THE EFFECT OF PROSTATE CANCER ON ENDURANCE EXERCISE CAPACITY IN THE RAT

by

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Abstract

Cancer patients have a reduced exercise capacity compared to age-matched healthy counterparts which contributes to premature fatigue. The reductions in exercise capacity are multifactorial and vary depending on the type of treatments and the specific cancer. Given that cancer treatments have been shown to impair cardiovascular and/or skeletal muscle function, it is difficult to determine if cancer itself reduces exercise capacity. We used a rat prostate tumor model to test the hypothesis that cancer independently reduces endurance exercise capacity. **Methods:** In male Copenhagen rats (COP/CrCrl), an initial treadmill test to exhaustion was used to determine endurance exercise capacity. Subsequently, the prostates of the rats were injected with either prostate carcinoma cells (R-3327 AT-1) in Matrigel (cancer: n = 9) or Matrigel only (sham: n = 7). Treadmill tests to exhaustion were repeated four and eight weeks post-surgery. **Results:** Time to exhaustion decreased over the course of the experimental protocol in both the sham and cancer groups. However, the overall reduction in time to exhaustion in the cancer group (-16.7 ± 1.9 min) was significantly greater ($p = 0.038$) than the sham group (-10.1 ± 2.2 min). Despite no differences in total body mass at the end of the experimental protocol, heart, left ventricle, and gastrocnemius muscle mass were significantly lower in the cancer group compared to the sham group ($p < 0.05$ for all). Moreover, within the cancer group heart and left ventricle mass, but not gastrocnemius mass, were significantly inversely correlated with prostate tumor mass. **Conclusion:** Endurance exercise capacity was reduced in rats with untreated prostate cancer to a greater extent than it was reduced in sham operated rats. Although multiple mechanisms likely contributed to the reduced exercise capacity, reductions in heart and gastrocnemius muscle mass likely played an important role.

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Dedication

I want to dedicate this thesis to my mom, Mary Dillon, and my dad, John Esau, who support me every day in everything I do. And, most importantly, I dedicate this thesis to my cat BooButt. None of this would have been possible without you.

Chapter 1 - Literature Review

Prevalence and Impact of Cancer

Currently in the United States, over 14 million people have a history of cancer (Miller *et al.*, 2016). These children and adults are considered cancer survivors by the American Cancer Society. 66.5% of people diagnosed with cancer survive for over five years after diagnosis and treatment, with the majority of deaths within this five year span coming from those over 65 years in age (Siegel *et al.*, 2016). In 2016 alone it is estimated that there will be 1,685,210 new cancer cases, and 595,690 cancer deaths (Siegel *et al.*, 2016). The financial burden of cancer in the U.S. in 2012 was over 70 billion USD, which is expected to continue to rise (Lee *et al.*, 2016).

For an individual, the cost of cancer is not only the cost of insurance and copays, but time away from work for treatment. Because diagnoses are specific and individualized, the exact cost per person can vary widely from very little to tens of thousands of dollars. The diagnosis of cancer also comes with far more than just a monetary or economic cost; the toll it has on the mental health of patients (and caregivers (Padmaja *et al.*, 2016)) can be immense. Fatigue is prevalent in cancer survivors and often linked to reduced quality of life, stemming from a reliance on caregivers, an inability to maintain employment, and difficulty leading a 'normal' life (Curt *et al.*, 2000). The increased psychological stress of these fatigue ramifications can lead to depression and anxiety (Chipperfield *et al.*, 2013), which can manifest physically with a suppressed immune system (Dhabhar, 2014), fatigue, and generally a lower quality of life (Chipperfield *et al.*, 2013). Distress and depression are associated with suppressed cytotoxic T-cell and Natural-Killer-Cell presence, which are associated with immune surveillance of tumors (Reiche *et al.*, 2004). This added stress lowers the body's ability to fight the tumor on its own.

Prostate Cancer

One in five men will be diagnosed with prostate cancer in his lifetime, making prostate cancer the most prevalent cancer in men; in 2016 alone an estimated 180,890 men will be diagnosed with prostate cancer (Siegel *et al.*, 2016). The most commonly diagnosed groups are men over 50, those of African-American heritage, and those with a family history of the disease (Attard *et al.*, 2016).

The signs and symptoms of prostate cancer vary depending on the progression of the disease and whether or not metastases have developed. In many cases, symptoms involve painful, irregular, and/or bloody urination with more advanced cases including pain in the lower back, hip, or femur. Each case is different and the disease progresses differently for each person, making prostate cancer treatment unique to the patient.

Prostate Cancer Treatment

Because the prostate is a sex organ, many prostate carcinomas initially grow in response to androgens such as testosterone (Attard *et al.*, 2016; Miller, 2016). Treatment usually includes androgen deprivation therapy (ADT) which is accomplished by pharmaceutical reductions in androgens using a hormone blocker, or physical castration by removing one or both testicles, or removal of the prostate (i.e., radical prostatectomy; Miller *et al.*, 2016). Reducing androgen levels in the body elicits several side effects such as erectile dysfunction, loss of libido, diminished bone density, loss of skeletal muscle mass, weight gain, insulin resistance, fatigue, and depression. Resistance training and aerobic training is often prescribed to combat these side effects, especially bone density, muscle mass, and weight gain changes (Mason, 2006).

After reducing the androgen levels in the body, prostate tumors may become 'castration resistant' where upon continued growth occurs despite little to no androgen content in the body. If a prostate tumor becomes castration resistant the treatment option has to change, as lowering levels of androgens in the body may no longer be sufficient to combat the disease. However, patients often remain on ADT in order to keep the tumor from further progression, and other adjuvant treatments with chemotherapy and/or radiation are included (Miller *et al.*, 2016).

Prostate Cancer and Exercise

According to the American College of Sports Medicine's extensive review of cancer and exercise (Schmitz *et al.*, 2010), it is safe for individuals with prostate cancer to exercise. Indeed, the majority of deaths from prostate cancer do not come directly from the cancer but result from cardiovascular disease later in life (Schmitz *et al.*, 2010). Exercise decreases the risk of death from cardiovascular disease, as the cardiovascular benefits of exercise are well documented (Lavie *et al.*, 2015). In addition, exercise decreases the risk of developing cancer and increases survivorship in prostate cancer patients (Antonelli *et al.*, 2009).

In preclinical studies during exercise prostate tumor blood flow can increase up to 200% when compared to resting conditions in the rat, leading to a 50% reduction in hypoxia within the tumor (McCullough *et al.*, 2014). Voluntary wheel running in mice has even been shown to decrease the risk of metastasis with a prostate tumor model (Jones *et al.*, 2012). Increasing the vascular density, decreasing hypoxia, and increasing apoptosis all act to 'normalize' the cell, leading to increased chemotherapy delivery and a better outcome (Betof *et al.*, 2015).

In human studies of prostate cancer, reductions in maximal oxygen consumption (VO_{2max}) are reported in men on ADT for more than three months compared to men on ADT for less than

three months (Wall *et al.*, 2015). This study had no healthy age-matched control group, so comparisons to the healthy population can not be made. While the authors attribute the reduction in exercise capacity to ADT, individuals who have been on ADT longer have also likely had prostate cancer longer.

In another study of exercise performance of men with prostate cancer, 6-minute walk distance and handgrip tests were reduced significantly compared to age-matched healthy controls (Alibhai *et al.*, 2015). While there was a prostate cancer group who was not on ADT (prostate cancer control group), no statistical comparisons were made with this group against either the control or the prostate cancer group on ADT (Alibhai *et al.*, 2015). This study also states that individuals on ADT have more aggressive cancer phenotypes, leading to more aggressive treatments aside from ADT. While ADT certainly plays a role in the reductions in performance, whether or not the cancer itself plays a role has yet to be investigated.

Cancer Cachexia

Cachexia is defined as weight loss of at least 5% that is not due to edema, occurring within a period of 12 months or less that is due to an underlying disease including cancer (Evans *et al.*, 2008). Cachexia is experienced by an estimated 50-80% of cancer patients (Argilés *et al.*, 2014) and is likely one of the contributing factors involved with the reduction in exercise capacity seen in cancer patients (Peel *et al.*, 2014). Along with decreased performance, cancer may cause exaggerated fatigue and a lower quality of life (Curt *et al.*, 2000; Cella *et al.*, 2001; Murphy, 2016). Because exercise seems to improve the tumor microenvironment (McCullough *et al.*, 2013), finding the cause of this cancer-related fatigue could help to improve outcomes in prostate cancer patients.

Markers of muscle atrophy, such as activin A, interleukin-6, tumor necrosis factor-α, and forkhead box O pathway (Tsujinaka *et al.*, 1996; Chen *et al.*, 2014; Sandri *et al.*, 2004) are upregulated with many cancers, including colon cancer compared to controls leading to reductions in gastrocnemius mass (Matsuyama *et al.*, 2015). However, reductions in food intake (anorexia) have also been shown with cancers (especially of the digestive system), leading to a reduction in body mass and skeletal muscle mass (Springer *et al.*, 2014; Matsuyama *et al.*, 2015). These reductions in body and muscle mass are likely due to the cancer itself, but how these affect performance has not been investigated.

Cardiac cachexia, a loss of cardiac muscle mass due to disease, and cardiac dysfunction are evident in many cancer survivors who have been on treatments known for cardiotoxicity (such as doxorubicin; Curigliano *et al.*, 2016). These instances of heart disease might not be caused by the treatments, they may only be exacerbating already existing heart disease (Murphy, 2016). Reductions in heart mass have been shown in colon cancer (Matsuyama *et al.*, 2015), a subcutaneous colon cancer model (Wysong *et al.*, 2011) and a liver tumor model (Springer *et al.*, 2014). Along with reductions in heart mass, Springer *et al.* (2014) found reductions in stroke volume, a measure of cardiac function, when compared to a sham group.

There could be differences in how tumors reduce the mass of cardiac skeletal muscle (Cosper & Leinwand, 2011), and there are still gaps in knowledge as to how much of an effect the cancer itself has on cachexia (Murphy, 2016). Finding mechanisms that contribute to reductions in muscle mass could help to combat the effects of the cancer could improve quality of life in cancer patients.

Experimental Tumor Cells

Using the correct tumor model is essential when researching cancer, and ethical considerations limit the ability to test cancer in human subjects. When comparing blood flow to a subcutaneous model of prostate cancer cells and blood flow to an orthotopic prostate tumor model during exercise in the rat, blood flow to the subcutaneous tumor was decreased by 25% during exercise whereas orthotopic prostate tumor flow was increased by 181% during exercise (Garcia *et al.*, 2016). Orthotopic tumors are also better predictors of clinical success (Killion *et al.*, 1998).

A comparison of a subcutaneous and peritoneal injection of colon cancer cells showed differences in multiple tissues between groups (Matsuyama *et al.*, 2015). The control group had significantly larger gastrocnemius masses compared to both the subcutaneous and peritoneal colon cancers. There were also differences between each type of cancer, as the subcutaneous model of colon cancer had significantly larger gastrocnemius masses when compared to the peritoneal model. Cardiac muscle mass was not different between the control and subcutaneous group, however the peritoneal group had significantly reduced cardiac mass compared to both the control and subcutaneous group (Matsuyama *et al.*, 2015). Importantly, these inconsistencies highlight how essential using the correct tumor model is with cancer research.

Within prostate tumor models, AT-1 cells from the Dunning R-3327 strain of prostate carcinoma cells tumor cells cultured in RPMI 1640 media with 10% Fetal Bovine Serum, 1% penicillin/streptomyocin, 2mM glutamine, and 250 μM dexamethasone in a 37^{o}C incubator have been used in past research as a viable model for rat prostate cancer (Dunning, 1963; McCullough *et al.*, 2013), producing prostate tumors in rats. These cells mimic the growth patterns of human prostate cancer (Isaacs *et al.*, 1978).

Chapter 2 - Introduction

Cancer patients have a reduced maximal exercise capacity (i.e., maximal oxygen consumption, VO_{2max}) and report exaggerated levels of fatigue (excessive tiredness) when compared to their age-matched healthy counterparts (Curt *et al.*, 2000; Peel *et al.*, 2014). Excessive fatigue is experienced by over 50% of cancer patients (Cella *et al.*, 2001; Wagner & Cella, 2004; Hofman *et al.*, 2007; Wang *et al.*, 2014; Charalambous & Kouta, 2016) and is related to the type of cancer (Birgegård *et al.*, 2005; Hofman *et al.*, 2007; Wang *et al.*, 2014) as well as both the type (Hofman *et al.*, 2007; Alibhai *et al.*, 2015) and duration (Wall *et al.*, 2015) of treatment. The exaggerated levels of fatigue in cancer patients impair the ability to perform activities of daily living which has been shown to lead to a reduced quality of life (Curt *et al.*, 2000; Morrow *et al.*, 2002; Charalambous & Kouta, 2016). Multiple mechanisms likely contribute to the reduced exercise capacity and exaggerated fatigue in cancer patients. For example, reductions in cardiac function (Chicco *et al.*, 2005; Springer *et al.*, 2014), anemia (Birgegård *et al.*, 2005), reduced cardiac and skeletal muscle mass (i.e., cachexia; Tisdale, 2009; Matsuyama *et al.*, 2015; Murphy, 2016), and increased fat mass (Galvão *et al.*, 2008) have all been reported in cancer patients. Given the clear link between fatigue and reduced quality of life in cancer patients (Curt et. al., 2000), identifying the specific mechanisms that contribute to the reduced exercise capacity and exaggerated fatigue would have immense clinical value. One specific hindrance, however, to identifying the causes and mechanisms of fatigue in this population is that various forms of cancer (Wysong *et al.*, 2011; Xu *et al.*, 2011; Matsuyama *et al.*, 2015) and cancer treatments (Arola *et al.*, 2000; Chicco *et al.*, 2005; van Norren *et al.*, 2009) by themselves have been shown to produce deleterious physiological effects.

One in five men will be diagnosed with prostate cancer in his lifetime, making prostate cancer the most common cancer in men (Siegel *et al.*, 2016). One of the most common treatments for prostate cancer is surgical or pharmacological androgen deprivation therapy (ADT) which itself has been implicated in multiple undesired side-effects including reduced muscle mass and bone density as well as increased fat mass (Cheung *et al.*, 2014; Rhee *et al.*, 2015; Nelson *et al.*, 2016). These side-effects likely contribute to the fact that prostate cancer patients receiving ADT have reduced 6-minute walk test performance and grip strength when compared to healthy controls (Alibhai *et al.*, 2015). Furthermore, prostate cancer patients on ADT for more than three months have a lower VO_{2max} compared to those on ADT for less than three months (Wall *et al.*, 2015). While ADT certainly contributes to the reductions in exercise capacity found in prostate cancer patients the effect of the prostate cancer itself may also play a role, but this has not been investigated.

The purpose of this study was to investigate the effect of untreated prostate cancer on endurance exercise capacity in an established rat prostate tumor model. We tested the hypothesis that time to exhaustion during submaximal treadmill running would be reduced following the development of prostate cancer compared to pre-cancer values. Additionally, to examine potential mechanisms by which prostate cancer may impact endurance exercise capacity in this model, we tested the hypothesis that prostate cancer would reduce cardiac function, cardiac and hindlimb skeletal muscle mass, as well as hindlimb skeletal muscle oxidative capacity.

Chapter 3 - Methods

All procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council Committee, Washington, D.C., rev. 2011). Eighteen immunocompetent male Copenhagen rats (COP/CrCrl, Charles River, Wilmington, MA) were used. The rats were housed at 23^{o}C using a 6 AM to 6 PM 12-hour light-dark cycle and provided rat chow and water ad libitum.

Experimental Protocol

All rats were acclimated to treadmill running on a custom built motor driven treadmill for approximately five days at 25 m/min at a 10% incline. In order to ensure the rats remained untrained, each acclimation lasted <5 minutes. Within three days of the final acclimation, endurance exercise capacity was assessed by measuring time to exhaustion during a graded submaximal treadmill test according to the methods described in detail by Copp *et al.* (2009) (see below).

After the initial endurance exercise capacity test, the rats were assigned to either a sham or cancer group. At least 48 hours of recovery was given until the sham or cancer surgery was performed (see below). Three weeks after surgery, the rats were re-familiarized (~3 times, <5 min each) to treadmill running and the endurance exercise capacity test was repeated four weeks post-surgery. At seven weeks post-surgery, rats were again re-familiarized (~3 times, <5 min each) to treadmill running and the final endurance exercise capacity test was completed at eight weeks post-surgery.

Following the final endurance exercise capacity test, rats were anesthetized (2-3% isofluorane O_2 balance) and the right carotid artery was isolated and cannulated for the advancement of a 2-Fr catheter-tipped pressure transducer (Millar Instruments, Houston, TX) into the left ventricle (LV) to measure the rate of LV pressure increase over time (Δpressure/Δtime) and the LV end-diastolic pressure (LVEDP). The pressure transducer was then withdrawn from the LV and systolic blood pressure was measured when the catheter tip was in the aorta. Thereafter the rats were euthanized by a thoracotomy under anesthesia (5% isofluorane O_2 balance) followed by removal of the heart to verify death. Subsequently, the left gastrocnemius, extensor digitorum longus (EDL), and soleus muscles, the heart, as well as a portion of the costal diaphragm muscle were dissected and weighed. The wall of the right ventricle (RV) was cut away and the RV wall and the LV (along with the intraventricular septum) were weighed separately. In addition, red and white portions of the gastrocnemius were isolated and dissected. The left femur was also dissected and the length was measured. All tissues were frozen at -80^{o}C for future analysis.

Endurance Exercise Capacity Protocol

The endurance exercise capacity test consisted of 15 minute stages starting at 25 m/min for 15 minutes at a 10% incline. The speed of the treadmill was increased by 5 m/min every 15 minutes (with the incline held constant) until the rat was unable or unwilling to run. Rats were motivated to run with bursts of high-pressure air aimed at the hind legs. An endurance exercise capacity test was deemed valid if a marked attenuation of the rat's righting reflex and/or a noticeable change in gait that is indicative of exhaustion prior to termination of the test was present (Copp *et al*., 2009). Time to exhaustion was measured to the nearest second. The work

performed (joules) during each test was calculated by multiplying the vertical distance run by the rat's body mass and then dividing by 9.81. No endurance exercise capacity test was repeated more than once, and tests were repeated in the same proportion by sham rats and cancer rats. Each test was completed between 8 AM and 12 PM by the same investigators in a room with the temperature maintained between 21 and 23°C, with no additional fans or cooling devices used. During each endurance exercise capacity test the investigators were blinded to the group and previous run times of the rat.

Prostate Cancer Model

The AT-1 cell line from the Dunning R-3327 strain of Copenhagen rat prostate carcinoma cells was used (Dunning, 1963). These cells have a high growth rate, low metastatic potential, and are similar to the growth patterns of human prostate cancer (Isaacs *et al.*, 1978). The cells were grown in RPMI 1640 media with 10% Fetal Bovine Serum, 1% penicillin/streptomyocin, 2 mM glutamine, and 250 μM dexamethasone in a 37^{o}C humidified incubator at 5% CO_2. When cells reached ~90% confluence, a sample of the cells were counted in a hemocytometer, and the rest of the viable cells were used to prepare a tumor cell stock solution with Matrigel. Matrigel enhances the chance the cancer cells will form a tumor and augments tumor growth (Kleinman & Martin, 2005). This solution was aliquoted into 0.1 mL syringes that each contained approximately 10^5 AT-1 cells**.** This model has been used previously to induce prostate cancer (McCullough *et al.*, 2014; Garcia *et al.*, 2016).

All procedures were performed under aseptic conditions. Rats were anesthetized (2-4% isofluorane O_2 balance) and the bladder/prostate complex was exposed through a small incision (<2cm) lateral to the midline of the abdomen. The ventral lobe of the prostate in the cancer group

were injected with the cell stock solution with cancer cells and 0.1mL of Matrigel using sterile insulin syringes (26G). The prostates of the rats in the sham group were injected with 0.1mL of Matrigel without cancer cells. Following the surgery, the incision was closed and rats were injected with buponorphrine (0.05 mL/kg) and acepromazine (0.04 mL/kg). Post-operative monitoring occurred daily for one week.

Citrate Synthase Activity

Citrate synthase activity was measured in the red and white portions of the gastrocnemius, soleus, and costal diaphragm muscles as a marker of oxidative capacity. The muscles were mechanically homogenized and analysis was completed by a spectrophotometer using the methods of Srere (1969). Briefly, 15 μl and 30 μl samples were diluted using 210 μl and 195 μl of tris buffer, respectively, and 15 μl of acetyl coenzyme A and 30 μl of DTNB were added to each sample. All samples were incubated in a spectrophotometer (Fisher Scientific, accuSkan GO) for 5 minutes at 30^{o}C. Readings were taken once per minute for five minutes and then 35 μl of oxaloacetate was added to all samples and immediately analyzed again. The citrate synthase activity was given in μmol/min/g wet weight. If the difference between the 15 μl and the 30 μl samples was larger than 15% the sample was re-analyzed until there was less than 15% difference.

Data Analysis

Statistical analyses were completed in Prism (version 7.0, Graphpad) data analysis software. Two-way ANOVAs with SNK post-hoc tests or unpaired t-tests were used to compare

group means as appropriate. Pearson Product Moment Correlations and regression analyses were used to quantify relationships between variables. Significance was accepted at $p < 0.05$.

Chapter 4 - Results

All eighteen rats completed the experimental protocol. In the cancer group, one rat developed an ectopic tumor rather than a prostate tumor and one rat did not develop a tumor. The data from these rats are shown in Appendix A but were excluded from the analyses resulting in final sample sizes of n = 7 and n = 9 for the sham and cancer groups, respectively. The average tumor mass in the cancer group was 9.8 ± 2.6 g (range: 0.2 to 19.5 g).

For body mass, there was a statistically significant main effect of time ($p < 0.001$), but there was not a statistically significant group effect ($p = 0.762$) or interaction ($p = 0.543$, Figure 1A). Body mass increased significantly in both groups from the initial to the four week test (sham: $p = 0.045$, cancer: $p = 0.006$) and was further increased at the eight week compared to the four week test in the sham group ($p = 0.039$) but not in the cancer group ($p = 0.461$). The overall increase in body mass over the course of the experimental protocol was not different between groups ($p = 0.139$, Figure 1B). When prostate tumor mass was subtracted from the eight week body mass of the rats in the cancer group, however, the overall increase in "non-tumor mass" was significantly lower in the cancer group compared to the sham group ($p = 0.029$, Figure 1C). Within the cancer group there was no correlation between tumor mass and eight week body mass ($r = -0.14$, $p = 0.725$).

Endurance Exercise Capacity

For time to exhaustion during the endurance exercise capacity test, there was a statistically significant main effect of time ($p < 0.001$) but there was not a statistically significant group effect ($p = 0.098$) or interaction ($p = 0.086$, Figure 2A). Time to exhaustion at four weeks for the sham group was not different from the initial value ($p = 0.971$), whereas time to

exhaustion at four weeks for the cancer group was significantly reduced from the initial value (↓18%, $p = 0.027$). Time to exhaustion at eight weeks was significantly reduced in both the sham and cancer groups from the initial values ($p < 0.001$ for both). When expressed as the change in time to exhaustion from the initial value, the cancer group had a significantly larger reduction compared to the sham group at four weeks ($p = 0.005$, Figure 2B) and at eight weeks ($p = 0.038$, Figure 2C).

For work performed during the endurance exercise capacity test, there was a statistically significant main effect of time ($p < 0.001$) but there was not a statistically significant group effect ($p = 0.098$, Figure 3A). The interaction between these variables was statistically significant ($p = 0.037$). There was no difference in the work performed between the initial and four week tests for either the sham ($p = 0.976$) or cancer group ($p = 0.118$). Work performed at eight weeks was significantly reduced from the initial values in both the sham and cancer groups ($p < 0.001$ for both). When expressed as the change in work from the initial value, the cancer group had a significantly larger reduction compared to the sham group at four weeks ($p = 0.011$, Figure 3B) and at eight weeks ($p = 0.045$, Figure 3C).

Cardiac Function

There were no statistically significant differences between the mean values for LVEDP (sham: 12 ± 1, cancer: 11 ± 2 mmHg, $p = 0.736$), LV Δpressure/Δtime (sham: 7614 ± 387, cancer: 7038 ± 311 mmHg/s, $p = 0.261$), or systolic blood pressure (sham: 122 ± 2, cancer: 114 ± 6 mmHg, $p = 0.271$) between groups. Within the cancer group, however, tumor mass was significantly inversely correlated to LV Δpressure/Δtime ($r = -0.71$, $p = 0.047$, Figure 3A) and

systolic blood pressure ($r = -0.89$, $p = 0.004$, Figure 3B). Tumor mass was not significantly correlated to LVEDP ($r = -0.14$, $p = 0.725$).

Cardiac and Skeletal Muscle Mass

Heart, LV, and gastrocnemius muscle mass were significantly lower in the cancer group compared to the sham group ($p < 0.05$ for all, Table 1). This was the case even when muscle mass was normalized to body mass ($p < 0.05$ for all). RV, EDL, and soleus muscle mass were not different between groups. Within the cancer group, tumor mass was significantly inversely correlated to heart mass ($r = -0.74$, $p = 0.022$, Figure 4A) and LV mass ($r = -0.85$, $p = 0.004$, Figure 4B). Tumor mass was not significantly correlated to gastrocnemius mass ($r = -0.15$, $p =$ 696, Figure 4C). Femur length was not different ($p = 0.237$) between the sham group (39.4 ± 0.5 mm) and cancer group (40.6 ± 0.7 mm) indicating there was no difference in the overall growth of the rats.

Citrate Synthase Activity

There were no differences in citrate synthase activity between the sham and cancer groups for any muscle or muscle part analyzed (Table 2).

Table 1. Individual Muscle Mass

	Sham (n = 7)			Cancer (n = 9)			P-Value
Absolute muscle mass (mg)							
Heart	820	±	17	723	±	30	0.021*
LV	601	±	15	498	±	20	0.001*
RV	219	±	14	215	±	18	0.861
Gastrocnemius	1864	±	32	1702	±	35	0.005*
Soleus	167	±	8	168	±	4	0.923
EDL	158	±	5	147	±	4	0.113
Normalized muscle mass (mg/g)							
Heart/BM	2.42	±	0.04	2.19	±	0.08	0.035*
LV/BM	1.77	±	0.02	1.54	±	0.05	0.002*
RV/BM	0.65	±	0.04	0.65	±	0.05	0.960
Gastrocnemius/BM	5.49	±	0.07	5.16	±	0.10	0.022*
Soleus/BM	0.50	±	0.01	0.50	±	0.02	0.731

Mean ± SEM. * = statistically significant difference between groups. LV (left ventricle), RV (right ventricle), extensor digitorum longus (EDL), BM (body mass).

Table 2. Citrate Synthase Activity

Citrate Synthase Activity (μmol/min/g)	Sham (n = 7)			Cancer (n = 9)			P-Value
Soleus	19.9	±	1.7	17.0	±	1.2	0.719
Red gastrocnemius	24.1	±	2.7	25.4	±	2.1	0.711
White gastrocnemius	8.5	±	0.4	7.41	±	0.3	0.270
Costal diaphragm	30.7	±	1.4	20.1	±	2.5	0.858

Mean ± SEM. There were no statistically significant differences between groups.

Figure 1. Body Mass

Body mass of the sham (n = 7) and cancer (n = 9) groups (A). There was no difference in the increase in body mass over the course of the experimental protocol between groups (B). When subtracting prostate tumor mass from body mass in the cancer group, the increase in non-tumor mass over the course of the experimental protocol was significantly lower in the cancer group compared to the sham group (C). Values are mean ± SEM. ‡ = significantly different from initial value, # = significantly different from initial and 4 week values. * = significantly different from sham.

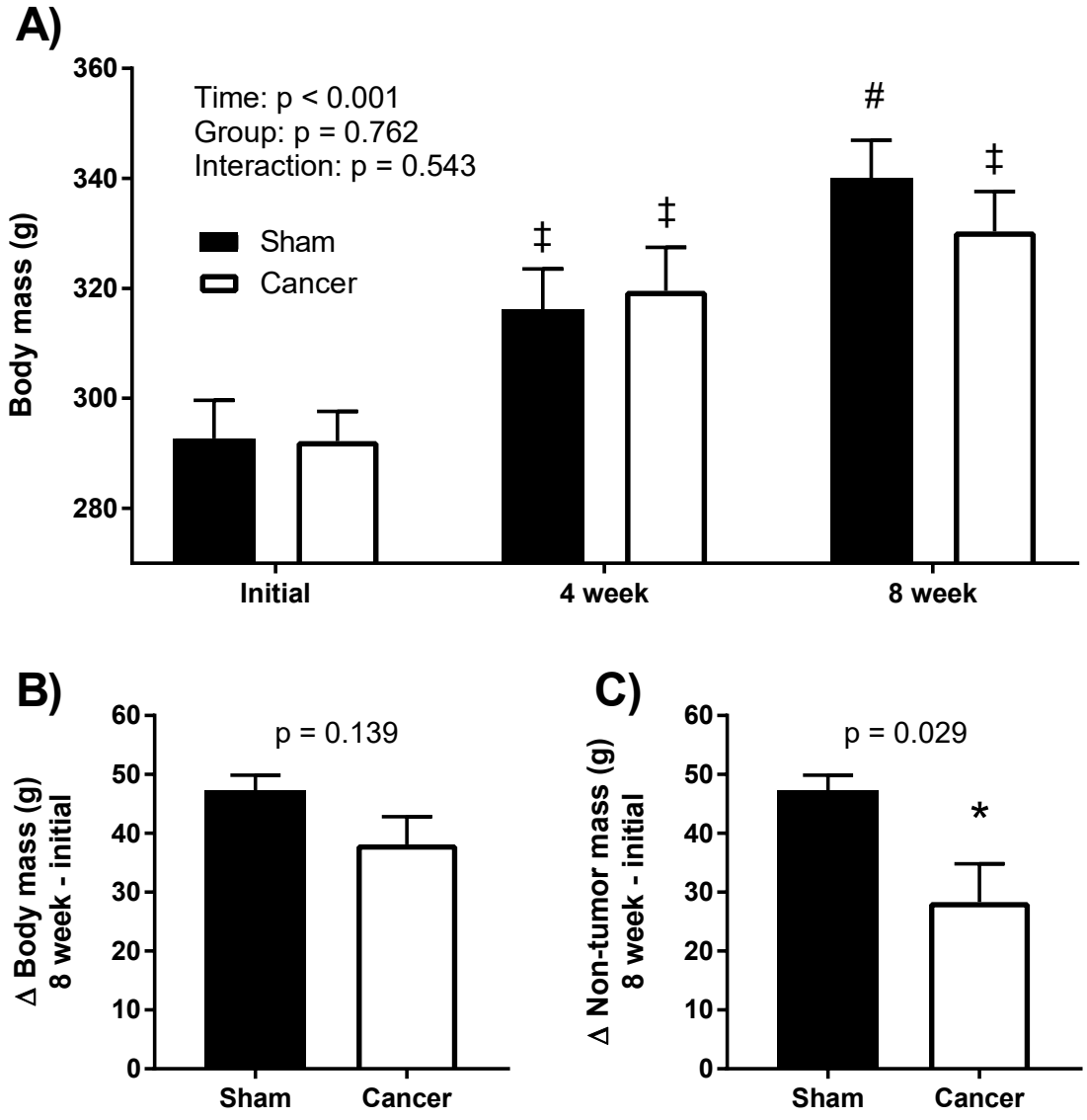


Figure 2. Time to Exhaustion

Time to exhaustion during the endurance exercise capacity tests for the sham (n = 7) and cancer (n = 9) groups (A). The cancer group had a significantly larger reduction in time to exhaustion from the initial value at four weeks (B) and eight weeks (C) compared to the sham group. Values are mean ± SEM. ‡ = significantly different from initial value, # = significantly different from initial and 4 week values. * = significantly different from sham.

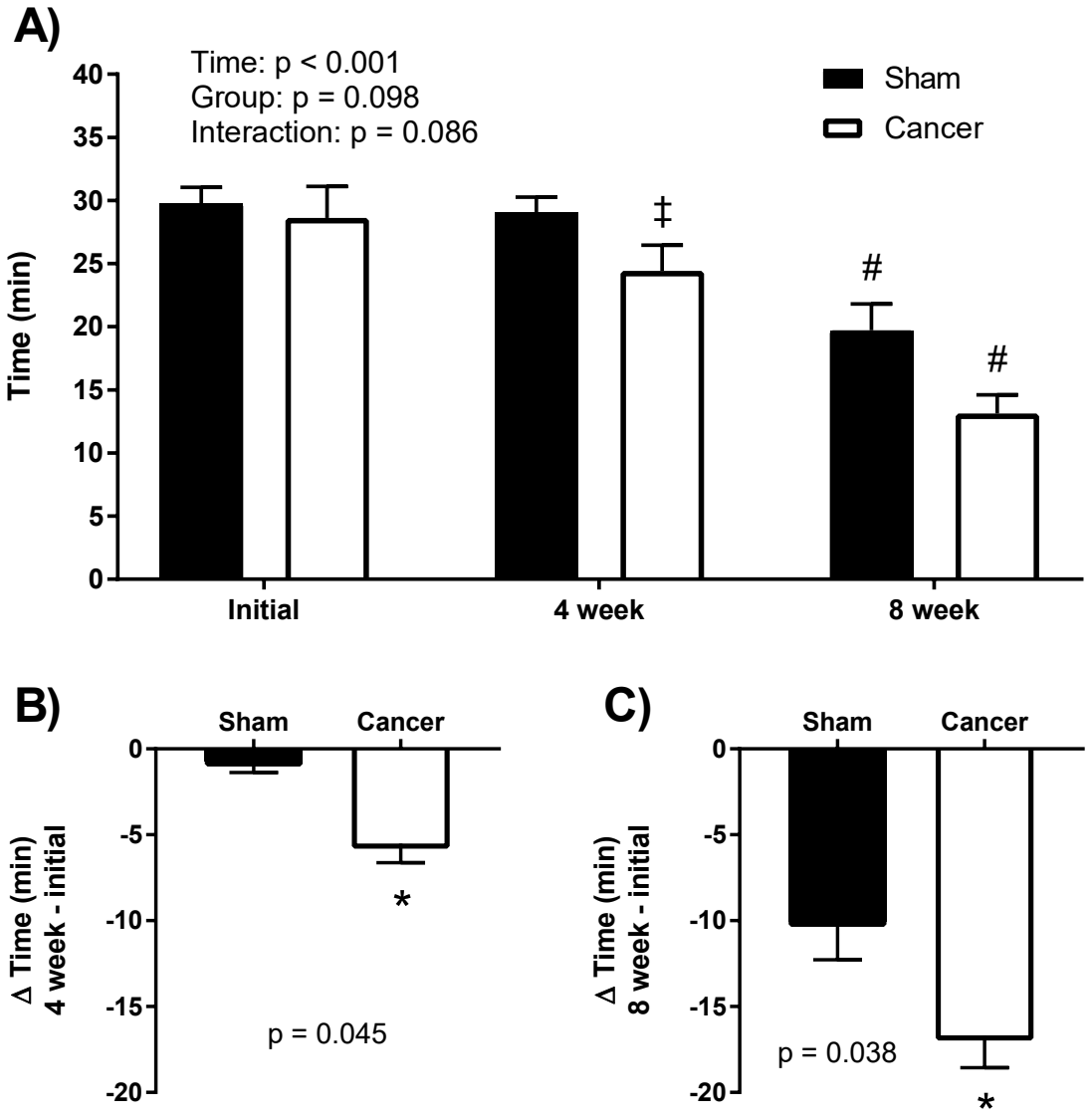


Figure 3. Work Performed during Endurance Exercise Capacity Test

Work performed during the enurance exercise capacity test for the sham (n = 7) and cancer (n = 9) groups at each time point (A). The cancer group had a significantly larger reduction in work performed from the initial value at four weeks (B) and eight weeks (C) compared to the sham group. Values are mean ± SEM. ‡ = significantly different from initial value, # = significantly different from initial and 4 week values, * = significantly different from sham.

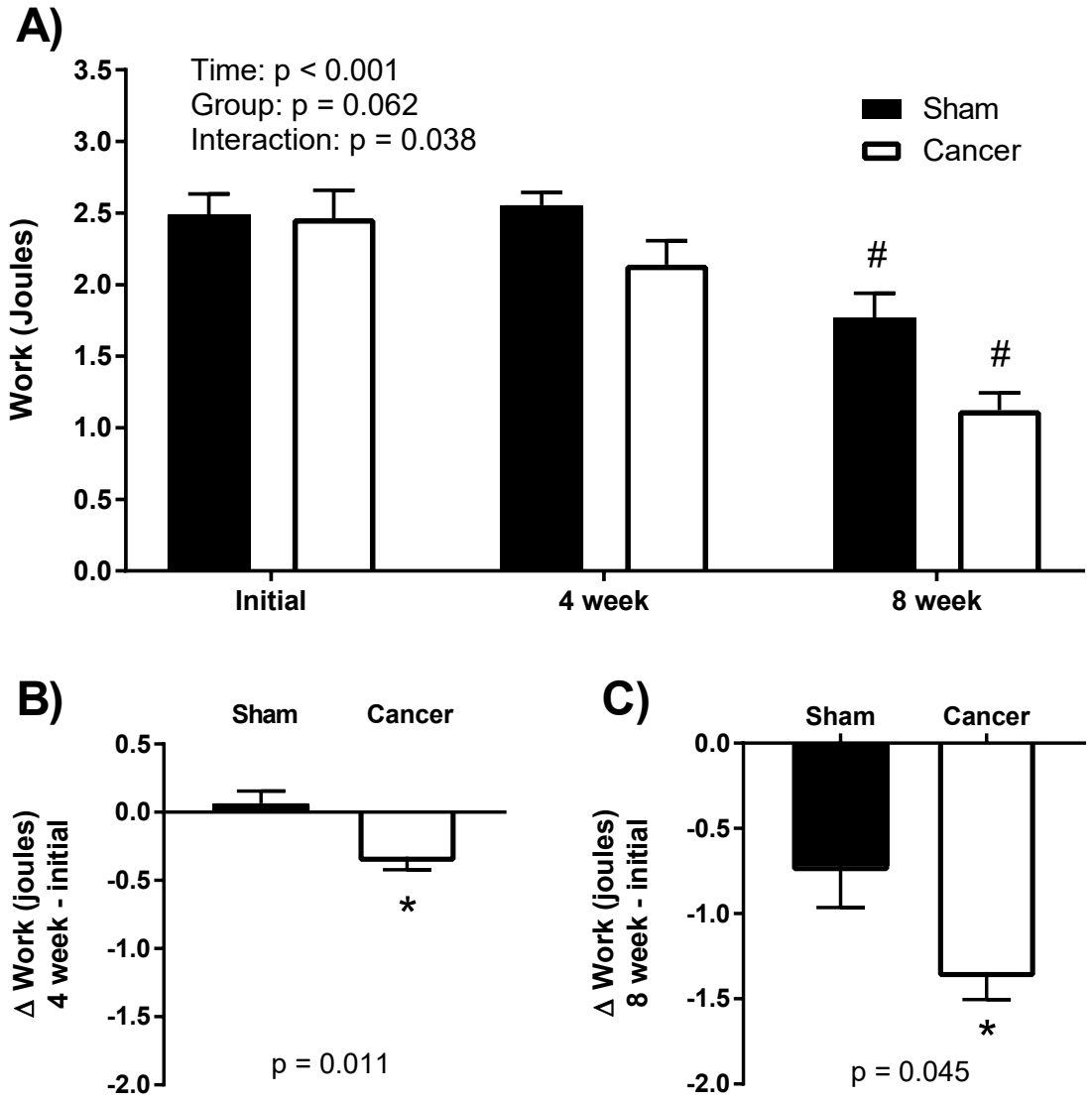


Figure 4. Correlations between Tumor Mass and Indexes of Cardiac Function

Within the cancer group (n = 9), tumor mass was significantly inversely correlated to LV Δpressure/Δtime (A) and systolic blood pressure (B). The open circles represent the mean and SEM of the sham group. The closed circles represent individual data from the cancer group.

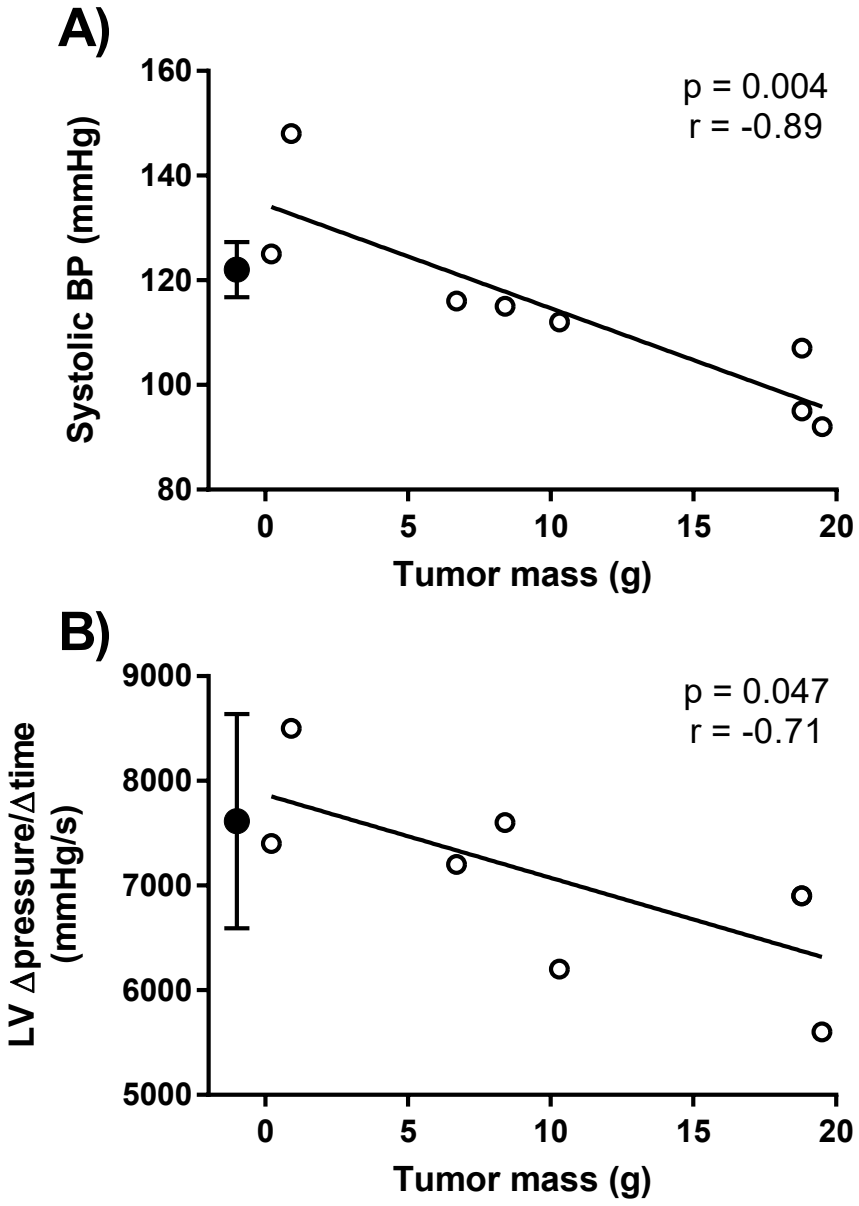
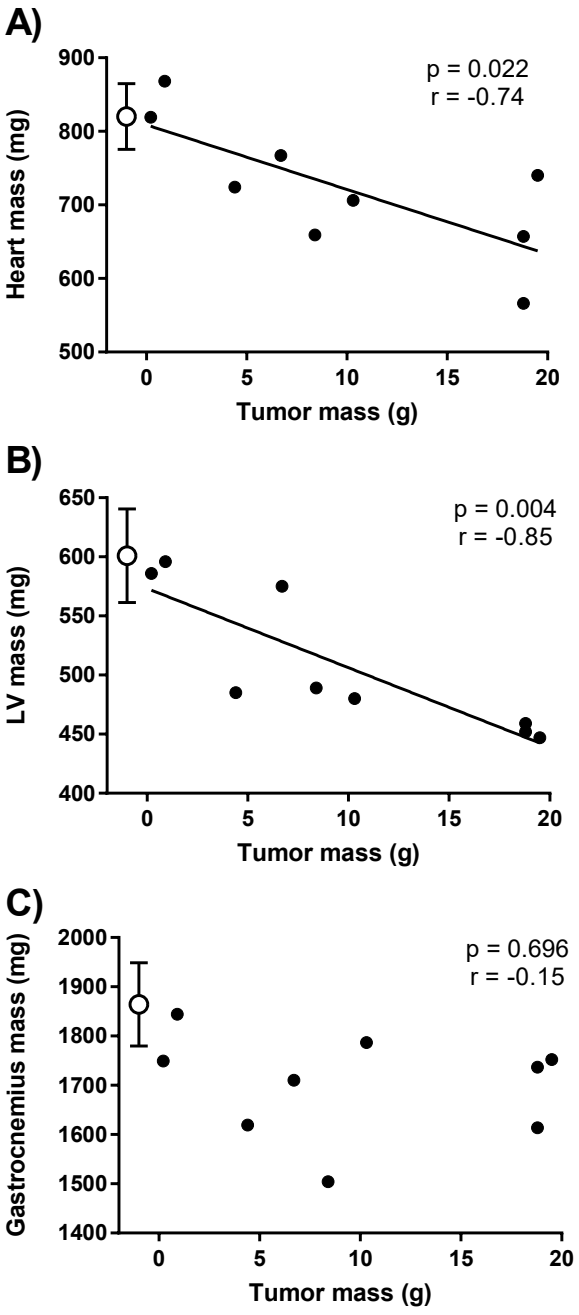


Figure 5. Correlations between Tumor Mass and Select Muscle Masses

Within the cancer group (n = 9), tumor mass was significantly inversely correlated to heart mass (A) and left ventricle (LV) mass (B), but not gastrocnemius mass (C). The open circles represent the mean and SEM of the sham group. The closed circles represent individual data from the cancer group.



Chapter 5 - Discussion

Consistent with our hypothesis we found that endurance exercise capacity was reduced in rats with prostate cancer, and this reduction was greater than that observed in rats without prostate cancer. Additionally, we found that the development of prostate cancer significantly reduced heart, LV, and gastrocnemius muscle mass in the cancer group compared to the sham group. In contrast, the development of prostate cancer did not reduce citrate synthase activity, an index of oxidative capacity, in the gastrocnemius, soleus, or costal diaphragm muscles. These findings are important because they are the first to show that prostate cancer itself reduces endurance exercise capacity.

We measured time to exhaustion using a standardized submaximal treadmill running protocol which has been shown to be reproducible within-rat for up to five weeks (Copp *et al.*, 2009). Unexpectedly, we found that time to exhaustion during the eight week test was decreased compared to the four week test in both the sham (↓32%) and cancer groups (↓45%). We calculated the work performed during the treadmill test to determine if the slightly greater increase in body mass could account for the reduced time to exhaustion in the sham group. That was not the case, however, because work performed was also reduced from eight weeks versus four weeks in both the sham (↓30%) and cancer (↓46%) groups. Because both the time to exhaustion and work performed during the endurance test were reduced from four weeks to eight weeks in the sham group, we cannot attribute the reductions in time to exhaustion and worked performed during this period in the cancer group entirely to the effects of prostate cancer. It appears, therefore, that the effect of prostate cancer on time to exhaustion and work performed in our study occurred primarily within the first four weeks following surgery.

The reason for the reduction in time to exhaustion and work performed in the sham group is unknown. A "learning" effect may have occurred in both groups following the second (or third in cases where repeated tests were necessary) endurance exercise tests such that the rats were not as motivated to run once sensations of fatigue were perceived. Regardless of the reason time to exhaustion and work performed during the endurance exercise tests decreased from eight weeks compared to four weeks in the sham group, the overall reductions in both time to exhaustion and work performed were significantly greater in the cancer group compared to the sham group. Importantly, the greater reductions in time to exhaustion and work performed in the cancer group are unlikely to be attribuatble to reductions in cage activity (and therefore deconditioning) because citrate synthase activity of the select hindlimb skeletal muscles investigated was not different between groups. If the development of prostate cancer had led to reduced spontaneous cage activity, we would have expected to see lower skeletal muscle citrate synthase activity levels in the cancer group.

Stroke volume is determined by the preload of the LV (LVEDP), afterload on the heart (systolic BP), and the contractility of the LV (LV Δpressure/Δtime). While we did not find any statistical differences between group means of these indexes in the present study, we did find that within the cancer group systolic BP and LV Δpressure/Δtime were significantly inversely correlated to tumor mass. Those inverse correlations between tumor mass and systolic BP and LV Δpressure/Δtime were found when the rats were anesthetized and the heart was not stressed which is consistent with the study of Springer *et al*. (2014) who found that stroke volume was reduced in a rat model of liver cancer compared to a sham group at rest. In our study, the fact that LV contractility was inversely correlated with tumor mass suggests to us that a reduced stroke volume likely contributed to the greater reductions in exercise capacity in the cancer

group compared to the sham group, particularly in the rats with the largest tumors. Future studies which examine the effects of prostate cancer on indexes of stroke volume during exercise are warranted.

Cachexia is defined clinically as a weight loss of at least 5% that is not due to edema occurring within a period of 12 months or less that is due to an underlying disease including cancer (Evans *et al.*, 2008) and cachexia has been estimated to affect 50-80% of cancer patients (Argilés *et al.*, 2014). In our study we found that heart, LV, and gastrocnemius muscle mass were lower in the cancer group compared to the sham group and this was true even when normalized to body mass (Table 1). Femur lengths were not different between groups, however, which suggests that prostate cancer did not affect the overall growth rate of the rats in the cancer group. Because body mass increased over the duration of the eight week protocol in both the cancer and sham groups we do not know if the lower heart, LV and gastrocnemius muscle masses in the cancer group reflect a true cachexia (i.e., a loss) or a growth retardation. Regardless, cachexia reflects an imbalance between the rate of protein synthesis and degradation (Murphy, 2016) and the lower cardiac and skeletal muscle mass found in our study indicates that such an imbalance existed within the cancer group.

We found that gastrocnemius muscle mass, but not soleus or EDL muscle mass, was lower in the cancer group compared to the sham group. The lower gastrocnemius mass in the cancer group compared to the sham group is similar to the finding of a Matsuyama *et al.* (2015) who reported lower gastrocnemius muscle mass in a murine model of colon cancer compared to control. Our finding that EDL mass was not different between groups is inconsistent with the finding of Gorselink *et al.* (2009) who reported that EDL mass was lower in tumor-bearing mice compared to non-tumor bearing mice. Taken together, these findings indicate that the type of

cancer, the host species, and/or muscle fiber-type composition may influence the presence of skeletal muscle cachexia. Our finding that heart and LV mass were lower in the cancer group compared to the sham group is consistent with studies that have reported lower heart mass in murine models of colon cancer (Matsuyama *et al.*, 2015) and liver cancer (Springer *et al.*, 2014) as well as in a subcutaneous colon cancer model (Wysong *et al.*, 2011). The fact that gastrocnemius mass was ~9% lower whereas LV mass was ~15% lower in the cancer group compared to the sham group in our study was surprising given the relative lack of information regarding the effects of various forms of cancer on cardiac cachexia compared to skeletal muscle cachexia (Murphy, 2016). Springer *et al.* (2014) found that cardiac muscle specifically was more impacted by catabolic stimuli than total body mass or total lean mass in a rat model of liver cancer. The significant inverse correlation between cardiac mass and tumor mass but not gastrocnemius mass and tumor mass in our study further supports the notion that prostate cancer had a greater effect on cardiac muscle mass than skeletal muscle mass. Despite this difference, however, the reduced gastrocnemius mass likely contributed to the reduced exercise capacity in the cancer group because more work per unit of muscle (thus increased recruitment) was performed in order for the rat to keep pace with the inclined treadmill. The lower heart and LV mass also likely contributed to the attenuated exercise capacity in the cancer group because a reduction in LV mass would result in a reduced stroke volume and therefore cardiac output during exercise.

There are two limitations of our study. First, the greatest effect of prostate cancer on endurance exercise capacity occurred within the first four weeks following surgery whereas the measurement of tumor, skeletal muscle and cardiac muscle mass was performed eight weeks following surgery. We chose eight weeks for the length of the study to ensure the rats would

develop substantial tumors. Valuable information would be gained from repeating this study and determining the effect of prostate cancer on skeletal and cardiac muscle mass four weeks following surgery. Second, we did not measure food consumption. The development of liver cancer has been shown reduce food intake compared to control (Springer *et al.*, 2014). In our study a reduction in food intake may have contributed to reduced protein synthesis, and therefore reduced cardiac and skeletal muscle mass in the cancer group compared to the sham group. However, as stated above the fact that femur length was not different between groups indicates that the overall growth rate of the cancer group was similar to the sham group and that the lower cardiac and skeletal muscle masses reflects an effect of prostate cancer itself.

In summary, endurance exercise capacity was reduced in rats with untreated prostate cancer to a greater extent than it was reduced in sham operated rats. Moreover, there were significant reductions in heart and LV mass in the cancer group compared to the sham group; within the cancer group, heart and LV mass were inversely correlated to tumor mass. The significant reduction in gastrocnemius muscle mass was not significantly inversely correlated with tumor mass. Although we did not investigate the mechanisms which specifically contributed to the reduced endurance exercise capacity in rats with prostate cancer the reduced cardiac and skeletal muscle mass likely contributed. Our findings have important implications for patients with prostate cancer given that the bulk of the available literature has focused on the effects of prostate cancer treatment (namely ADT) and its role in the exaggerated fatigue and reduced exercise capacity in this population.

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Appendix A - Data for Rats Excluded from Final Analysis

	No Tumor	Ectopic Tumor
Body mass (g)		
Initial body mass	282	269
8 week body mass	335	331
Endurance exercise capacity		
Initial time (min)	35.62	34.45
4 week time (min)	23.22	29.77
8 week time (min)	27.15	26.17
Δ pre-test 8 week time (min)	-8.47	-8.28
Initial work (joules)	3.03	2.74
4 week work (joules)	2.50	2.02
8 week work (joules)	2.40	2.53
Δ Initial 8 week work (joules)	-0.63	-0.21
Tissue mass (mg)		
Tumor mass	N/A	2.1
Heart	819	761
LV	595	590
RV	171	224
Soleus	162	159
EDL	150	155
Gastrocnemius	1783	1834
Citrate synthase activity (μmol/min/g)		
Soleus	22.1	17.5
Red gastrocnemius	29.5	29.1
White gastrocnemius	10.1	8.4
Costal diaphragm	30.3	34.8

Left ventricle (LV), right ventricle (RV), extensor digitorum longus (EDL), body mass (BM).