

University of Northern Colorado

## Scholarship & Creative Works @ Digital UNC

---

Master's Theses

Student Research

---

5-2020

### EFFECTS OF EXERCISE ON MYOGENIC REGULATORY FACTORS IN CANCER CACHEXIA-INDUCED SKELETAL MUSCLE WASTING

Kelsey Daehlin

daeh2617@bears.unco.edu

Follow this and additional works at: <https://digscholarship.unco.edu/theses>

---

#### Recommended Citation

Daehlin, Kelsey, "EFFECTS OF EXERCISE ON MYOGENIC REGULATORY FACTORS IN CANCER CACHEXIA-INDUCED SKELETAL MUSCLE WASTING" (2020). *Master's Theses*. 163.

<https://digscholarship.unco.edu/theses/163>

This Dissertation/Thesis is brought to you for free and open access by the Student Research at Scholarship & Creative Works @ Digital UNC. It has been accepted for inclusion in Master's Theses by an authorized administrator of Scholarship & Creative Works @ Digital UNC. For more information, please contact [Jane.Monson@unco.edu](mailto:Jane.Monson@unco.edu).

UNIVERSITY OF NORTHERN COLORADO

Greeley, Colorado

The Graduate School

EFFECTS OF EXERCISE ON MYOGENIC REGULATORY  
FACTORS IN CANCER CACHEXIA-INDUCED  
SKELETAL MUSCLE WASTING

Kelsey Anne Daehlin

College of Natural and Health Sciences

School of Sport and Exercise Science

Exercise Physiology

May 2020

This Thesis by: Kelsey Anne Daehlin

Entitled: *Effects of Exercise on Myogenic Regulatory Factors in Cancer Cachexia-Induced Skeletal Muscle Wasting*

has been approved as meeting the requirement for the Degree of Master of Science in College of Natural Health Sciences in School of Sport and Exercise Science, Program of Exercise Physiology

Accepted by the Thesis Committee:

---

David S. Hydock, Ph.D., Chair

---

Reid Hayward, Ph.D., Committee Member

Accepted by the Graduate School

---

Cindy Wesley  
Interim Associate Provost and Dean  
The Graduate School and International Admissions

## ABSTRACT

Daehlin, Kelsey. *Effects of Exercise on Myogenic Regulatory Factors in Cancer Cachexia-Induced Skeletal Muscle Wasting*. Unpublished Master of Science thesis, University of Northern Colorado, 2020.

Cancer cachexia is a complex multiorgan syndrome that affects bodily systems such as liver, brain, fat tissue, and skeletal muscle. It is a major factor that decreases quality of life in cancer patients and is related to about 20% of cancer deaths, however exercise has shown to be effective in helping combat the muscle wasting effects that cancer cachexia has on the body. Cachexia characterized by skeletal muscle wasting which may be caused by downregulation of positive myogenic regulatory factors such as MyoD and myogenin, and upregulation of negative myogenic regulatory factors such as myostatin and MuRF-1. While it is known that exercise is effective in combatting skeletal muscle wasting, little is known about how different modes of exercise affect cachexia. The purpose of this study was to determine the effects of an endurance training protocol, a resistance training protocol, and a combined endurance and resistance training protocol in cancer cachexia on specific positive myogenic regulatory factors such as MyoD and myogenin, and on negative myogenic regulatory factors myostatin and MuRF-1.

Six week old male Balb/c mice were randomly assigned to one of the following groups: sedentary+control (SED+Control), sedentary+tumor (SED+Tumor), treadmill training+tumor (TM+Tumor), resistance training+tumor (RT+Tumor), and combined treadmill training and resistance training+ tumor (COMBO+Tumor). TM mice were

trained on a motorized treadmill 5 days per week, RT mice had their food and water raised to an end height of 18 cm as a resistance training model, and the COMBO group used both protocols. The exercise protocols started at 6 weeks of age. At 11 weeks old, mice in the tumor groups were inoculated with C26 cells that caused cancer cachexia and were allowed to remain sedentary (SED, normal cage activity) if in SED+Tumor group; the rest of the groups continued with the exercise protocol according to their assigned group. Mice were then sacrificed at 14 weeks of age, and the gastrocnemius was excised. Western blotting was then performed to quantify the expression of MyoD, myogenin, myostatin, and MuRF-1. A one-way ANOVA found a significant difference between groups for myostatin expression ( $F=4.383$ ,  $P<0.05$ ), and Tukey's *post hoc* testing revealed that SED+Tumor had a significantly higher myostatin expression than TM+Tumor, RT+Tumor, and COMBO+Tumor. No significant group differences were observed for MyoD ( $F=2.389$ ,  $p>0.05$ ), myogenin ( $F=.799$ ,  $p>0.05$ ), or MuRF-1 expression ( $F=1.03$ ,  $p>0.05$ ). The main finding of this study was that exercised tumor bearing animals had significantly less myostatin than sedentary tumor bearing mice suggesting that any mode of exercise could have a protective effect on skeletal muscle. Because the decrease was evident regardless of exercise mode, including endurance training, resistance training, or combined endurance and resistance training may help combat the negative effects of cachexia through the suppression of the negative myogenic regulatory factor myostatin.

## TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION .....	1
Statement of Purpose	
Research Hypotheses	
Significance of Study	
II. REVIEW OF LITERATURE.....	6
Cancer Cachexia	
Cachexia and Exercise	
Mitochondria	
Myogenic Differentiation Protein 1	
Myogenic Differentiation Protein 1 and Exercise	
Myogenin	
Myogenin and Exercise	
Myostatin	
Myostatin and Exercise	
Muscle Ring Finger 1	
Muscle Ring Finger 1 and Exercise	
Conclusion	
III. METHODS.....	19
Experimental Design	
Animals and Animal Care	
Exercise Training Protocol	
C26 Cell Culture Inoculation	
Biochemical Analysis	
Statistical Analyses	
IV. RESULTS.....	25
V. DISCUSSION.....	30
Conclusion and Further Applications	
REFERENCES .....	36

APPENDIX

A. Institutional Review Board Approval.....

## LIST OF FIGURES

	PAGE
Figure 1. Experimental Design .....	21
Figure 2. MyoD expression levels in the Gastrocnemius and representative immunoblots of MyoD and GAPDH.....	26
Figure 3. Myogenin expression levels in the Gastrocnemius and representative immunoblots of myogenin and GAPDH.....	27
Figure 4. Myostatin expression levels in the Gastrocnemius and representative immunoblots of myostatin and GAPDH.....	28
Figure 5. MuRF-1 expression levels in the Gastrocnemius and representative immunoblots of MuRF-1 and GAPDH.....	29

## CHAPTER I

### INTRODUCTION

Cancer cachexia is a complex multiorgan syndrome that affects bodily systems such as liver, brain, fat, and skeletal muscle. It is a disease that is mainly characterized by skeletal muscle wasting that ultimately decreases that ability for cancer patients to perform normal activities of daily living (Argiles, Busquets, Lopez-Soriano, Costelli, & Penna, 2012; Porporato, 2016). The decreased muscle mass also causes a lower tolerance to treatments such as chemotherapy and radiation and a higher mortality rate in cancer patients (Smith & Lin, 2013). Muscle wasting occurs when there is a higher rate of catabolism and a lower rate of anabolism (Porporato, 2016; Tisdale, 2009). By examining the specific mechanisms in which skeletal muscle mass decreases in cancer cachexia, ways can be found to decrease the rate of muscle loss and increase the quality of life in cancer patients.

The Colon-26 (C26) tumor is a type of colon cancer that is known to induce cancer cachexia and skeletal muscle wasting. It has been shown that the C26 tumor is known to decrease skeletal muscle cross sectional area in both oxidative and glycolytic muscle fibers (Aulino et al., 2010). The C26 tumor also increases protein degradation in skeletal muscle. However, exercise is known to help combat cancer cachexia. It has been shown that exercise has a protective effect on skeletal muscle with cancer cachexia, the research in this area is limited and has not focused on one mode of exercise.

Exercise has been shown to be effective in combatting the muscle wasting effects of cachexia, but little is known about how different modes of exercise affect this disease (Hardee, Counts, & Carson, 2017). Because skeletal muscle is mostly made up of proteins, there is a delicate balance between protein synthesis and protein degradation (Bowen, Schuler, & Adams, 2015). Exercise in general is known to upregulate the anabolic pathways and downregulate the catabolic pathways in skeletal muscle, which may lead to muscle hypertrophy while decreasing muscle atrophy. In a study that included both an endurance and a resistance training protocol with cancer cachexia, it was found that anabolic stimulus was increased in the cancer groups that included any mode of exercise (Khamoui et al., 2016). Exercise is known to help increase muscle mass and have a protective effect on the mitochondria of skeletal muscle which leads to less apoptosis of muscle cells (Bowen et al., 2015).

Skeletal muscle wasting in cancer cachexia is caused by several mechanisms that are still being studied. Muscle wasting may be due to downregulation of positive myogenic regulatory factors myogenic differentiation protein 1 (MyoD) and myogenin, and upregulation of negative myogenic regulatory factors such as myostatin and MuRF-1. It has been noted that exercise has a protective effect on skeletal muscle which can help to upregulate myogenic regulatory factors positive myogenic regulatory factors and decrease the expression of negative myogenic regulatory factors.

MyoD and myogenin being positive regulators of skeletal muscle can be upregulated with exercise. Satellite cells are known to proliferate when there is skeletal muscle damage and aid in skeletal muscle hypertrophy (Meggeney, Kablar, Garret, Andersen, & Rudnicki, 1996). These two positive myogenic regulatory factors are both

known to be expressed during satellite cell proliferation and differentiation. MyoD is associated with satellite cell activation and the regeneration of skeletal muscle (Lenk, Schuler, & Adams, 2010) and is known to be sensitive to mechanical stimulus, such as exercise regardless of mode (Legerlotz & Smith, 2008). A large amount of MyoD is released when satellite cells proliferate. Myogenin is known to become upregulated especially in resistance exercise that causes muscle damage therefore leading to skeletal muscle hypertrophy. Myogenin is more specifically known to be expressed when satellite cells are in the first stage of proliferation and then decline once the muscles are no longer in the stage where muscle hypertrophy is not taking place (Ishido, Kami, & Masuhara, 2004).

Because myostatin and muscle ring finger 1 (MuRF-1) are negative regulators of skeletal muscle, they are both known to become downregulated with exercise. Increased myostatin levels are associated with increased muscle degradation. Myostatin is one of the more studied myogenic regulatory factors in cancer cachexia and tends to be upregulated in cachectic groups as compared to control groups (Liu et al., 2008). With exercise, myostatin becomes downregulated which allows for skeletal muscle hypertrophy. MuRF-1 is one of the main ligases whose upregulation is associated with increased degradation of skeletal muscle mass (Bowen et al., 2015). Exercise, regardless of mode, is known to downregulate MuRF-1 leading to less degradation of skeletal muscle (Bowen et al., 2015). This has been shown both with voluntary and involuntary exercise. By knowing how these myogenic regulatory factors change with exercise and cancer cachexia, it can be determined which pathways should be targeted to help prevent skeletal muscle wasting.

## **Statement of Purpose**

The purpose of this study was to determine the effects of an endurance training protocol, a resistance training protocol, or a combined endurance and resistance training protocol in cancer cachexia on the positive myogenic regulatory factors MyoD and myogenin and the negative myogenic regulatory factors myostatin and MuRF-1. This study examined both positive and negative myogenic regulatory factors to increase the overall understanding of the mechanisms of cancer cachexia.

## **Research Hypotheses**

- H1 The C26 tumor will cause myostatin and MuRF-1 to be upregulated in the sedentary tumor group when compared to the sedentary control group.
- H2 The C26 tumor will cause myogenin and MyoD to be downregulated in the sedentary tumor group when compared to the sedentary control group.
- H3 MuRF-1 and Myostatin will be downregulated in the C26 tumor groups that are resistance trained, endurance trained, and in the combo group.
- H4 MyoD and myogenin will become upregulated in the C26 tumor groups that are resistance trained, endurance trained, and in the combo group.
- H5 The combo group will cause the largest change in myogenic regulatory factors, upregulating MyoD and myogenin more than the other groups and downregulating myostatin and MuRF-1 more than the other groups.

## **Significance of Study**

Cancer cachexia affects many cancer patients and is related to many cancer deaths. Models examining exercise as a mechanism to prevent muscle wasting in cancer cachexia are not well studied. By determining which myogenic regulatory factors are affected by cancer cachexia and exercise, it can be determined how to best prevent the loss of skeletal muscle mass and increase the ability of cancer patients to keep up with their everyday activities of daily living. Increasing a patient's muscle mass can also help

increase the types of treatments that cancer patients can tolerate. When patients lose a large amount of muscle mass through cancer cachexia, the cross sectional area of skeletal muscle is known to be decreased. This ultimately reduces the amount of force production a patient can produce leading to a decrease in activities of daily living. Some of these specific myogenic regulatory factors that are affected by cancer cachexia include MyoD, myogenin, myostatin, and MuRF-1. Knowing if these factors are upregulated or downregulated with exercise and cachexia will allow for more specific treatments which can help maintain a better balance of the myogenic regulatory factors and improve the overall quality of life in cancer patients.

CHAPTER II  
LITERATURE REVIEW  
**Cancer Cachexia**

Cancer cachexia is a major factor that decreases quality of life in a large number of cancer patients (Porporato, 2016). Patients are typically considered pre-cachectic if they lose less than or equal to five percent of their body mass, and they are categorized as cachectic if they have lost between five and twenty percent of their body mass. When a patient loses more than twenty percent of their body mass, they are then considered to be in the refractory stage of cachexia (Fearon et al., 2011).

Cachexia is known as a muscle wasting syndrome that is caused by the increase in muscle degradation and decrease in muscle anabolism (Porporato, 2016). Skeletal muscle consists of a high amount of proteins with a delicate balance between protein synthesis and degradation (Bowen et al., 2015). Because cachexia is typically characterized by a significant amount of muscle wasting, looking at the specific mechanisms through which skeletal muscle wasting occurs, ways of preventing skeletal muscle loss will become prevalent. The increase in the ubiquitin-proteasome pathway and decrease in mixed muscle synthesis are two of the main pathways that lead to an increase in muscle wasting (Brown et al., 2018). Significant losses in body mass can majorly reduce the types of treatments that the patient can receive as well as reduces the response to anti-tumor treatments (Fearon et al., 2011; Klimek et al., 2010) thus highlighting the importance of managing cachexia. This particular condition also decreases patient quality of life

through causing decreased functionality and ambulation, as well as increases the rate of mortality (Brown et al., 2018). Some of the proposed mechanisms of skeletal muscle wasting are an increase in mitochondrial degradation, decreased levels of the positive myogenic regulatory factors MyoD and myogenin, and an increase in levels of the negative myogenic regulators myostatin and MuRF-1.

### **Cachexia and Exercise**

Exercise is known to help increase muscle mass in both healthy and diseased individuals (Hardee et al., 2017). Different types of endurance training have been shown to help increase food intake and decrease tumor size; however, it cannot be said directly whether it was the smaller tumors and increased exercise that helped increase the muscle mass (Lenk et al., 2010). Resistance exercise, even though minimally studied with cancer cachexia is known to cause an anabolic stimulus which leads to increased signaling that causes muscle hypertrophy. In a study that used both resistance and endurance exercise, it was found that absolute strength (grip strength normalized to body weight) was significantly decreased in mice with the C26 tumor (measured at -7% in the control and -21% in the mice with the C26 tumor) (Khamoui et al., 2016). The C26 mice also had significantly lower gastrocnemius and plantaris mass when compared to the controls; however, the post-training values for both resistance and aerobic training tended to be increased compared to the pre-test values (+10%) (Khamoui et al., 2016). With aerobic training, it has been shown that phosphorylated mTOR to total mTOR ratio was increased by 32% in mice with the C26 tumor compared to those with the C26 tumor that were sedentary indicating that exercise helps regulate this pathway (Khamoui et al., 2016). With exercise, it was found that both the resistance trained and endurance trained mice

had reduced body mass when compared to the control group (Khamoui et al., 2016).

Body mass may have been decreased in the exercised mice due to a volume of exercise that was set too high for mice with the C26 tumor.

### **Mitochondria**

Skeletal muscle mitochondria are known to go through phospholipid bilayer remodeling with cancer cachexia which causes them to become dysfunctional (de Castro et al., 2019). In a study done on mice, there was a disruption in the morphology of the mitochondria as well as an increase in mitophagy in skeletal muscle, but no changes were seen in the number of copies of mitochondrial DNA. This means that selective degradation of mitochondria was not seen in the cachectic group compared to the weight-stable group (de Castro et al., 2019). When looking at the mitochondria in a group of rats with cancer cachexia, it was seen that there was a 25% and 45% decrease in the functionality of mitochondria at three and four weeks after injection of the tumor, respectively (Brown et al., 2017). Mitochondrial reactive oxygen species were also found to double one week after tumor injection and continued to stay elevated through the third week (Brown et al., 2017). This means that there would be more apoptosis of muscle cells with an increased level of reactive oxygen species.

The mitochondrial proteins that code for mitochondrial fission were found to be elevated meaning that Drp1 was lowered by 45% by week 4 and Fis 1 was increased by 80% in the fourth week of the cachectic group. This was being measured in comparison to the control group that received PBS as a placebo. (Brown et al., 2017). In this study, it was also noted that in week 1, there was an increase in reactive oxygen species in the mitochondria, and it was not until week 4 that there was a decrease in muscle mass

(Brown et al., 2017). This suggests that the onset of cachexia does not start until week 4, and that targeting the mitochondria could be helpful in preventing muscle loss. In cachectic patients compared to weight stable cancer patients, mitochondrial area was found to be increased ( $p=0.01$ ) due to swelling of the mitochondria and not due to an increase in the number of mitochondria.

Using a skeletal muscle photomicrograph, it was shown that triads became remodeled in the patients considered to be cancer cachectic (greater than 5% body weight loss) (de Castro et al., 2019). Furthermore, in a study conducted using mice that were injected with a strain of Lewis lung carcinoma that induced cachexia, it was found that on average the cachectic rats lost 11.3% of their body weight (Brown et al., 2017). In rats that went through the four week progression with the tumor, it was found that the weights of the gastrocnemius, plantaris, quadriceps, and soleus were 15 to 20% lower than those in the control group (Brown et al., 2017). Overall with cancer cachexia, there is a decrease in mitochondria and mitochondrial function and an increase in reactive oxygen species leading to apoptosis of muscle cells.

### **Myogenic Differentiation Protein 1**

MyoD which is more formally known as myogenic differentiation protein 1 is known to regulate muscle differentiation and belongs to the family of myogenic regulatory factors (Ishido et al., 2004). MyoD is known to be important in muscle differentiation in the process of embryogenic myogenesis; it is known to be under different regulatory control during development than during muscle regeneration (Kitzmann & Fernandez, 2001). MyoD can be a marker of satellite cell activation due to its early release during the proliferation and differentiation during the cell cycle

(Legerlotz & Smith, 2008; Megeney et al., 1996). In a study done using a knockout model, MyoD-null mice showed less muscle regeneration than those expressing MyoD (Megeney et al., 1996). In a study using a tumor bearing model of cachexia in mice, muscle weights and MyoD expression with tumor bearing models were decreased by up to 45.3% compared to the control group (Liu et al., 2008). While the mechanism is not well understood, it is believed that when there is an increase in myostatin, MyoD becomes inhibited (Liu et al., 2008). It is not yet known, however, whether it is the response in the satellite cells or the response in the myonuclei that is responsible for the upregulation of MyoD (Legerlotz & Smith, 2008).

Both myonuclei and satellite cells are known to express MyoD. Satellite cells release a large amount of MyoD when they are activated, and MyoD is highly sensitive to both increased and decreased mechanical stress (Legerlotz & Smith, 2008). When satellite cells start to proliferate, there is a large increase in the amount of MyoD present which means that if there is a stimulus to cause muscle growth, there should also be an increase in MyoD expression (Lenk et al., 2010).

MyoD has also been shown to be linked to the acetylcholine receptors (AChR) in the myotubes (Dutton, Simon, & Burden, 1993). These are known to be downregulated in muscles that are cachectic (Brown et al., 2018). In a different study using muscle denervation, it was found that when there was denervation of the muscle with an electrical stimulus, MyoD is upregulated. This may seem contradictory to the other data reported, but it is believed that MyoD is increased due to the body trying to preserve skeletal muscles and keep them from atrophying (Legerlotz & Smith, 2008).

### **Myogenic Differentiation Protein 1 and Exercise**

In a study that used exercise-trained mice in a tumor bearing model, it was found that there was no significant difference in MyoD expression between the mice that were exercise trained and the mice that remained sedentary (Khamoui et al., 2016). Because satellite cells contain high levels of MyoD, it should be expected that with exercise, there will be an increase in the amount of MyoD that is expressed. With resistance exercise, MyoD has been shown to increase five times within a twelve hour period in the gastrocnemius (Legerlotz & Smith, 2008). It was also found that twenty-four hours after six consecutive training days, MyoD levels returned to normal (Legerlotz & Smith, 2008). There are conflicting data showing either upregulated or downregulated MyoD with exercise; however in these studies that show the conflicting data, there is not one specific mode of exercise used which likely contributes to these discrepancies (Legerlotz & Smith, 2008). Since MyoD is specific to satellite cells and muscle regeneration, the mode of exercise most commonly showing increases in MyoD would be high load and include eccentric contractions (Legerlotz & Smith, 2008). MyoD expression is likely to become more detectable with any type of exercise intervention because MyoD is sensitive to any type of mechanical stimulation. (Legerlotz & Smith, 2008).

### **Myogenin**

Myogenin is a myogenic regulatory factor that is involved in the process of skeletal muscle differentiation and repair. It has been shown that when mice are deficient in myogenin, skeletal muscles do not develop correctly (Hasty et al., 1993). When mice develop with a myogenin deletion, they tend to be smaller in size as well as having altered gene expression (Meadows, Cho, Flynn, & Klein, 2008). Myogenin tends to be

expressed more in the slow oxidative muscles (Hughes et al., 1993) and is known to be one of the last proteins that is expressed during muscle differentiation and proliferation (Megeny et al., 1996). However, in a study that looked at satellite cell proliferation and skeletal muscle hypertrophy, it was found that myogenin is expressed at the beginning of satellite cell proliferation (Ishido et al., 2004). In another study that examined mitochondrial activity and myogenin, it was seen that when the mitochondria become damaged, there is then a decreased expression of myogenin (Rochard et al., 2000). This means that in patients or mice with cancer cachexia, there may be a decrease in myogenin expression due to mitochondrial damage. With damaged mitochondria there tends to be a decrease in circulating hypertrophic factors. While it is well known that myogenin is involved in myogenic differentiation, it is still not well known exactly what role myogenin plays in adult cells. It has also been shown that myogenin is increased with a stimulus and then drops after the muscles no longer have stimulus to hypertrophy meaning that once the stimulus goes away, the levels of myogenin will decrease (Ishido et al., 2004).

### **Myogenin and Exercise**

In a study that was previously mentioned, it was found that there was not a significant difference in the exercised C26 mice compared to the exercised control mice in myogenin mRNA and protein levels (Khamoui et al., 2016). However, there was a difference in the resistance trained mice where there was a 126% increase in myogenin mRNA when compared to 150% increase in controls. Myogenin was increased in a study that used bouts of resistance exercise; with a second bout of resistance exercise, myogenin continued aid in increasing muscle mass. In another study that deleted

myogenin in mice, it was found that mice with no myogenin accumulated lactate faster with endurance exercise and reached exhaustion quicker than those with myogenin. However, over time, metabolism shifted and the performance abilities of the mice increased so that the myogenin knockout mice could run faster and longer without accumulating as much lactate. This could possibly mean that myogenin negatively regulates performance ability by leading to possible changes in skeletal muscle metabolism (Flynn, Meadows, Fiorotto, & Klein, 2010). In a study that used mice and exercise, it was found that myogenin tended to accumulate with exercise mostly in the fast glycolytic fibers (Hughes et al., 1993). It has also been found that with endurance exercise, myogenin expression is typically increased (Siu, Donley, Bryner, & Alway, 2004). While the main function of myogenin in adult muscle is that it is part of the satellite cell cycle, it is believed that myogenin may be involved in more than just this pathway but more research is needed to determine how else myogenin functions (Siu et al., 2004).

### **Myostatin**

Myostatin is also a negative regulator of muscle growth and is a member of the transforming growth factor- $\beta$  family and is known to be significantly increased in cachectic tumor models (Liu et al., 2008). Inhibiting myostatin in animals has been shown to significantly increase muscle mass as well as help to preserve muscle mass in cases of muscle wasting diseases such as cancer cachexia (Smith & Lin, 2013). The myostatin signaling pathways shows how myostatin inhibitors work by causing muscles to hypertrophy by increasing the amounts of myofibrillar proteins (Smith & Lin, 2013). Myostatin works by attaching to the Activin A receptor type IIB (ActRIIb) which then

activates the transcription factors SMAD2 and SMAD3, and atrogene expression then becomes upregulated and autophagy of skeletal muscle is increased (Miyamoto, Hanna, Zhang, Baba, & Lenz, 2016). The mechanisms that are mainly used for myostatin inhibition look mostly at the receptors of myostatin and stop the receptors from binding myostatin (both the SMAD receptors and the ActRIIB receptors are the most understood) (Miyamoto et al., 2016). In a study using healthy controls and patients with untreatable cancer, it was found that myostatin inhibition was well tolerated by all subjects, and all subjects showed an increase in total muscle volume (Smith & Lin, 2013).

In a study done using rats in a cardiac failure model of cancer cachexia, it was found that when myostatin levels were increased in the gastrocnemius by 140% when compared to the controls. In animals that were considered to be cachectic but not having heart failure, a significant difference was not found in the expression of myostatin (Lenk et al., 2009). In a study using the Yoshida AH-130 hepatoma which is known to increase cancer myostatin expression, levels of myostatin were found to be increased. The difference, however, was not shown to induce a significant difference in circulating myostatin levels until day 7. This led the researchers to believe that using the Yoshida AH-130 hepatoma is not a reliable way to look at muscle wasting in cachexia (Costelli et al., 2008). It has been speculated that TNF- $\alpha$  may be a player in perturbations in myostatin (Costelli et al., 2008) as it was shown that there has been a decrease in the ActIIB receptor activity. It has also been shown that with myostatin deficiency, not only is protein synthesis increased but protein degradation also becomes increased which could also contribute to the muscle mass increasing in size (Mendias, Kayupov, Bradley, Brooks, & Claflin, 2011; Smith & Lin, 2013). In a study using tumor-bearing mice, it was

found that knockout mice lacking myostatin had a decreased tumor weight compared to the wild type mice with myostatin. This points to the fact that myostatin inhibition could also be helpful in decreasing tumor size (Miyamoto et al., 2016).

### **Myostatin and Exercise**

In general, it is well known that myostatin levels decrease with both endurance and resistance training. This concept has been applied in a few studies that included looking at myostatin and exercise, but there are fewer studies that have explored what happens to myostatin levels with exercise and cachexia or other muscle wasting diseases. In a study that used mice and both aerobic and resistance training, it was found that myostatin levels were decreased significantly (37%) with either type of exercise (Hittel et al., 2010). This study also looked at insulin resistance with the decrease in myostatin. It was found that there was an 87% decrease in insulin-stimulated phosphorylation of AKT in the mice that were treated with myostatin. This suggests that there could be an important link between inactivity and insulin resistance with conditions such as obesity (Hittel et al., 2010).

### **Muscle Ring Finger 1**

Muscle Ring finger 1 (MuRF-1) is known as a negative regulator of skeletal muscle growth. MuRF-1 and muscle atrophy F box/atrogen 1 are associated with diseases that include muscle atrophy (Bodine & Baehr, 2014). The more MuRF-1 gene present, the higher the occurrence of atrophy. When transcription of skeletal muscle-specific E3 ubiquitin ligases become upregulated, they cause MuRF-1, Maxfb, and atrogen-1 to be transcribed (Lenk et al., 2010). MuRF-1 and atrogen-1 are two of the main ligases that identify proteins that need to be degraded in skeletal muscle in order to decrease skeletal

muscle atrophy (Bowen et al., 2015). Upregulation of MuRF-1 is known to occur in cancer cachexia (Pigna et al., 2016). Deubiquitinases have also been found to be elevated with cachexia to help regenerate the free ubiquitin from the ubiquitin chain (Bowen et al., 2015). MuRF-1 as well as MAFbx have been found to be upregulated when the ubiquitin proteasome pathway becomes upregulated (Bowen et al., 2015). In a model where the hind limbs of the rat were unloaded, MuRF-1 was found to be upregulated with muscle atrophy (Bodine & Baehr, 2014). MuRF-1 is known to interact with key proteins of the sarcomere such as titin, myosin light chain, myosin heavy chain, and nebulin. While this is known, it is not well identified how MuRF-1 interacts with these proteins. In a study done using dexamethasone treatment it was found that myosin heavy chain can be preserved if MuRF-1 is downregulated, meaning if MuRF-1 can become downregulated there may be a protective effect for the sarcomeres in skeletal muscle (Clarke et al., 2007). MuRF-1 is downstream of atrogin 1 and FoxO proteins which become upregulated with the autophagy pathways. When autophagy becomes upregulated, MuRF-1 becomes upregulated leading to increased degradation of skeletal muscle (Miyamoto et al., 2016). MuRF-1 regulates E3 ubiquitin ligase activity, and therefore if MuRF-1 becomes upregulated, E3 ubiquitin ligase activity becomes upregulated and causes skeletal muscle to be degraded through that pathway (Miyamoto et al., 2016).

### **Muscle Ring Finger 1 and Exercise**

Because MuRF-1 is known as a negative regulator of skeletal muscle, it is known to decrease with increased exercise. In a study done using patients with chronic heart failure it was found that those with prescribed exercise were able to decrease levels of MuRF-1. This led to the overall conclusion that an increase in exercise can aid in

blocking the ubiquitin proteasome pathway which would then decrease the amount of muscle atrophy taking place overall (Gielen et al., 2012). In a study using voluntary wheel running in a C26 tumor model, it was shown that as exercise downregulates MuRF-1. With a decrease in MuRF-1 expression there was also a decrease in skeletal muscle degradation (Pigna et al., 2016).

### **Conclusion**

Cancer cachexia is known to severely decrease skeletal muscle mass to the point where it is difficult for cancer patients to perform normal activities of daily living. Overall, it is well known that exercise helps increase muscle mass regardless of the mode. Exercise protocols included with cancer cachexia have been found to be an effective way of combatting some of the negative side effects of cancer cachexia. With exercise, myogenic regulatory factors such as MyoD and myogenin are known to become upregulated, while myostatin and MuRF-1 become downregulated. By including exercise in treatment for cancer cachexia, it is possible that the positive myogenic regulatory factors, MyoD and myogenin may become upregulated while the negative regulators such as myostatin and MuRF-1 become downregulated. If myogenin and MyoD become upregulated, there is an increase in the anabolic pathways leading to muscle hypertrophy. When myostatin and MuRF-1 are downregulated, they decrease the amount of skeletal muscle wasting. By understanding what happens to the positive myogenic regulatory factors such as MyoD and myogenin as well as the negative myogenic regulatory factors such as myostatin and MuRF-1 in cancer cachexia and how they can become up- or downregulated with exercise, treatments can be more targeted and increase a patient's quality of life.

## CHAPTER III

### MATERIALS AND METHODS

#### **Experimental Design**

The effects of exercise were looked at using various exercise approaches that spanned over eight weeks. Male Balb/c mice (n=60) were randomly assigned to one of five groups: sedentary control group (Sed+Control), sedentary and tumor group (Sed+Tumor), treadmill training and tumor group (TM+Tumor), resistance training and tumor group (RT+Tumor), and combined treadmill training and resistance training and tumor group (COMBO+Tumor). Mice were randomly assigned to groups at six weeks of age. The mice assigned to the tumor bearing groups were injected with  $1 \times 10^6$  C26 tumor cells. The sedentary control group did not receive C26 cells to act as the non-tumor control. Upon sacrifice the gastrocnemius was harvested, weighed, and prepared for biochemical analysis.

#### **Animals and Animal Care**

All procedures used in this study complied with the Animal Welfare Act guidelines and were approved by the University of Northern Colorado Institutional Animal Care and Use Committee (IACUC). Male Balb/c mice (The Jackson Laboratory; Bar Harbor, ME; stock #000651; n = 60) were housed at the University of Northern Colorado Animal Research facility. They were given chow and water *ad libitum* and housed one per cage in a 12:12-hour light-dark cycle in a temperature-controlled facility.

### Exercise Training Protocol

For the endurance training protocol, animals that were randomly selected to the TM+tumor group were housed in cages with a standard height of 12.5 cm for the full eight week intervention. All animals assigned to the TM+tumor group were acclimatized to the treadmill for 3 days before the protocol started. Acclimation to the treadmill was done by gradually increasing the speed of the treadmill by 10-15 m/min for 20 min. The full running protocol for the intervention included a 5 minute warm up at a grade of 5% with a speed of 15 m/min. This was then followed by a period of running at 15 m/min with a 5% grade. The mice ran 5 days per week for the duration of eight weeks. This was done during their dark cycle under red lights.

For the resistance training protocol, the mice that were randomly selected to the RT+tumor group were housed in a standard cage (12.5 cm). Cage height was progressively increased from the starting height of 12.5 cm. This forced the mice to stand on their hind limbs in order to reach the food and water. When the exercise training protocol began, mice were moved to cages with a height of 15.5 cm for the first week. The next week, the cage height was increased to 18 cm which was maintained for the rest of the eight week intervention. For the first three weeks of the intervention, body mass, food, and water were monitored every day to ensure the mice were able to reach their food and water. After the third week, food and water were measured three days per week while body mass was measured one day per week throughout the exercise protocol.

Animals assigned to the COMBO+tumor group followed both the endurance and resistance training protocols. The mice were only removed from their cages for treadmill training sessions. Figure 1 shows a visual representation of the experimental design.

<b>Groups</b>	<b>n</b>
Sed+Control	12
Sed+Tumor	12
RT+Tumor	12
TM+Tumor	12
COMBO+Tumor	12

### **Timeline**

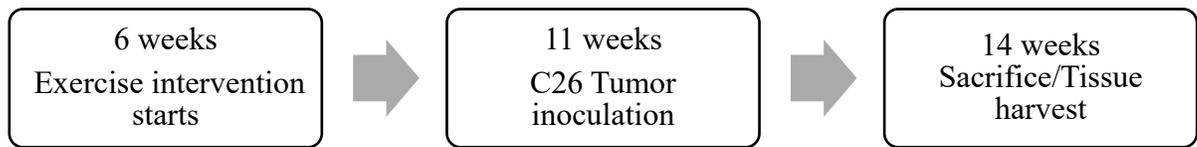


Figure 1. Experimental design

Note. Sed, sedentary; TM, treadmill trained, RT, resistance trained, COMBO, treadmill and resistance trained

### **C26 Cell Culture Inoculation**

The colon-26 (C26, DCTD Tumor Repository, National Cancer Institute; Frederick, MD) cells were suspended in Roswell Park Memorial Institute (RPMI) 1640 complete growth medium containing 10% FB Essence, 1% L-glutamine, and 1% streptomycin/penicillin and grown at 37°C in an incubator with an atmosphere that contained 5% CO<sub>2</sub>. The cells were counted using a commercial cell counter (Invitrogen; Carlsbad, CA). On the day that the cells were implanted, they were dissociated using a trypsin-EDTA solution, and cell concentration was determined as cells/m<sup>2</sup>. The cells were then resuspended in sterile PBS to be used for tumor cell inoculation. Mice were anesthetized prior to the injection using isoflurane which was administered through inhalation. This was maintained throughout the time needed to perform the tumor cell inoculation. The cells were injected between the scapulae subcutaneously and was done by using a 25-gauge needle with a sterile 1-mL syringe.

After tumor cell inoculation animals and tumors were monitored daily up until sacrifice. This included the relative tumor size, loss in body mass, tumor ulceration, and body score condition. Body weight was measured daily, and once the tumors were palpable, tumor size was measured daily. Upon sacrifice, the tumors were also removed and weighed.

## **Biochemical Analysis**

### **Tissue Preparation**

Gastrocnemius muscles were put into Eppendorf microcentrifuge tubes and frozen in liquid nitrogen upon sacrifice. Muscles were then transported back to the biochemistry lab and stored at -80°C until analysis.

### **Homogenate Preparation**

A 0.100 g portion of each gastrocnemius sample once weighed was placed in a glass tissue homogenizer, and homogenized in a homogenizing buffer (250 mM Sucrose, 100 mM KClm, 5 mM EDTA, 20 mM Tris [pH 6.8]). Homogenates were then centrifuged at 3,000 g and 4°C for 10 minutes. The supernatant was then removed and placed in a separate microcentrifuge tube. Samples were then sonicated and centrifuged at 10,000 g and 4°C for another 10 minutes. The supernatant was removed and placed in a new microcentrifuge tube. 100 µL of supernatant was combined with 100 µL of 2X Laemmli sample buffer.

### **Western Blotting**

Western blotting was used to quantify the expression of MyoD, myogenin, myostatin and MuRF-1 in the gastrocnemius. All antibodies were from Santa Cruz Biotechnology, Inc. After samples were thawed, they were boiled for two minutes and

then put on ice for approximately 10 minutes. Next, 20  $\mu$ L of each sample was vortexed and loaded onto 4-20% Invitrogen Tris-Glycine precast polyacrylamide gels (Thermo Fischer Scientific, Waltham, MA). Once the gels were loaded, they were run at 125 V at 40 mA until the proteins migrated to the bottom of the gel which took approximately two and a half hours.

Proteins were then transferred from the gel to PVDF membranes. This process was done over 90 minutes at 25 mV and 100 mA. The transfer was ensured by presence of the SeeBlue Plus2 protein ladder on the membrane (Novex, LifeTechnologies). PVDF membranes were then rinsed in deionized water 3 times for 5 minutes and then blocked for 30 minutes using 10 mL of Superblock (Thermo Fischer Scientific, Waltham, MA). They were then rinsed again deionized water 3 times for 5 minutes and incubated overnight for 15-18 hours with the primary antibody. The next day membranes were washed in TBST for 5 minutes 4 times then rinsed with deionized water for 5 minutes 3 times and incubated in the appropriate secondary antibody for 60 minutes. The membranes were again washed in TBST 4 times for 5 minutes and rinsed in deionized water 3 times for 5 minutes. The membranes were then prepared for imaging.

Membranes were incubated in enhanced chemiluminescence (ECL) substrate to initiate the imaging process. Imaging was done using a C-Digit imaging system (Li-Cor: Lincoln, NE), and protein bands were quantified using ImageJ software (NIH: Bethesda, MD). After the membranes were imaged, they were stripped using 10 mL stripping buffer (Invitrogen) 2 times for 20 minutes, washed in 20 mL of TBST 4 times for 5 minutes, and rinsed in 20 mL of deionized water three times for two minutes. The process of membrane blocking and primary antibody incubation was then repeated. Each membrane

was imaged with each of the four primary antibodies as well as GAPDH which was used to normalize the amount of protein in each sample.

### **Statistical Analyses**

Data are presented as mean $\pm$ SEM, and all statistical analyses were performed using GraphPad Prism. A one-way analysis of variation (ANOVA) was used to identify the differences in myogenic regulatory factors between groups. When a significant F-value was observed, Tukeys *post hoc* test was performed to determine where the significant differences existed. Significance was set at the  $\alpha=0.05$  level.

## CHAPTER IV

### RESULTS

The purpose of this study was to determine the effects of an endurance training protocol, a resistance training protocol, and a combined endurance and resistance training protocol in cancer cachexia on the myogenic regulatory factors MyoD, myogenin, myostatin, and MuRF-1. This study examined both positive and negative myogenic regulatory factors to determine which pathways are more or less affected by cancer cachexia in exercise.

As shown in Figure 2, there were no significant group differences for increases in the expression of MyoD ( $p>0.05$ )

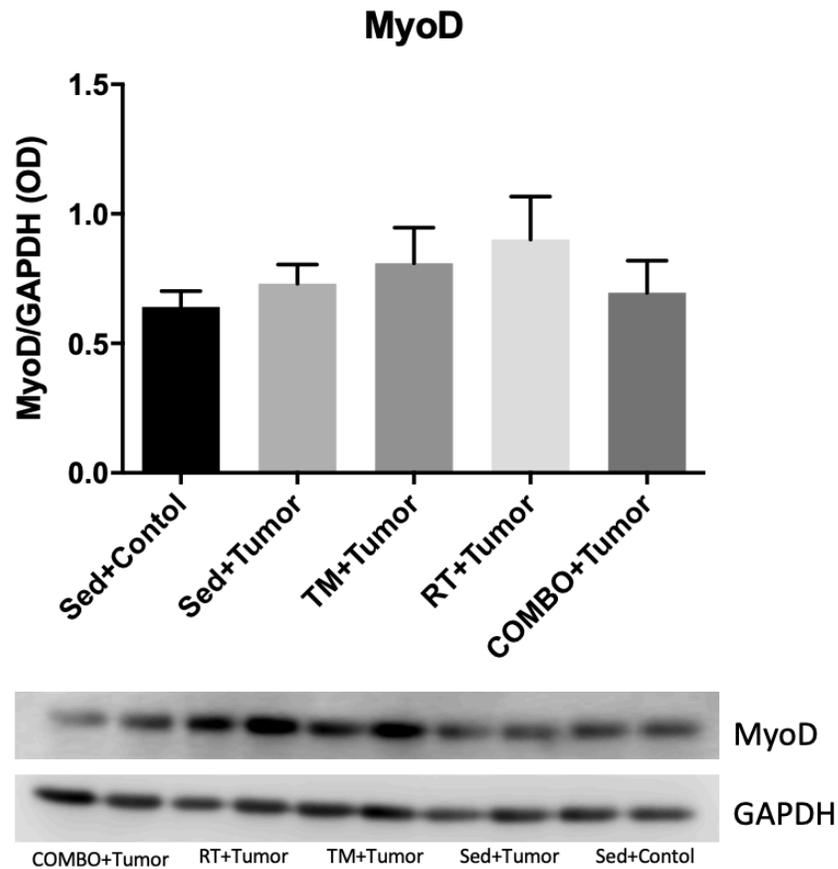


Figure 2. MyoD expression levels in the Gastrocnemius and representative immunoblots of MyoD and GAPDH. OD=optical density, Sed+control, n=12, Sed+Tumor, n=12, TM+Tumor, n=12, RT+Tumor, n=12, COMBO+Tumor, n=12. Values are means  $\pm$  SEM.

As shown in Figure 3, there were no significant group differences in the expression levels of myogenin ( $p > 0.05$ ).

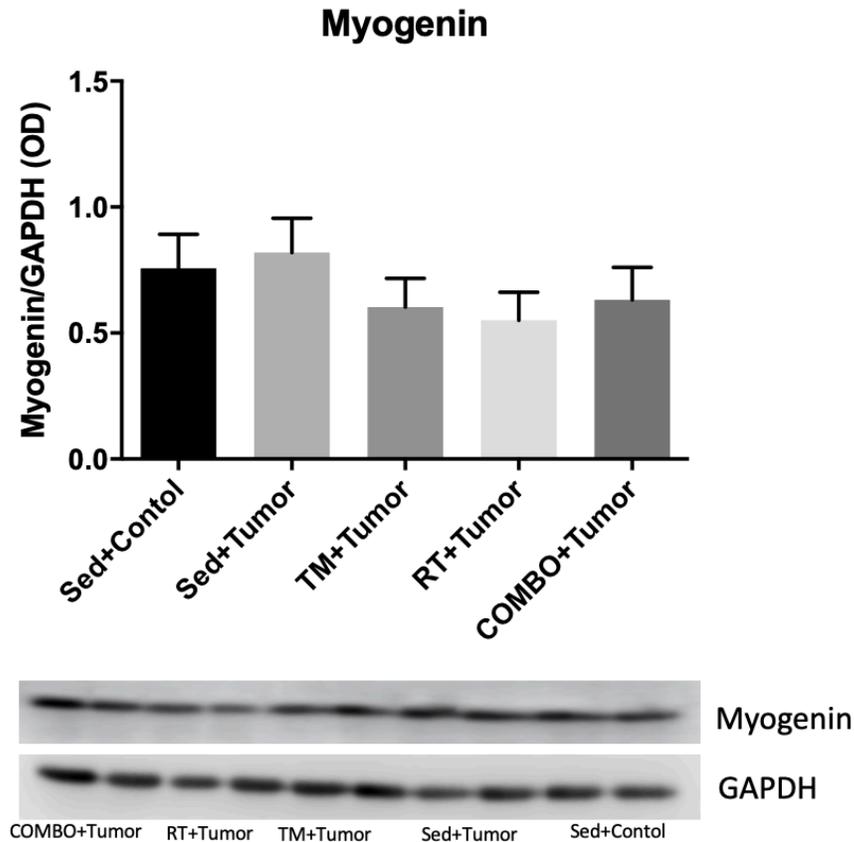


Figure 3. Myogenin expression levels in the Gastrocnemius and representative immunoblots of myogenin and GAPDH. OD=optical density, Sed+control, n=12, Sed+Tumor, n=12, TM+Tumor, n=12, RT+Tumor, n=12, COMBO+Tumor, n=12. Values are means  $\pm$  SEM.

As shown in Figure 4, there was a significant group difference in myostatin expression ( $p < 0.05$ ). *Post hoc* testing revealed that there was significantly lower myostatin expression in the Sed+Tumor group when compared to each of the exercise tumor groups. More specifically, the significant differences were found between Sed+Tumor and TM+Tumor, Sed+Tumor and RT+Tumor, and Sed+Tumor and COMBO+Tumor ( $p < 0.05$ ). The largest difference was observed between the Sed+Tumor and COMBO+Tumor group ( $p < 0.01$ ).

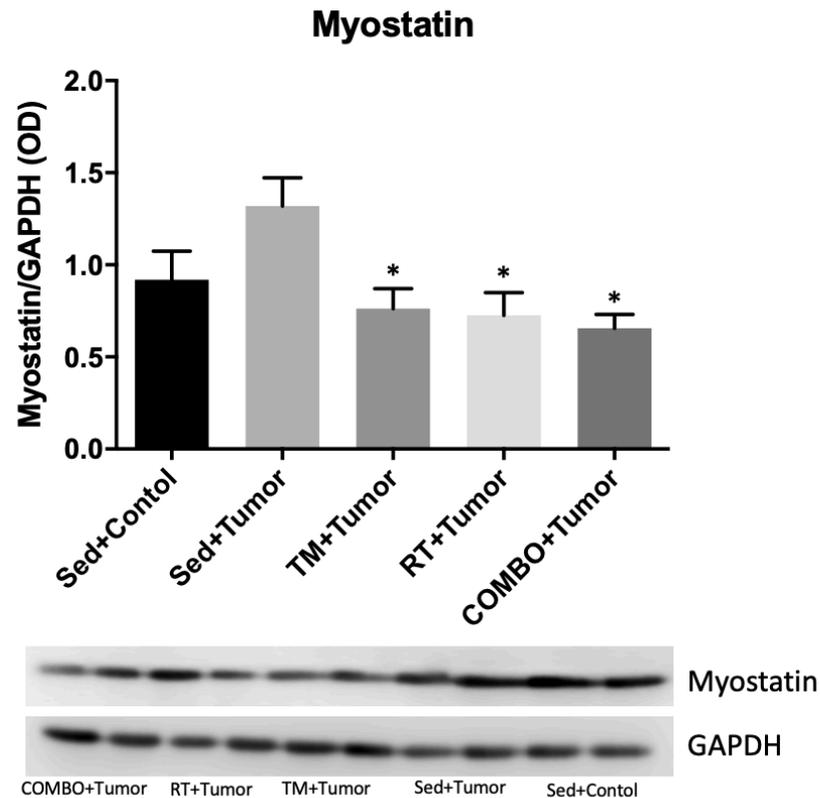


Figure 4. Myostatin expression levels in the Gastrocnemius and representative immunoblots of myostatin and GAPDH. OD=optical density, Sed+control, n=12, Sed+Tumor, n=12, TM+Tumor, n=12, RT+Tumor, n=12, COMBO+Tumor, n=12. Values are means  $\pm$  SEM.  $p < 0.05$   
\*significantly different from Sed+Tumor

As shown in Figure 4, there were no significant group differences in the expression of MuRF-1 ( $p > 0.05$ ). However, there was a trend showing an increased expression of myostatin and MuRF-1 in the Sed+Tumor group as compared to the exercise groups. There was also a trend showing a decreased amount of MuRF-1 in the exercised groups as compared to the SED+Tumor group. For both of the negative myogenic regulatory factors, myostatin and MuRF-1, the COMBO+Tumor group looks to have the largest decrease in protein expression.

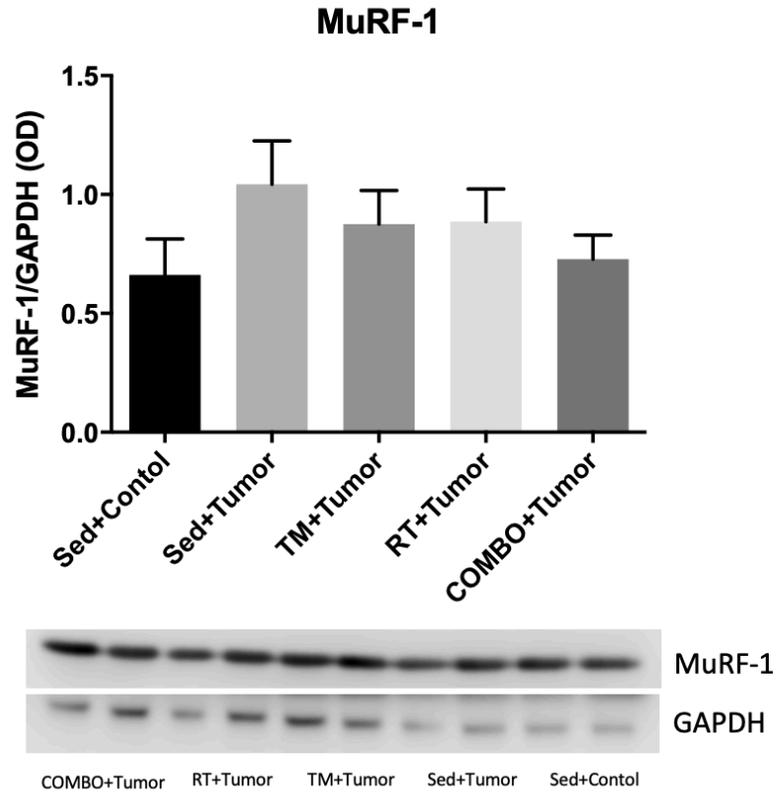


Figure 4. MuRF-1 expression levels in the Gastrocnemius and representative immunoblots of MuRF-1 and GAPDH. OD=optical density, Sed+control, n=11, Sed+Tumor, n=12, TM+Tumor, n=12, RT+Tumor, n=12, COMBO+Tumor, n=11. Values are means  $\pm$  SEM

## CHAPTER V

### DISCUSSION

This study examined the effects of an endurance training protocol, a resistance training protocol, and a combined endurance and resistance training protocol in cancer cachexia on the positive myogenic regulatory factors MyoD and myogenin, as well as the negative myogenic regulatory factors myostatin and MuRF-1. The main finding of this study revealed that SED+Tumor had significantly higher myostatin than TM+Tumor, RT+Tumor, and COMBO+Tumor. This means that any type of exercise led to significant decreases in myostatin expression with cancer cachexia. It has been well established that exercise leads to metabolic changes that typically increase the positive regulators of muscle growth such as MyoD and myogenin and decrease the negative regulators of muscle growth such as myostatin and MuRF-1.

This study did not find significant differences in the levels of MyoD. This could be due to a number of factors, one being that there was either too little or too much stimulus. In Figure 2, it is shown that while there are no significant group differences, the group that had a trend toward the highest amount of MyoD expression was the resistance training group. This finding is consistent with data found in other experiments where MyoD was shown to increase with mechanical stimulation as well as with stimulation that is known to induce satellite cell proliferation (Legerlotz & Smith, 2008). It would be expected that MyoD would increase in response to resistance exercise, as that is one way

in which satellite cells in skeletal muscle are proliferated (Megeny et al., 1996). In a different arm of this study that was performed it was found that the only significant differences in cross sectional area of the gastrocnemius were between the Sed+control group and the Sed+tumor group (Wood, 2019). This means that while there was not a significant decrease in muscle mass, there was also not a significant increase in muscle mass. Overall there may not have been enough stimulus to induce muscle hypertrophy during cachexia.

This study also found that there were no group differences in expression of myogenin. In Figure 3, a trend toward the levels of myogenin being decreased was observed in the exercise groups compared to both the Sed+Control and the Sed+Tumor group. While there were no significant group differences, myogenin may not have been upregulated due to the fact that there could have been more mitochondrial damage than expected due to cancer cachexia. In other studies, it has been found that damaged mitochondria could cause myogenin not to be expressed (Megeny et al., 1996). This would also go along with the fact that myogenin is involved in the satellite cell cycle. Therefore, if the muscles are not hypertrophying, then the myogenic regulatory factors that are involved in that process will not likely be upregulated (Ishido et al., 2004). It has also been shown that when muscles stop hypertrophying and are in a stage of maintenance, myogenin tends to not be expressed (Ishido et al., 2004). As stated before, there may not have been enough stimulus to lead to skeletal muscle hypertrophy, or the cachexia could have led to a significantly lower baseline of the positive myogenic regulatory factors; meaning it would be more difficult for there to be an overall increase

compared to the Sed+Control group in MyoD and myogenin expression. In future studies, mitochondrial damage should be taken into consideration.

This study found significant decreases in the levels of myostatin that were expressed in the exercised tumor groups when compared to the sedentary tumor group. Tukey's *post hoc* testing revealed that the significant group differences were between the Sed+Tumor group and each of the exercise groups. The largest decrease was shown in the COMBO+Tumor group suggesting that the combination training approach had a trend toward the largest effect on myostatin expression. This finding is similar to findings in other research, in that the downregulation of myostatin does tend to have a protective effect on skeletal muscles with cancer cachexia (Smith & Lin, 2013). While myostatin is known to be upregulated in cachectic models, it is known to decrease with exercise. This study confirms that with any mode of exercise, myostatin will be downregulated. While the downregulation of myostatin was most prevalent in the combined endurance and resistance training group, there was also a significant difference between the Sed+Tumor and TM+Tumor groups as well as a significant difference between Sed+Tumor and RT+Tumor groups. This shows that there is a chance to downregulate myostatin with any mode of exercise. This is consistent with others who used both endurance and resistance training to promote a significant decrease in myostatin expression (Hittel et al., 2010).

The final myogenic regulatory factor that was examined was MuRF-1. While there were no significant group differences found, there does seem to be a trend toward MuRF-1 being decreased with exercise as shown Figure 5. It is well known that when MuRF-1 is upregulated, muscle atrophy is stimulated (Bowen et al., 2015). While the decrease of MuRF-1 was not significant, it was also found that there were no significant

decreases in skeletal muscle mass of the gastrocnemius (Wood, 2019). This is consistent with another study that used wheel running as a way to decrease MuRF-1 levels (Pigna et al., 2016). MuRF-1 is downregulated with exercise under normal control conditions; however it also tends to be upregulated with cancer cachexia (Bodine & Baehr, 2014). This study did look at both cancer cachexia and exercise. While there were not significant MuRF-1 decreases with exercise, it was shown that there was an overall protective effect on the gastrocnemius with the exercise protocols. This was shown though through the fact that there was not a significant decrease in the cross-sectional area of the gastrocnemius, which was observed in a separate arm of this study (Wood, 2019).

While there were no significant differences in the positive myogenic regulatory factors with exercise, there was a significant decrease in one of the negative myogenic regulatory factors with exercise and a trend toward a decrease the other negative myogenic regulatory factor. While trying to stimulate hypertrophy in skeletal muscles during cancer cachexia is important, it may be just as important to protect the skeletal muscles from atrophy. With cancer cachexia it is prevalent that muscle metabolism is changed and the positive myogenic regulatory factors decrease in expression and the negative myogenic regulatory factors increase in expression. It is now a matter of understanding how exactly these pathways become affected in cancer cachexia with exercise, as it has been shown that exercise can prove beneficial in decreasing the amount of skeletal muscle wasting. It may be difficult for there to be a significant increase in the positive myogenic regulatory factors such as MyoD and myogenin as both are involved in the satellite cell activation and the hypertrophy of skeletal muscle. If there is not enough stimulus for satellite cells to proliferate, then changes in MyoD and myogenin

will not be prevalent enough to be detected. The currently accepted mechanism behind cancer cachexia is shown through a decrease in cross sectional area of the myofibers and not necessarily a decrease in muscle myofiber number (Ishido et al., 2004). As far as the negative myogenic regulatory factors go, there was a significant decrease in the amount of myostatin expression and a trend toward a decrease in the level of MuRF-1. With these factors showing a decrease expression with exercise, it is shown that the negative regulators of skeletal muscle are easier to manipulate than the positive regulators of skeletal muscle growth. It also shows that while the exercise was not enough for the skeletal muscles to hypertrophy, the decreases in the negative myogenic regulatory factors did have a protective effect. The combination group may have showed the largest decrease because they did have the greatest overall volume of work suggesting that those muscles got the largest amount of stimulus. This could have been a main factor in why the combination group showed the largest decrease in both myostatin and MuRF-1 expression.

### **Conclusion and Further Applications**

While cancer cachexia is known to affect a large portion of the cancer population, it is important to find the underlying mechanisms of skeletal muscle wasting. Exercise, while it is not a treatment, has been shown to negate some of the effects of cancer cachexia such as muscle wasting (Porporato, 2016). Overall, the current study showed that the negative regulators of skeletal muscle growth may be just as important to take into consideration as the positive regulators of skeletal muscle growth. Further research should look more into specific modes of exercise with cancer cachexia. A combination of endurance and resistance training led largest affect in this study, it could be important to

increase volume of the endurance and resistance training protocols. This will help ensure that the type of exercise performed led to the specific changes in the myogenic regulatory factors, and that it was not just the increased volume in the combination group that led to these changes.

This study could also have clinical applications leading to the inclusion of exercise protocols in cancer patients with cachexia could help increase quality of life. Exercise interventions could lead to less skeletal muscle wasting allowing patients to have an easier time performing activities of daily living. The less skeletal muscle mass that a patient loses, typically the better the prognosis (Lenk et al., 2010). This suggests that if there is an exercise approach that can help decrease the expression of negative myogenic regulatory factors, it should be taken into consideration as part of the patient's treatment.

## REFERENCES

- Argiles, J. M., Busquets, S., Lopez-Soriano, F. J., Costelli, P., & Penna, F. (2012). Are there any benefits of exercise training in cancer cachexia? *Journal of Cachexia, Sarcopenia, and Muscle*, 3, 73-76
- Aulino, P., Berardi, E., Cardillo, V. M., Rizzuto, E., Perniconi, B., Ramina, C., . . . Coletti, D. (2010). Molecular, cellular and physiological characterization of the cancer cachexia-inducing C26 colon carcinoma in mouse. *BMC Cancer*, 10(1), 363. doi:10.1186/1471-2407-10-363
- Bodine, S. C., & Baehr, L. M. (2014). Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogen-1. *American journal of physiology. Endocrinology and metabolism*, 307(6), E469–E484. <https://doi.org/10.1152/ajpendo.00204.2014>
- Bowen, T. S., Schuler, G., & Adams, V. (2015). Skeletal muscle wasting in cachexia and sarcopenia: molecular pathophysiology and impact of exercise training. *Journal of cachexia, sarcopenia and muscle*, 6(3), 197–207. <https://doi.org/10.1002/jcsm.12043>
- Brown, J. L., Lee, D. E., Rosa-caldwell, M. E., Brown, L. A., Perry, R. A., Haynie, W. S., . . . Greene, N. P. (2018). Protein imbalance in the development of skeletal muscle wasting in tumour-bearing mice. *Journal of Cachexia, Sarcopenia and Muscle*, 9(5), 987-1002. doi:10.1002/jcsm.12354
- Brown, J. L., Rosa-Caldwell, M. E., Lee, D. E., Blackwell, T. A., Brown, L. A., Perry, R. A., . . . Greene, N. P. (2017). Mitochondrial degeneration precedes the development of muscle atrophy in progression of cancer cachexia in tumour-bearing mice. *Journal of Cachexia, Sarcopenia, and Muscle*, 8(6), 926–938. doi:10.1002/jcsm.12232
- Clarke, B. A., Drujan, D., Willis, M. S., Murphy, L. O., Corpina, R. A., Burova, E., . . . Glass, D. J. (2007). The E3 ligase MuRF1 degrades myosin heavy chain protein in dexamethasone-treated skeletal muscle. *Cell Metabolism*, 6(5), 376-385. doi:10.1016/j.cmet.2007.09.009
- Costelli, P., Muscaritoli, M., Bonetto, A., Penna, F., Reffo, P., & . . . Fanelli, F. R. (2008). Muscle myostatin signaling is enhanced in experimental cancer cachexia. *European Journal of Clinical Investigation*, 38: 531-538. doi:10.1111/j.1365-2362.2008.01970.x

- de Castro, G. S., Simoes, E., Lima, J. D., Ortiz-Silva, M., Festuccia, W. T., Tokeshi, F., ... Seelaender, M. (2019). Human Cachexia Induces Changes in Mitochondria, Autophagy and Apoptosis in the Skeletal Muscle. *Cancers*, 11(9), 1264. doi:10.3390/cancers11091264
- Dutton, E. K., Simon, A. M., & Burden, S. J. (1993). Electrical activity-dependent regulation of the acetylcholine receptor  $\delta$ -subunit gene, MyoD, and myogenin in primary myotubes. *Proceedings of the National Academy of Sciences of the United States of America*, 90(5), 2040-2044. doi:10.1073/pnas.90.5.2040
- Fearon, K., Strasser, F., Anker, S. D., Bosaeus, I., Bruera, E., Fainsinger, R. L., ... Baracos, V. E. (2011). Definition and classification of cancer cachexia: an international consensus. *The Lancet Oncology*, 12(5), 489-495. doi:10.1016/s1470-2045(10)70218-7
- Flynn, J. M., Meadows, E., Fiorotto, M., & Klein, W. H. (2010). Myogenin regulates exercise capacity and skeletal muscle metabolism in the adult mouse. *PloS one*, 5(10), e13535. <https://doi.org/10.1371/journal.pone.0013535>
- Gielen, S., Sandri, M., Kozarez, I., Kratzsch, J., Teupser, D., Thiery, J., ... Adams, V. (2012). Exercise training attenuates MuRF-1 expression in the skeletal muscle of patients with chronic heart failure independent of age: The randomized leipzig exercise intervention in chronic heart failure and aging catabolism study. *Circulation*, 125(22), 2716-2727. doi:10.1161/CIRCULATIONAHA.111.047381
- Hardee, J. P., Counts, B. R., & Carson, J. A. (2017). Understanding the Role of Exercise in Cancer Cachexia Therapy. *American journal of lifestyle medicine*, 13(1), 46-60. doi:10.1177/1559827617725283
- Hasty, P., Bradley, A., Morris, J. H., Edmondson, D. G., Venuti, J. M., Olson, E. N., & Klein, W. H. (1993). Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene. *Nature*, 364(6437), 501-506. doi:10.1038/364501a0
- Hittel, D. S., Axelson, M., Sarna, N., Shearer, J., Huffman, K. M., & Kraus, W. E. (2010). Myostatin decreases with aerobic exercise and associates with insulin resistance. *Medicine and science in sports and exercise*, 42(11), 2023-2029. doi:10.1249/MSS.0b013e3181e0b9a8
- Hughes, S. M., Taylor, J. M., Tapscott, S. J., Gurley, C. M., Carter, W. J., & Peterson, C. A. (1993). Selective accumulation of MyoD and Myogenin mRNAs in fast and slow adult skeletal muscle is controlled by innervation and hormones. *Development*, 1993, 118: 1137-1147.

- Ishido, M., Kami, K., & Masuhara, M. (2004). Localization of MyoD, myogenin and cell cycle regulatory factors in hypertrophying rat skeletal muscles. *Acta Physiologica Scandinavica*, 180(3), 281-289. doi:10.1046/j.0001-6772.2003.01238.x
- Khamoui, A. V., Park, B., Kim, D., Yeh, M., Oh, S., Elam, M. L., ... Kim, J. (2016). Aerobic and resistance training dependent skeletal muscle plasticity in the colon-26 murine model of cancer cachexia. *Metabolism*, 65(5), 685-698. doi:10.1016/j.metabol.2016.01.014
- Kitzmann, M., & Fernandez, A. (2001). Crosstalk between cell cycle regulators and the myogenic factor MyoD in skeletal myoblasts. *Cellular and Molecular Life Sciences*, 58(4), 571-579. doi:10.1007/PL00000882
- Klimek, M. E., Aydogdu, T., Link, M. J., Pons, M., Koniaris, L. G., & Zimmers, T. A. (2010). Acute inhibition of myostatin-family proteins preserves skeletal muscle in mouse models of cancer cachexia. *Biochemical and Biophysical Research Communications*, 391(3), 1548-1554. doi:10.1016/j.bbrc.2009.12.123
- Legerlotz, K., & Smith, H. K. (2008). Role of MyoD in denervated, disused, and exercised muscle. *Muscle & Nerve*, 38(3), 1087-1100. doi:10.1002/mus.21087
- Lenk, K., Schuler, G., & Adams, V. (2010). Skeletal muscle wasting in cachexia and sarcopenia: molecular pathophysiology and impact of exercise training. *Journal of Cachexia, Sarcopenia, and Muscle*, 1, 9-21. Doi: 10.1007/s13539-010-0007-1
- Lenk, K., Schur, R., Linke, A., Erbs, S., Matsumoto, Y., Adams, V., & Schuler, G. (2009). Impact of Exercise training on myostatin in the myocardium and skeletal muscle in a chronic heart failure model. *European Journal of Heart Failure*, 11, 342-348. Doi: 10.1093/eurjhf/hfp020
- Liu, C., Yang, Z., Liu, C., Wang, R., Tien, P., Dale, R., & Sun, L. (2008). Myostatin antisense RNA-mediated muscle growth in normal and cancer cachexia mice. *Gene Therapy*, 15(3), 155-160. doi:10.1038/sj.gt.3303016
- Meadows, E., Cho, J., Flynn, J. M., & Klein, W. H. (2008). Myogenin regulates a distinct genetic program in adult muscle stem cells. *Developmental Biology*, 322(2), 406-414. doi:10.1016/j.ydbio.2008.07.024
- Megeney, L. A., Kablar, B., Garret, K., Andersen, J. E., & Rudnicki, M. A. (1996). MyoD is required for myogenic stem cell function in adult skeletal muscle. *Genes and Development*, 10, 1173-1183

- Mendias, C. L., Kayupov, E., Bradley, J. R., Brooks, S. V., & Claflin, D. R. (2011). Decreased specific force and power production of muscle fibers from myostatin-deficient mice are associated with a suppression of protein degradation. *Journal of applied physiology (Bethesda, Md. : 1985)*, *111*(1), 185–191. <https://doi.org/10.1152/jappphysiol.00126.2011>
- Miyamoto, Y., Hanna, D. L., Zhang, W., Baba, H., & Lenz, H. J. (2016). Molecular Pathways: Cachexia Signaling-A Targeted Approach to Cancer Treatment. *Clinical cancer research: an official journal of the American Association for Cancer Research*, *22*(16), 3999–4004. doi:10.1158/1078-0432.CCR-16-0495
- Pigna, E., Berardi, E., Aulino, P., Rizzuto, E., Zampieri, S., & ... Moresi, V. (2016). Aerobic Exercise and Pharmacological Treatments Counteract Cachexia by Modulating Autophagy in Colon Cancer. *Scientific Reports*, 1–14. <https://doi.org/10.1038/srep26991>
- Porporato, P. E. (2016). Understanding cachexia as a cancer metabolism syndrome. *Oncogenesis*, *5*(2), e200-e200. doi:10.1038/oncsis.2016.3
- Rochard, P., Rodier, A., Casas, A., Cassar-Malek, I., Marchal-Victorion, S., Daury, L., . . . Cabello, G. (2000). Mitochondrial activity is involved in the regulation of myoblast differentiation through myogenin expression and activity of myogenic factors. *Journal of Biological Chemistry*, *275*(4), 2733-2744. doi:10.1074/jbc.275.4.2733
- Siu, P. M., Donley, D. A., Bryner, R. W., & Alway, S. E. (2004). Myogenin and oxidative enzyme gene expression levels are elevated in rat soleus muscles after endurance training. *Journal of Applied Physiology*, *97*(1), 277-285. doi:10.1152/jappphysiol.00534.2004
- Smith, R. C., & Lin, B. K. (2013). Myostatin inhibitors as therapies for muscle wasting associated with cancer and other disorders. *Current opinion in supportive and palliative care*, *7*(4), 352–360. doi:10.1097/SPC.0000000000000013
- Tisdale, M. J. (2009). Mechanisms of cancer cachexia. *Physiol Rev.* *89*(2): 381-410.
- Wood, N. (2019). The Effects of Exercise Training on Cachexia in Mice Bearing the Colon-26 Carcinoma (Unpublished doctoral dissertation). University of Northern Colorado, Greeley, CO.

APPENDIX A  
INSTITUTIONAL ANIMAL CARE &  
USE COMMITTEE APPROVAL



IACUC Memorandum

To: Dr. Reid Hayward  
From: Laura Martin, Director of Compliance and Operations  
CC: IACUC Files  
Date: 07/09/17  
Re: IACUC Protocol 1511CE-RH-R-18 Annual Renewal Approval

---

The UNC IACUC has reviewed your annual renewal request for animal use protocol 1511CE-RH-R-18.

The committee's review was based on the requirements of the Government Principles, the Public Health Policy, the USDA Animal Welfare Act and Regulations, and the Guide for the Care and Use of Laboratory Animals, as well as university policies and procedures related to the care and use of live vertebrate animals at the University of Northern Colorado.

Based on the review, the IACUC has determined that all review criteria have been adequately addressed. The PI/PD is approved to perform the experiments or procedures as described in the identified protocol for an additional year.