

UNIVERSITY OF NORTHERN COLORADO | Cancer Rehabilitation Institute

NAME _____ **DATE** _____

PROJECT TITLE : Exercise Interventions to Attenuate the Negative Side-Effects of Cancer Treatments

University of Northern Colorado Cancer Rehabilitation Institute

Reid Hayward, Ph.D., Director
Phone Number: 970-351-1821
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The University of Northern Colorado Cancer Rehabilitation Institute (UNCCRI) and the School of Sport and Exercise Science support the practice of protection of human subjects participating in research. The following information is provided for you to decide whether you wish to participate in either the standard UNCCRI program or, if recruited, specific research investigations. You should be aware that even if you agree to participate, you are free to withdraw at any time without affecting opportunities for participation in other programs offered by this department.

This program is involved with the assessment of your body composition, pulmonary function, cardiovascular endurance, muscular strength and endurance, range of motion, and flexibility. Skinfold calipers and a commercially available bioelectrical impedance scale will be used to measure body composition (body fat percentage). Pulmonary function will be measured using maximum exhalation into a sterile mouthpiece. Measuring oxygen consumption on a motor-driven treadmill will assess your cardiorespiratory capacity. Assessment of muscular strength and endurance will occur through the use of weights, dumbbells, a handgrip dynamometer, and other established tests. Flexibility and range of motion will be measured by the modified sit-and-reach test and the reaching test. Baseline measurements such as: heart rate, blood pressure, height, weight, and circumference measurements will be taken for risk stratification and safety during your participation. You may be asked to wear a fitness tracking device on your wrist to measure activitiy level, heart rate, and sleep quality. Forms to be completed for the program include cancer history, medical history, lifestyle/activity questionnaire, and psychological tests such as depression scales, quality of life, fatigue and cognitive functioning. Blood may be drawn with your permission at various time points during your participation. Once all of the tests are completed, results will be analyzed and an exercise prescription will be written. You may then have the option of participating in a three month exercise intervention based on your testing results. The expected benefits associated with your participation in this program include information regarding your level of physical fitness and lifestyle changes necessary to improve your health and quality of life.

Please Initial _____

If you are recruited and agree to participate in a specific research investigation, additional exercise, psychological, and/or cognitive tests may be administered. Your optional three month exercise intervention may also differ, but the expected benefits should still include improved health and quality of life. All participants at UNCCRI will be under the direction of the UNCCRI Director and Clinical Coordinator but other persons will be associated or assist with the data collection. Your participation is solicited, although strictly voluntary. The obtained data may be used in reports or publications but your identity will not be associated with such reports. We at UNCCRI take mental distress that may accompany health issues seriously and will attempt to support you with counseling referrals and information on local cancer support groups if this is an issue. Our staff is required to report evidence of clear and imminent danger.

This research should not result in physical injury, however, some soreness may occur and some of the fitness tests can be uncomfortable. Additionally, with the blood draws you may feel temporary discomfort. The duration of the discomfort is short. Please give your consent with full knowledge of the nature and purpose of the procedures, the benefits that you may expect, and the discomforts and/or risks which may be encountered. We appreciate your assistance.

Participation is voluntary. You may decide not to participate in this study and if you begin participation, you may still decide to stop and withdraw at any time. Your decision will be respected and will not result in loss of benefits to which you are otherwise entitled. Having read the above and having had an opportunity to ask any questions, please sign below if you would like to participate in this research. A copy of this form will be given to you to retain for future reference if requested. If you have any concerns about your selection or treatment as a research participant, please contact Nicole Morse, IRB Administrator, Office of Sponsored Programs, Kepner Hall, University of Northern Colorado Greeley, CO 80639; 970-351-1910.

The University of Northern Colorado Cancer Rehabilitation Institute would like to share your contact information with the UNC Foundation and Development Office for purposes of marketing and communication. By signing below you agree to allow us to share your contact information with the UNC Foundation and Development Office. NO MEDICAL INFORMATION WILL BE SHARED. If you would like to opt out, please check this box ☐ .

_____ _____

Signature of Subject Agreeing to Participate — Date

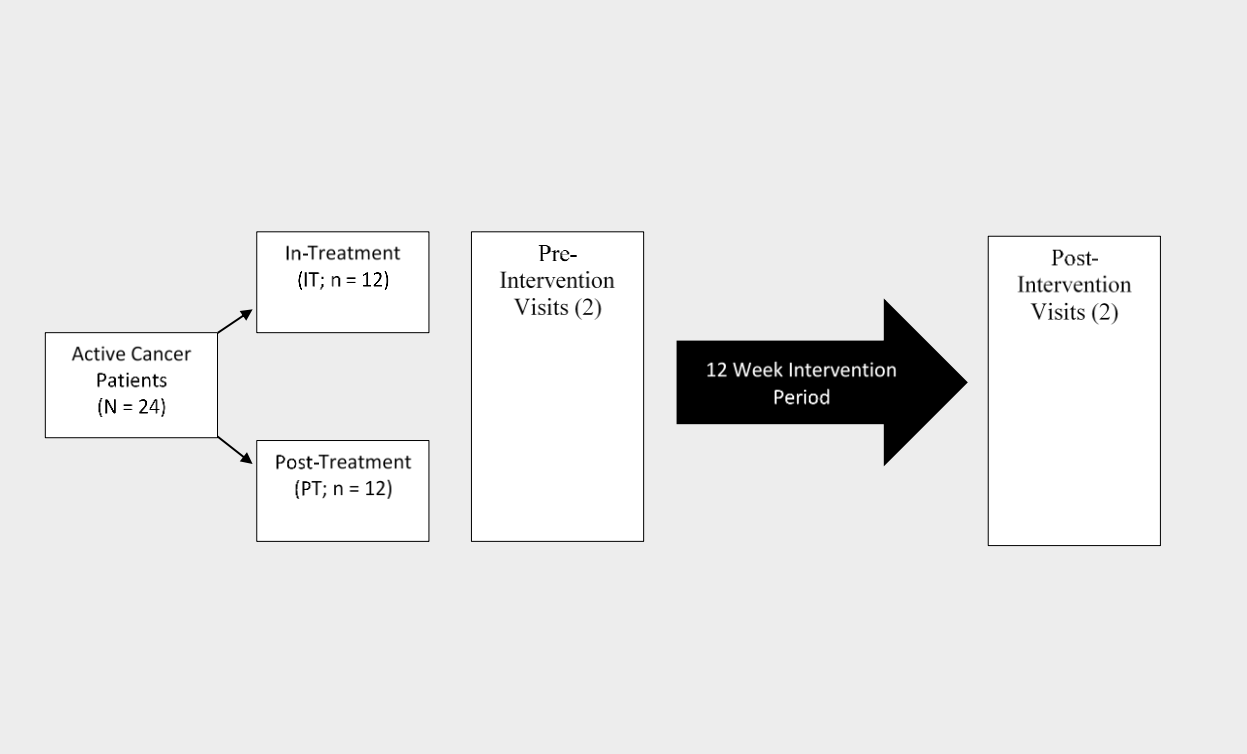
By signing this consent you certify you are at least 18 years of age.

_____ _____

Signature of Researcher — Date

APPENDIX C

STUDY PARTICIPATION TIMELINE


Active Cancer Patients (N = 24)
In-Treatment (IT; n = 12)
Post-Treatment (PT; n = 12)
Pre-Intervention Visits (2)
12 Week Intervention Period
Post-Intervention Visits (2)

APPENDIX D

EXERCISE ASSESSMENT PROTOCOL

1) Vital sign acquisition
    a. Blood pressure
    b. Heart rate
    c. Oxygen saturation
2) Body composition analysis
    a. InBody 770 (InBody USA, Cerritos, CA)
3) Balance assessment: Limits of Stability
    a. Bertec Balance Software (Bertec Corporation, Columbus, OH)
4) Spirometry
    a. MIR Spirolab III (Medical International Research USA, Inc, New Berlin, WI)
5) Cardiorespiratory endurance
    a. UNCCRI Treadmill Protocol (Shackelford et al., 2017)
6) Estimated one-repetition maximum assessment
    a. Chest press
    b. Lat pull-down
    c. Leg press
    d. Seated row
    e. Shoulder press
    f. Leg extension
    g. Leg curl
7) Muscular endurance assessment
    a. 30 second sit-to-stand
8) Flexibility
    a. Modified sit-and-reach
9) Quality of life survey (completed prior to assessment)
    a. Functional Assessment of Cancer Therapy (FACT) survey (Cella et al., 1993)

APPENDIX E

PRELIMINARY CONTROL PARTICIPANT DATA

Table A1

Preliminary Control Data

	Pre (n = 3)	Post (n = 3)	P value
ANC (K cells/µl)	0.73 ± 0.22	0.99 ± 0.38	NS
Rate of Activation (% change from rest)	99 ± 46%	235 ± 195%	NS
24 Hour Functional Capacity (% CD16 expression from hour 0)	80 ± 16%	59 ± 37%	NS
48 Hour Functional Capacity (% CD16 expression from hour 0)	20 ± 4%	25 ± 22%	NS

Values represent sample mean (± standard deviation) of the given population. NS= No significant difference was found between Pre and Post values after 12 weeks.