EFFECTS OF PROSTATE CANCER AND EXERCISE TRAINING ON LEFT VENTRICULAR FUNCTION AND CARDIAC AND SKELETAL MUSCLE MASS

Dryden R. Baumfalk1, Alexander B. Opoku-Acheampong1, Jacob T. Caldwell1, Carl J. Ade1,2, Steven W. Copp1, Timothy I. Musch1,3, Bradley J. Behnke1,2

1Department of Kinesiology, Kansas State University, Manhattan, KS 66506
2Johnson Cancer Research Center, Kansas State University, Manhattan, KS 66506
3Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506

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Corresponding author:

Dryden Baumfalk
Department of Kinesiology
Kansas State University
139A Justin Hall
Manhattan, KS 66506
Tel: 785-532-0766
E-mail: dryden32@ksu.edu
ABSTRACT

Prostate cancer was found to reduce cardiac and left ventricle (LV) masses in association with diminished exercise capacity in rats. We tested the hypothesis that exercise training will mitigate prostate cancer-induced cardiac and skeletal muscle atrophy and improve LV function versus sedentary tumor-bearing counterparts. **Methods:** Copenhagen rats (n=39; ~5 mo. old), randomized into four groups; exercise-trained tumor-bearing (EXTB) or control (EXCON) and sedentary tumor-bearing (SEDTB) or control (SEDCON). Dunning R-3327 prostate cancer cells were injected orthotopically in 19 of the 39 animals. Treadmill exercise training was performed for 60 min/day for ~30 days. Animals underwent echocardiography to examine ventricle dimensions pre-cancer injection or exercise (PRE) and 15 (Post 1) and 32-35 (Post 2) days post-cancer cell injection with tissues collected after Post 2. LV TNF-α and IL-6 concentrations were measured post-mortem. **Results:** Cardiac and LV mass of SEDTB animals were lower than all groups (p<0.05). Tumor mass was negatively correlated with LV mass in EXTB (-0.75, p<0.02) and SEDTB animals (-0.72, p<0.02). EXCON group had higher stroke volume Post 2 assessment compared to both sedentary groups (p<0.05), but not EXTB animals. No difference in LV [IL-6] or [TNF-α] were found between the cancer groups. **Conclusion:** The current investigation demonstrates prostate cancer, independent of anti-cancer treatment, significantly reduces cardiac mass, and LV mass as well as locomotor muscle masses. However, moderate intensity exercise training can mitigate cardiac and skeletal muscle atrophy with prostate cancer and preserve the cardiac phenotype (i.e., mass and function) to that of the healthy sedentary group.

**New & Noteworthy**

This study demonstrates the atrophic effects of prostate cancer on cardiac and skeletal muscle mass independent of anti-cancer treatment(s) that can be mitigated with moderate intensity exercise. These findings have important implications for potentially improving the quality of life as well as therapeutic outcomes for prostate cancer patients.
Introduction

Fatigue is one of the most common cancer-related symptoms leading to an inability to perform activities of daily living, resulting in reduced quality of life in over 50% of cancer patients (8, 26). Although mechanisms of fatigue with cancer are multifaceted, they are often attributed to the adverse effects of treatment, as fatigue is common in cancer patients both during and after treatment(s) (19, 39). Despite up to 40% of cancer patients reporting fatigue at the time of diagnosis (26), the effects of cancer solely on fatigue, and potential pathophysiological mechanisms, has received relatively scant attention. Given cancer-related fatigue can compromise the completion of anti-cancer treatment regimes, it is clinically important to understand how cancer, independent of any conventional treatment(s), affects determinants of exercise capacity (e.g., cardiac mass and function and potentially skeletal muscle mass).

In men, prostate cancer is the most frequently diagnosed non-skin cancer accounting for 20% of all new non-skin cancer cases in the United States (3). Upon diagnosis of prostate cancer, many patients receive pharmacological or surgical androgen deprivation therapy (ADT), which is associated with reductions in muscle mass and bone density (21, 72), and increased cardiovascular disease (16, 42), all of which can contribute to fatigue and frailty of patients (38). Other adjuvant therapies, such as radiation therapy or chemotherapy, can elicit and/or exacerbate cardiovascular dysfunction (16, 55). In the human cancer patient, it is difficult to delineate the mechanisms of fatigue from cancer versus adjuvant therapies, as it is unethical to withhold conventional treatment (e.g. chemotherapy and radiation therapy) to study the independent effects of cancer (10, 70). Thus, pre-clinical animal models, with and without cancer, are invaluable to investigate the tumor microenvironment and underlying mechanisms of discord (36, 77), and cancer-related fatigue independent of treatment (15). Recent evidence suggests
prostate cancer induces whole heart and left ventricle (LV) atrophy (4) which were associated with reduced endurance exercise capacity in rats (15). Exercise training in healthy populations can increase both cardiac and skeletal muscle mass (22) as well as potentially mitigate atrophy associated with cancer and anti-cancer therapies (11, 24, 74). With cancer, there are multiple beneficial effects of exercise within the tumor microenvironment including mitigation of tumor hypoxia (36, 75), increased infiltration of immune cells (75) and a shift towards vascular normalization (53) that may contribute to increased delivery of chemotherapeutic agents (53).

However, beyond the tumor microenvironment there is a dearth of information regarding effects of exercise training on cancer related cardiac atrophy and function, despite a significant portion of cancer patients presenting a cardiac comorbidity at diagnosis. Therefore, determining whether exercise training may mitigate cancer-induced cardiac atrophy, independent of treatment, is important to combat cancer-related cardiotoxicity associated with many adjuvant therapies.

Habitual exercise can decrease the morbidity and mortality of many diseases (22, 25) and is recognized as a fundamental component of cancer patient care programs (6, 24, 46) to attenuate complications, such as fatigue or loss of aerobic capacity (5, 56, 72). Despite exercise prescription, the beneficial effects of aerobic exercise training on heart function and structure in prostate cancer populations, independent of therapy, are limited (2, 5). Therefore, the purpose of the current set of investigations was to determine if aerobic exercise training (also referred to as aerobic exercise therapy) can prevent heart and skeletal muscle atrophy that has been previously shown across various types of cancer (2, 15, 39). Further, to determine whether prostate cancer, independent of treatment, impacts left ventricular mechanics and/or function. We hypothesized that prostate cancer-induced cardiac atrophy is mitigated with exercise training, and that left ventricular function (assessed with 2-D echocardiography) will be preserved in the exercise
trained tumor-bearing rat compared to its sedentary counterpart. Further, that exercise training will preserve locomotor skeletal muscle mass and oxidative capacity in an established pre-clinical model of prostate cancer. To investigate potential mechanisms of dysfunction within the heart, we measured two cytokines of which circulating concentrations have been shown to be impacted with both cancer and exercise; specifically, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Although there is a paucity of data on the effects of exercise training with cancer on the expression of these cytokines within heart tissue, based upon the diminished basal IL-6 production after exercise training (48, 18), we hypothesized that IL-6 concentrations within the left ventricle with cancer would be diminished with exercise training. Further, given the ability of regular exercise training to suppress TNF-α (for review see: Peterson and Pedersen, (49)) we hypothesized that, with prostate cancer, exercise training will reduce TNF-α concentrations within the left ventricle.

Methods

Animals

The procedures performed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee 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). Male immunocompetent Copenhagen rats (n=39, ~5 mo. Old; COP/CrCrl: Charles River Wilmington, MA) were used in this study. Animals were housed in a temperature-controlled room (23°C) on a 12:12-h light-dark cycle, with water and standard rat chow provided ad libitum.
Orthotopic Model of Cancer

The cell line utilized in this study was the Dunning R-3327 AT-1 strain of rat prostate adenocarcinoma cells, characterized by a high growth rate, low metastatic potential, and similar growth characteristics as human prostate cancer (28). AT-1 cells were cultured in RPMI-1640 media (GE Healthcare Life Sciences, Marlborough, MA) containing 10% fetal bovine serum (FBS; RMBIO, Missoula, MT), 2 mM L-glutamine (Fisher Scientific, Hampton, NH), 100 mM sodium pyruvate (Thermo Fisher Scientific, Waltham, MA), 1% penicillin/streptomycin (Thermo Fisher Scientific), and 0.025 mM dexamethasone (Cayman Chemical, Ann Arbor, MI) and incubated at 37˚C with 5% CO₂. Once cells reached ~80-90% confluence, a sample of the cells was counted via hemocytometer to calculate proper dilution (100,000 cells/ml) of the viable cells for a tumor cell stock solution placed in physiological salt solution (PSS). This solution was aliquoted such that each 0.1 ml increment contained ~1x10⁴ AT-1 cells. These methods have been used previously to induce orthotopic prostate tumors (23, 32).

In tumor-bearing (TB) rats, animals were anesthetized (2-5% isoflurane, O₂ balance) and a small incision of ~1 cm or less was made in the abdomen, lateral of the midline. Under aseptic conditions, the bladder/prostate complex was exposed, the ventral lobe of the prostate isolated, and 10⁴ AT-1 cells were injected in 0.1 ml physiological saline solution (pss) using a sterile 26G insulin syringe. To prevent leakage of cells to the tissue surrounding the prostate, a sterile cotton tipped applicator was placed alongside the needle during removal. Immediately following injection, the abdominal wall was closed with sterile 3–0, polyglycolic acid coated suture (DemeTECH, Miami Lakes, FL) and the overlying skin/fascia was closed with 3–0 nylon monofilament (DemeTECH, Miami Lakes, FL) and sealed with skin adhesive (3M, Vet-Bond). Rats were administered 0.05 mg/kg buprenorphine (Patterson Veterinary, Boone, IA) and 0.5
mg/kg acepromazine (Patterson Veterinary, Boone, IA) S. C. for analgesia and sedation, respectively, and isoflurane was withdrawn. Initial recovery from surgery was complete within 48 hours and daily postoperative monitoring of the animals was performed until animals were placed into sedentary or exercise trained groups ~7 days post-injection. As our main comparison was within cancer groups, non-manipulated control animals were used to compare cancer to healthy counterparts. There was no difference in the weight gained in time-matched control versus injected (i.e., tumor-bearing; TB) animals the week following surgery (change in 7-day weight; control, $3.5 \pm 0.6$ g, TB, $3.5 \pm 0.8$ g, $P>0.05$). Animals were separated into sedentary (SED) and exercise-trained (EX) groups, as described below, 7 days after cell injection and no difference in weight gained was observed within these groups during the week after injection (EXTB, $3.3 \pm 0.9$ g, SEDTB, $3.7 \pm 1.4$ g, $P>0.05$).

**Echocardiographic assessment of LV function**

Three transthoracic echocardiographic evaluations were performed with a commercially available 2D ultrasound system (Logiq S8; GE Medical Systems, Milwaukee, WI) with an 18 MHz linear transducer (L8-18i) by a trained sonographer. For primary analysis, the first evaluation “Pre” exercise training and/or cancer (Pre) was performed the day preceding tumor injection, and post cancer cell injection and/or exercise were performed 15 (Post 1) and 32-35 (Post 2) days after the onset of exercise training. The Post 1 measure reflects the acute cancer state (i.e., prior to any palpable tumors) whereas the Post 2 measure reflects an overt cancerous state with palpable tumors in all animals. All system settings and parameters used for echocardiographic evaluation remained unchanged throughout the experimental protocol for a given animal. Echocardiographic data were collected and stored on a local hard drive and analyzed using the manufacturer’s dedicated software for imaging analysis. For measures, rats
were anesthetized with 5% isoflurane/O\textsubscript{2} balance, placed on a heating pad (42°C) and maintained at 2% isoflurane/O\textsubscript{2} balance for the duration of the study to limit anesthesia effects on heart function (51, 63). Hair was removed from the sternum using a depilatory agent (Nair, Johnson &Johnson, New Brunswick, NJ) prior to any measurements. Two-dimensional guided M-mode images were obtained from parasternal short-axis views of the left (ventricle) LV at the level of the mitral leaflets in line with previous studies (13, 47). The following LV dimensions were measured: Left-ventricular end-diastolic (LVEDD) and end-systolic dimensions (LVESD) these values were used to estimate volumes using the Teichholz formula (67). LV posterior wall thicknesses (PWT) at end-diastole (PWT\textsubscript{D}) and end-systole (PWT\textsubscript{S}). Myocardial function (stroke volume, ejection fraction, fractional shortening) was evaluated using mean values from a minimum of three cardiac cycles that were used for analysis during each visit (65). A representative image collected for the non-invasive cardiac measures during systole and diastole is shown in Figure 1.

**Exercise Training**

All rats were habituated to treadmill exercise prior to Pre-measures, during which each rat walked on a motor-driven treadmill for ~ 5 min/day at 15 m/min (0° incline) for 3-5 days. Rats were then randomly assigned to an exercise-trained control (EXCON) (n=10), sedentary control (SEDCON) (n=10), exercise-trained tumor-bearing (EXTB) (n=10), and sedentary tumor-bearing (SEDTB) (n=9) groups. In exercise-trained assigned animals, after the habituation period and Pre-measures, the incline was raised to 15° for the duration of the training period while the 15 m/min speed was maintained. During the first 3 weeks of training, the time of exercise training was increased by 10 min every 3 days, until 60-min duration was reached by the 3rd week. The EXCON and EXTB rats continued to exercise 5 days/week for 60 min/day for
the remainder of the 32-35 day training period. This training program was adapted from McCullough et al. (36) to represent a moderate-intensity of exercise training, eliciting ~60-70% of maximal aerobic capacity response from animals of similar age and body mass, as previously described (40, 41). This moderate intensity of exercise was chosen, versus a higher intensity of exercise, as the former is well-tolerated by rats, and there is evidence that the latter may enhance tumor metastases (11). Both control and tumor-bearing rats underwent echocardiography and were euthanized after Post-2 measures were completed, with a minimum of 24h between the last bout of exercise for the exercise groups before Post 2 measures to avoid potential effects of acute exercise on reported measures. After the Post 2 ultrasound imaging, while under anesthesia (5% isoflurane, O₂ balance) all rats were euthanized by a thoracotomy followed by removal of the heart. Subsequently, the right ventricle was removed from the left ventricle and intraventricular septum, and the tumor, prostate (when delineation from tumor was possible), soleus muscle, plantaris muscle, gastrocnemius muscle (subdivided into red and white portions) were immediately excised, weighed, flash-frozen in liquid nitrogen, and stored at -80°C for future analyses. The right femur was removed, cleaned of connective tissue and remnant muscle, weighed and measured before being stored at -80°C.

**Sandwich enzyme-linked immunosorbent assay (ELISA) of Left Ventricle Tissue of IL-6 and TNF-α**

Left ventricle tissue sections of approximately 50 mg were homogenized in 5 volumes of phosphate buffer containing protease inhibitor cocktail (Abcam; Cat. # ab65621, Cambridge, UK) with all sample cytokine levels normalized to total protein content (10 µg) in each well. The products for ELISA determinations of their respective contents in LV tissue: interleukin-6 (IL-6) (Abcam; Cat. # ab100772, Lot # GR3209521-1, Cambridge, UK), and tumor necrosis factor
(TNF-α) (Abcam; Cat. # ab46070 Lot # GR3221546-2, Cambridge, UK). All assays were performed according to the manufacturer's instructions, with wells developed with tetramethylbenzidine and measured at 450 nm. The protein content of each well was quantified using a standard curve constructed with known amounts of protein. All samples were assayed in duplicate, and measurements are expressed as the mean values are expressed as pg/ug of protein loaded.

**Citrate Synthase Activity**

To determine training efficacy, and potential effects of cancer on muscle oxidative capacity, the soleus muscle and red portion of the gastrocnemius muscle were used for determination of citrate synthase activity. This mitochondrial enzyme is a marker of muscle oxidative potential and was analyzed according to the method of Sere (44). In brief, 15 µl and 30 µl samples were diluted using 210 µl and 195 µl of tris buffer, respectively. In addition, 15 µl of acetyl coenzyme A (Cayman Chemical, Ann Arbor, MI), and 30 µl of DTNB (Thermo Fisher Scientific, Waltham, MA) were added to each sample. Samples were incubated in a spectrophotometer (accuSkan GO; Fisher Scientific, Hampton, NH) for 5 min at 30°C before readings. Following incubation, readings were collected with the spectrophotometer at 412 nm once per minute for 5 min followed by the addition of 30 µl of oxalacetic acid (Sigma-Aldrich, St. Louis, MO) to all samples and immediately analyzed again. Citrate synthase enzyme activity is reported as μmol/min/g wet weight of sample tissue.

**Data Analysis**

Prism (version 7.4, Graphpad software, INC., La Jolla, CA) data analysis software was used for all statistical analyses. Statistical comparisons were made with either one-way repeated measure analysis of variance (ANOVA), or two-way repeated-measure ANOVA with Holm-
Sidak post hoc tests used as appropriate to assess statistical differences between groups for all measures. Within the two tumor-bearing groups, Pearson correlations and linear regression analyses were performed to quantify relationships between tumor mass and select tissues. A p≤0.05 was set for statistical significance with data reported as mean±SEM.

**Results**

Body mass increased in both sedentary (SEDCON and SEDTB) and exercise-trained (EXCON and EXTB) groups across the ~40-day period of experiments, with significant differences between control and tumor-bearing groups (Figure 2). However, there were no significant differences in weight between EX and SED for control or tumor-bearing groups at any time point (Figure 2). Tumor mass and tumor burden were not different between EXTB vs. SEDTB groups (Table 1). There were no differences in femur lengths across all groups (in mm, EXCON, 39.2 ± 0.09; SEDCON, 39.0 ± 0.11; EXTB, 39.10 ± 0.1; SEDTB= 39.0 ± 0.12, p>0.3).

**Echocardiographic Assessment of LV Function**

Left ventricle measures pre-to post-exercise intervention and/or cancer were used to analyze potential changes in heart function. There were differences found between groups for a number of measures of ventricular function over time, with all groups demonstrating similar baseline (i.e., Pre) parameters (Table 2). At Post 2, LVEDD, LVESD, and SV were higher in the EXCON rats compared to both SEDTB and SEDCON (Table 2), but not different versus that of the EXTB group (p>0.05). Longitudinal measures in the EXTB group appeared to trend upward for both LVEDD and SV from Pre to Post 2 (Table 2). There were minimal within group changes between Pre and Post 1, with the exception that EXCON group demonstrated significant increases in LVEDD and SV (Table 2). Posterior wall thickness in diastole and systole (PWT_D,
PWTS respectively) were unchanged for all groups Pre-Post 1 measures. With Pre to Post 2 measures, EXTB had significantly greater PWTD compared to SEDTB group (Table 2).

Cardiac and Skeletal Muscle Mass and Skeletal Muscle Citrate Synthase Activity

Absolute mass of the heart, LV, gastrocnemius muscle, soleus muscle, and plantaris muscle were all greater in the EXCON group versus both TB groups (Table 1). Heart and left ventricle masses were also greater in the EXCON compared to the SEDCON animals (Table 1). Cardiac tissue mass was normalized to body mass and femur length (FL, Table 1) to account for possible differences in growth between groups (78). When normalized to body mass or FL, significant differences were found between groups for the heart and LV (see Figure 3).

Compared to sedentary counterparts, EXTB had greater absolute heart and LV mass (Table 1) as well as when normalized to BW and FL (Figure 3). However, there was no significant difference in RV mass normalized to body mass or femur length between groups (Table 1). Skeletal muscle tissue mass was also normalized to both body mass and femur length with significant differences between EXCON and SEDCON when compared to the SEDTB for gastrocnemius, soleus, and plantaris muscles (Table 1).

Regression analysis of the combined tumor-bearing groups cardiac mass to tumor mass, and skeletal muscle mass to tumor mass, showed no initial regression (p>0.05). However, within the EXTB bearing group, heart mass, LV mass, and body mass were all negatively correlated with tumor mass whereas only LV mass was negatively correlated with tumor mass in the SEDTB (Figure 4). In the SEDTB group, there were no significant correlations between skeletal muscle mass and tumor mass (Figure 5). Contrastingly, in the EXTB group, there were significant negative correlations with tumor mass for both gastrocnemius mass and plantaris muscle mass, with a trend (p=0.09) in the soleus muscle (Figure 5).
Despite smaller absolute masses, prostate cancer did not affect citrate synthase activity of the locomotor skeletal muscles measured in the SEDTB versus SEDCON (Table 3). Skeletal muscle citrate synthase activity was greater in both exercise-trained groups versus sedentary counterparts, confirming the efficacy of the training program. There were no differences in skeletal muscle citrate synthase activity between exercise-trained groups (Table 3).

**Sandwich enzyme-linked immunosorbent assay (ELISA) of Left Ventricle Tissue**

Inflammatory markers tumor necrosis factor alpha (TNF-α) and interleukin six (IL-6) were measured post-exercise and/or cancer intervention in the left ventricle tissue of all groups using ELISA to compare concentrations of each, as shown in Figure 6 (A, B). The EXCON group had higher values for both TNF-α and IL-6 in LV muscle compared to other groups, with concentrations of TNF-α ~50% higher versus both SEDCON and SEDTB groups (and ~40% higher than the EXTB group. IL-6 had similar pattern with EXCON having values ~60% higher versus both EXTB and SEDTB groups and ~35% higher than the EXTB group (p< 0.006). Assay results had intra-assay variability of 4.5 and 4.1 percent for TNF-α and IL-6 respectively. Levels of TNF-α and IL-6 both were positively correlated (p< 0.01) with LV mass across all groups (Figure 6, D). In tumor-bearing animals only IL-6 concentration was positively correlated with tumor mass (Figure 6, E).

**Discussion**

Determination of the effects of prostate cancer on cardiac mass and function as well as skeletal muscle mass, and the ability of exercise training to mitigate the effects of cancer on these parameters is clinically important. The primary finding of the current investigation is the mitigation of significant atrophic effects of prostate cancer on the heart and skeletal muscle with exercise training. This is important as these results show the underlying effects of cancer,
independent of treatment. Specifically, with prostate cancer LV mass was negatively correlated
with prostate tumor mass, in both EXTB and SEDTB animals, to which there were no
differences between EXTB and SEDCON animals. These findings demonstrate the mitigating
effects of aerobic exercise on cancer-related cardiac atrophy. Hence, cancer-related cardiac
atrophy is likely an underlying potential cause of fatigue which may be further exacerbated by
ADT or adjuvant therapies (13, 63).

Prostate Cancer and Atrophy

The study of prostate cancer in the absence of treatment is clinically important to
understanding the underlying mechanisms of the disease in patients prior to treatment. Both
atrophy and cachexia from various forms of cancer may occur in pre-clinical animal models (15,
45, 77) and humans (7, 39). Although the tumor-bearing animals in the current study were not
cachexic (5% loss of body mass without signs of edema, (19)) there was significant atrophy of
the heart, LV, and skeletal muscle in the sedentary group. There are several potential
mechanisms of atrophy that may have occurred between groups through necrotic and/or
apoptotic pathways, as discussed in recent reviews (12, 61). For example, Forkhead boxO
(FoxO) expression in muscle increases with cancer and has been implicated as an important
signaling pathway involved in cancer-induced skeletal muscle atrophy (29, 31). In cardiac tissue
two different E3 ligases i.e., atrogin-1/muscle atrophy F-box(MAFbx), and muscle ring finger-1
(MuRF-1), (both of which are upregulated via FoxO) are implicated in cancer-related cardiac
atrophy (1, 39, 80). Further, a reduction in spontaneous physical activity of the SEDTB group
could also induce muscle atrophy through disuse. Although spontaneous activity was not
measured in the current study, there were no differences in locomotor muscle oxidative capacity
between the SEDCON and SEDTB groups (Table 3), suggesting gross alterations in spontaneous physical activity between sedentary groups was not present (17).

Aerobic Exercise Training and Atrophy

Aerobic exercise training is known to be efficacious in increasing LV mass in health as well as multiple disease states (2, 20, 22). Hence, the elevated cardiac masses normalized to both body weight and FL were expected in the EXCON group after training, consistent with previous research (43, 73, 76). The increase in cardiac mass observed in the EXCON group was not as prominent in the EXTB animals, which may be due to the aforementioned mechanisms of cardiac atrophy with cancer. However, with exercise training the tumor-bearing group was able to demonstrate positive adaptations, or prevent maladaptation, and maintain values comparable to the sedentary, healthy control animals (e.g., heart-mass/FL ratio, LV-mass/FL ratio). Further, with exercise training and cancer, there was both increased LV hypertrophy and wall thickness, as well as an increased SV versus the SEDTB counterparts. The lower LVEDD (Table 2.) and LV mass (Figure 3) of the SEDTB group was likely responsible for the lower SV versus the EXTB and EXCON animals.

Cardiac atrophy is associated with reduced time to exhaustion in pre-clinical prostate cancer models (15), and could be attributable to modifications in the ubiquitin proteasome system (UPS) (45, 71). There is considerable evidence that both acute and regular exercise affect circulating cytokine levels and provide a mitigating effect on multiple pathways of muscle wasting (81). For example, the elegant work of Bente Pederson and others have demonstrated increases in circulating IL-6 from the contracting skeletal muscle during acute exercise, considering this myokine an ‘exercise factor’ (48) which may have beneficial effects on multiple tissues including attenuating TNF-α production. Further, although basal IL-6 levels decrease
with regular exercise training, TNF-α levels are attenuated from presumably both IL-6 dependent and independent mechanisms (for review see: Peterson and Pedersen, [49]). Given the role these cytokines play in skeletal muscle atrophy and muscle wasting in cachectic patients [81], it was hypothesized that exercise training would reduce both IL-6 and TNF-α concentrations in the left ventricle of the tumor-bearing animals versus their sedentary counterparts. Exercise training has also been demonstrated to prevent rises in heart and skeletal muscle FoxO levels during doxorubicin treatment [31], and reduces the expression of MAFbx and Murph-1 in heart failure (1). These findings were hypothesized to be due to the chronic attenuation of inflammatory markers, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) [1, 2]. Further, Padrão and colleagues [45] demonstrated that exercise training can mitigate cardiac cachexia and remodeling in a pre-clinical model of urothelial carcinoma [45] with several potential mechanisms by which exercise training may mitigate cancer-induced cachexia speculated.

Contrary to our hypotheses, exercise training did not alter LV [IL-6] or [TNF-α] in the cancer groups. Interestingly, [IL-6] was only elevated in the EXCON group. Importantly, all measures were performed at least 24h after the last bout of exercise to avoid any potential transient changes in these cytokines during exercise. There is scant data on IL-6 within the heart with training in healthy subjects, but our data is not consistent with Serra et al who found no change in myocardial IL-6 or TNF-α protein expression in exercise training [58]. Importantly, Serra and colleagues did not observe any training induced hypertrophy of the heart (i.e., no change in HW/BW, LV/BW, or RV/BW [58]), whereas with our more intense training protocol increased these parameters in the exercise training groups.

Interestingly, we found a significant positive correlation between LV mass and concentration of both TNF-α and IL-6 across groups. Previous research has demonstrated that
TNF-α induces a hypertrophic stimulus in adult cardiac myocytes (79), and is associated with myocardial hypertrophy (6), albeit typically associated with pathophysiological conditions (e.g. heart failure: (6, 33). Conversely, the elevated IL-6 levels in this study are not necessary for cardiac hypertrophy (30). Given IL-6 has both pro-and-anti-inflammatory properties (54) it is possible that IL-6 in the cardiac myocytes is acting as an anti-inflammatory myokine to offset, in a paracrine manner, negative pro-inflammatory effects of TNF-α (58). A recently meta-analysis demonstrates the high variability in [TNF-α] responses to exercise training (59). Thus, the lower levels of TNF-α in the tumor bearing groups compared to the exercise control group does not rule out the ubiquitin proteasome system being a possible major contributor to apoptosis. Although TNF-α has previously been associated with the UPS and cardiac cachexia (1, 61, 66, 69, 80), given the multifaceted pathways that can upregulate the UPS (61) it may not be as prevalent with atrophy occurring with prostate cancer.

Left Ventricular Function in Prostate Cancer and Exercise

In contrast to our hypothesis, there were no changes in many parameters from the non-invasive measures of cardiac function and mechanics in the EXTB versus SEDTB groups, despite significant differences in heart and LV mass between groups. Echocardiography evaluation of LV function has been a valuable tool in both clinical and pre-clinical investigations due to its non-invasive nature and ability to track within subject longitudinal changes in both animals, (9, 13, 35, 47, 64) and humans (27, 60). Non-invasively measured SV was elevated in the EXCON and EXTB animals versus their corresponding sedentary groups, which was likely a function of the larger LVEDD (Table 2.) and LV mass (Figure 3). It is important to note that the measures of LV function herein reflect a basal, non-stressed condition, from which subtle impairments in function may not be detected. However, there is clear evidence that endurance
capacity is decreased with prostate cancer associated with cardiac atrophy (15), and that any manifestation of altered mechanics should be investigated during exercise.

Limitations

Several limitations from this study should be addressed. Food consumption was not measured and could lead to decreased levels of protein synthesis, and ultimately contribute to the cardiac or skeletal muscles atrophy in the tumor-bearing groups compared to controls (62, 68). However, the continued increase in mass (including non-tumor mass of all groups) and that femur length was not different between groups suggests growth rates of the cancer group were of similar proportion to the control groups indicating normal growth patterns (57). Although the difference in average tumor weights was ~30% between groups, this was not significant and reflects a difference in tumor burden (tumor mass:body mass) of less than 1% (Table 1). This similar tumor-burden coupled with the orthotopic model of cancer (vs. ectopic models) also strengthens the translational aspects of prostate cancer effects on the heart and skeletal muscles as the tumor is matched to its host tissue (i.e., prostate tumor in prostate) (32). This is particularly important with exercise as the orthotopic model also mitigates possible confounding differences versus ectopic models on site-specific tumor blood flow with exercise, as previously shown (23). Estimations of volume of the left ventricle using the Teicholz formula (67) had minor differences due to the assumptions from one axis used in this study and, thus, dimensions were primarily used for statistical analysis. Given the primary comparison in the current study was between the tumor-bearing cancer groups, we chose to use non-manipulated animals as controls to compare cancer to healthy subjects. Although prostate blood flow is identical between non-manipulated (50) and sham-operated (37), we cannot rule out any adverse effects of the surgery on outcomes. It should be noted that weight gain for the initial seven days after
injection of the cells was identical in the tumor-bearing versus control groups (see methods).

Lastly, the length of time and exercise modality requisite to induce significant increases in cardiac structure and function are debated in healthy humans as well as in clinical and pre-clinical studies (52, 74, 76). From animal studies, the ideal length of training is typically 6-8 weeks of constant load moderate intensity exercise to induce an exercise phenotype (34). Due to the growth rate of these cancer cells, the entire duration of the study could not be extended beyond 5-6 weeks due to potential tumor size limitation and ethical treatment of the animals. Hence, a longer period of training may have been needed to induce functional and mechanical changes in the LV between the EXTB and SEDTB groups.

Conclusions

In summary, this investigation demonstrates that prostate cancer, independent of any adjuvant therapy, in an orthotopic prostate tumor model induces atrophy of cardiac, and select locomotor skeletal muscles. Furthermore, these reductions in muscle mass were negatively correlated with tumor mass, primarily among the EXTB animals. Although there are multiple potential contributing mechanisms to these reductions in muscle masses with cancer in tumor-bearing animals, exercise training mitigates gross changes in cardiac mass, and may benefit the patient and further support the importance of including exercise as a fundamental component of cancer patient care. Information garnered herein provide further insights into possible mechanisms of fatigue occurring in cancer patients that could be exacerbated by concomitant treatment. Given the increasing acknowledgement that different cardiac phenotypes (i.e., culmination of echocardiographic structural and functional data) are associated with distinct clinical outcomes (14), we also demonstrate that moderate-intensity exercise in the prostate cancer subject can maintain a relatively healthy LV phenotype (i.e. not different than the healthy,
non-manipulated sedentary group). Thus, this study demonstrates additional positive benefits to adopting an exercise regime after the diagnosis of prostate cancer. Lastly, the levels of TNF-α found in the left ventricle tissue were inconsistent with previous literature that indicated possible contributions from the UPS, with the present investigation promotes further investigation into the apoptotic mechanisms by which prostate cancer independently reduces cardiac and locomotor skeletal muscle mass, and further the role of exercise in the attenuation of aforementioned reductions.
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Conflicts of Interest: No conflicts of interest Financial or otherwise, are declared by the author(s).
Figure Captions

Figure 1. Representative echocardiography images
A representative 2D image of the rat heart at the level of the papillary muscle in diastole (top) and systole (bottom) are presented. The bottom panel has the analysis of the left ventricle dimensions as follows; 1= end-diastolic dimension of left ventricle, 2= end-diastolic posterior wall thickness, 3= end-systolic dimension of left ventricle, 4= end-systolic posterior wall thickness, 5= time between end-diastole to end-systole.

Figure 2. Change in body mass
Body mass for both exercise and sedentary control groups were significantly higher (#, p<0.05 than both tumor bearing-groups, but not between groups. Tumor-bearing groups had no significant differences in body mass between groups. Both Control and Tumor-bearing groups had significant increases in mass from Pre-Post 2 (*, p<0.05).

Figure 3. Cardiac Muscle
The heart, left ventricle (LV), and right ventricle (RV) were compared between exercise and sedentary control (EXCON, SEDCON) and tumor bearing groups (EXTB, SEDTB) (Two way-ANOVA and Holm-Sidak post hoc tests). #, p<0.05 vs. SEDTB; *, p<0.05 vs. EXTB; $, p<0.05 vs SEDCON.

Figure 4. Cardiac Muscle Correlation
Within the tumor-bearing groups (closed circles) tumor mass was negatively correlated with heart mass (B), left ventricle mass (D), and body mass (F) in the exercise-tumor-bearing group (EXTB). However, within the sedentary group only left ventricle mass was negatively correlated with tumor mass (C). There were no significant correlations between body mass (E) or heart mass (A) vs tumor mass for SEDTB animals. The sedentary and exercise control groups are presented as open circles representing the mean and SEM (n=10) for each group, of which are
shown only for comparison purposes, and are not factored into the correlation or regression calculations.

**Figure 5. Skeletal Muscle Correlation**

Within the tumor bearing groups (closed circles) tumor mass was negatively correlated with gastrocnemius mass (B), plantaris mass (F), and a trend (P=0.09) for soleus mass (D) in the exercise-trained tumor-bearing group (EXTB). The Sedentary and Exercise control groups are presented as open circles representing the mean and SEM (n=10) for each group, of which are shown only for comparison purposes and are not factored into the correlation or regression calculations.

**Figure 6. Elisa Results**

Within the tumor bearing groups, tumor necrosis factor alpha (TNF-α, A) and interleukin 6 (IL-6, B) were elevated in the exercise control group compared to the other three groups (total n=38, one excluded animal as statistical outlier.) in left ventricle tissue; #, p<0.05 vs. SEDTB; *, p<0.05 vs. EXTB; $, p<0.05 vs SEDCON. With all groups, ventricle mass was positively correlated with elevated levels of both TNF-α (C) and IL-6 (D). Within the both tumor bearing groups, tumor mass was positively correlated with increases in IL-6 (F), but not with TNF-α (E).
Figure 1.
Figure 2. Change in Body Mass

![Bar graph showing change in body mass over time for different groups.](image-url)
Figure 3. Cardiac Muscle

A. Heart mass/Body mass (mg/g)

B. Heart mass/Femur length mg/mm

C. Left ventricle/Body mass (mg/g)

D. Left ventricle/Femur length mg/mm

E. Right ventricle/Body mass (mg/g)

F. Right ventricle/Femur length mg/mm
Figure 4. Cardiac Muscle Correlations

A. SEDCON vs. SEDTB

\[ p = 0.52 \]
\[ r = -0.24 \]
\[ r^2 = 0.06 \]

B. EXCON vs. EXTB

\[ p < 0.04 \]
\[ r = -0.68 \]
\[ r^2 = 0.51 \]

C. SEDCON vs. SEDTB

\[ p < 0.02 \]
\[ r = -0.75 \]
\[ r^2 = 0.56 \]

D. EXCON vs. EXTB

\[ p < 0.02 \]
\[ r = -0.72 \]
\[ r^2 = 0.53 \]

E. SEDCON vs. SEDTB

\[ p = 0.47 \]
\[ r = -0.27 \]
\[ r^2 = 0.07 \]

F. EXCON vs. EXTB

\[ p < 0.04 \]
\[ r = -0.67 \]
\[ r^2 = 0.45 \]
Figure 5. Skeletal Muscle Correlations

A. SEDCON
   SEDTB
   \( p < 0.02 \)
   \( r = -0.65 \)
   \( r^2 = 0.43 \)

B. EXCON
   EXTB
   \( p = 0.98 \)
   \( r = 0.01 \)
   \( r^2 < 0.001 \)

C. SEDCON
   SEDTB
   \( p = 0.61 \)
   \( r = 0.19 \)
   \( r^2 = 0.04 \)

D. EXCON
   EXTB
   \( p = 0.09 \)
   \( r = -0.55 \)
   \( r^2 = 0.30 \)

E. SEDCON
   SEDTB
   \( p = 0.78 \)
   \( r = -0.39 \)
   \( r^2 = 0.01 \)

F. EXCON
   EXTB
   \( p = 0.01 \)
   \( r = -0.76 \)
   \( r^2 = 0.58 \)
Figure 6. ELISA Results

A. TNF-α Concentration (pg/ug)

B. IL-6 Concentration (pg/ug)

C. LV mass (g) vs TNF-α (pg/ug)

D. LV mass (g) vs IL-6 (pg/ug)

E. Tumor mass (g) vs TNF-α (pg/ug)

F. Tumor mass (g) vs IL-6 (pg/ug)
Table 1. Cardiac and muscle mass characteristics

<table>
<thead>
<tr>
<th></th>
<th>Exercise Control (n=10)</th>
<th>Sedentary Control (n=10)</th>
<th>Exercise Tumor-Bearing (n=10)</th>
<th>Sedentary Tumor-Bearing (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Absolute Mass (g)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Heart</td>
<td>0.95±0.01&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.81±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.79±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64±0.01</td>
</tr>
<tr>
<td>Left Ventricle</td>
<td>0.73±0.02&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.62±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.58±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47±0.01</td>
</tr>
<tr>
<td>Right Ventricle</td>
<td>0.22±0.01</td>
<td>0.20±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21±0.01</td>
<td>0.18±0.02</td>
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<tr>
<td>Gastrocnemius</td>
<td>2.33±0.05&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>2.09±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.88±0.07</td>
<td>1.78±0.07</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.16±0.003&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.15±0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14±0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12±0.003</td>
</tr>
<tr>
<td>Plantaris</td>
<td>0.28±0.004&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.26±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21±0.01</td>
</tr>
<tr>
<td><strong>Muscle Mass Normalized to</strong></td>
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<tr>
<td><strong>Body Mass (mg/g)</strong></td>
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<tr>
<td>Gastrocnemius/Body mass</td>
<td>6.7±0.1</td>
<td>6.2±0.3</td>
<td>6.3±0.2</td>
<td>6.3±0.2</td>
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<td>Soleus/Body mass</td>
<td>0.44±0.01</td>
<td>0.43±0.01</td>
<td>0.46±0.01</td>
<td>0.43±0.02</td>
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<tr>
<td>Plantaris/ Body mass</td>
<td>0.80±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.81±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.82±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.74±0.02</td>
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<td><strong>Tumor/Body mass (%)</strong></td>
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<td>-</td>
<td>3.0±0.5</td>
<td>2.5±0.4</td>
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<td><strong>Mass Normalized to Femur</strong></td>
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<tr>
<td><strong>Length (FL) (mg/mm)</strong></td>
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<tr>
<td>Gastrocnemius/ FL</td>
<td>59.7±1.1&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>53.8±2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.3±1.7</td>
<td>45.7±1.8</td>
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<td>Soleus/ FL</td>
<td>3.9±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6±0.1&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Plantaris/ FL</td>
<td>7.1±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.8±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.3±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.4±0.2</td>
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Abbreviations: FL, Femur Length
Data are mean±SEM and were compared with Two-way ANOVA.
a= p<0.05 vs. Sedentary Tumor-Bearing
b= p<0.05 vs. Sedentary Control
c= p<0.05 vs. Exercise Tumor-Bearing
e= p≤0.10 vs. Sedentary Tumor-Bearing
Table 2. Echocardiographic measures

<table>
<thead>
<tr>
<th>Left Ventricle Measures</th>
<th>Exercise</th>
<th>Sedentary</th>
<th>Exercise</th>
<th>Sedentary</th>
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<td>Control</td>
<td>Control</td>
<td>Tumor-Bearing</td>
<td>Tumor-Bearing</td>
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<td></td>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=9)</td>
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<tr>
<td>Pre-Measure</td>
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<tr>
<td>LVEDD (cm)</td>
<td>0.71±0.01</td>
<td>0.72±0.02</td>
<td>0.72±0.02</td>
<td>0.70±0.02</td>
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<tr>
<td>LVESD (cm)</td>
<td>0.45±0.02</td>
<td>0.42±0.02</td>
<td>0.44±0.01</td>
<td>0.41±0.02</td>
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<tr>
<td>LVEDV Teich (ml)</td>
<td>0.83±0.04</td>
<td>0.85±0.07</td>
<td>0.83±0.03</td>
<td>0.79±0.05</td>
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<td>LVESV Teich (ml)</td>
<td>0.23±0.03</td>
<td>0.19±0.02</td>
<td>0.21±0.02</td>
<td>0.18±0.02</td>
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<td>PWT_D (cm)</td>
<td>0.15±0.02</td>
<td>0.13±0.01</td>
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<td>PWT_S (cm)</td>
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<td>0.24±0.01</td>
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<td>0.23±0.01</td>
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<tr>
<td>SV (ml)</td>
<td>0.60±0.02</td>
<td>0.65±0.06</td>
<td>0.61±0.03</td>
<td>0.60±0.06</td>
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<tr>
<td>FS (%)</td>
<td>37.4±2.1</td>
<td>41.8±1.5</td>
<td>39.1±1.3</td>
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<tr>
<td>EF (%)</td>
<td>72.5±2.3</td>
<td>77.8±1.7</td>
<td>74.8±1.5</td>
<td>76.9±2.2</td>
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<tr>
<td>Post 1 Measure</td>
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<tr>
<td>LVEDD (cm)</td>
<td>0.77±0.02</td>
<td>0.75±0.02</td>
<td>0.74±0.02</td>
<td>0.72±0.01</td>
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<tr>
<td>LVESD (cm)</td>
<td>0.46±0.01</td>
<td>0.47±0.02</td>
<td>0.46±0.02</td>
<td>0.47±0.01</td>
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<tr>
<td>LVEDV Teich (ml)</td>
<td>1.00±0.05</td>
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<td>0.84±0.02</td>
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<tr>
<td>LVESV Teich (ml)</td>
<td>0.25±0.03</td>
<td>0.25±0.03</td>
<td>0.23±0.02</td>
<td>0.26±0.02</td>
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<tr>
<td>PWT_D (cm)</td>
<td>0.14±0.01</td>
<td>0.14±0.01</td>
<td>0.13±0.02</td>
<td>0.14±0.01</td>
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<tr>
<td>PWT_S (cm)</td>
<td>0.24±0.01</td>
<td>0.24±1.5</td>
<td>0.23±1.4</td>
<td>0.21±0.01</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>0.73±0.03</td>
<td>0.70±0.03</td>
<td>0.68±0.05</td>
<td>0.59±0.01</td>
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<tr>
<td>FS (%)</td>
<td>40.6±2.2</td>
<td>37.8±1.5</td>
<td>39.2±1.4</td>
<td>35.0±1.2</td>
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<tr>
<td>EF (%)</td>
<td>76.1±2.2</td>
<td>73.2±1.7</td>
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<td>69.8±1.5</td>
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<td>Post 2 Measure</td>
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<td>LVEDD (cm)</td>
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<td>LVESD (cm)</td>
<td>0.50±0.02</td>
<td>0.43±0.02</td>
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<td>LVEDV Teich (ml)</td>
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<td>0.93±0.05</td>
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<td>LVESV Teich (ml)</td>
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<td>0.21±0.02</td>
<td>0.24±0.02</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td>PWT_D (cm)</td>
<td>0.16±0.01</td>
<td>0.13±0.01</td>
<td>0.17±0.03</td>
<td>0.13±0.01</td>
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<tr>
<td>PWT_S (cm)</td>
<td>0.25±0.01</td>
<td>0.24±0.01</td>
<td>0.25±0.02</td>
<td>0.23±0.02</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>0.78±0.04</td>
<td>0.65±0.05</td>
<td>0.69±0.03</td>
<td>0.58±0.03</td>
</tr>
<tr>
<td>FS (%)</td>
<td>35.4±1.3</td>
<td>40.1±1.6</td>
<td>38.5±1.4</td>
<td>38.4±2.5</td>
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<tr>
<td>EF (%)</td>
<td>70.0±1.2</td>
<td>75.9±1.9</td>
<td>74.1±1.7</td>
<td>73.7±2.5</td>
</tr>
</tbody>
</table>

Abbreviations: LVEDD, left ventricle end-diastolic dimension; LVESD, left ventricle end-systolic dimension; PWT_D, posterior wall thickness end-diastole; PWT_S, posterior wall thickness end-systole SV, Stroke volume; FS, fractional shortening; EF, ejection fraction; v/s, velocity or radial shortening per second.

Data are mean±SEM and were compared with Two-way ANOVA.
SV Post 2 was compared with unpaired Student’s t-tests
a= p<0.05 vs. Sedentary Tumor-Bearing
b= p<0.05 vs. Sedentary Control
c= p≤0.10 vs. Exercise Tumor-Bearing
d= p≤0.10 vs. Sedentary Control
e= p≤0.10 vs. Sedentary Tumor-Bearing
f= p<0.05 Pre vs. Post 1
g= p<0.05 Pre vs. Post 2
Table 3. *Skeletal Muscle Citrate Synthase Activity (µmol/min/g)*

<table>
<thead>
<tr>
<th></th>
<th>Exercise Control (n=9)</th>
<th>Sedentary Control (n=9)</th>
<th>Exercise Tumor-Bearing (n=9)</th>
<th>Sedentary Tumor-bearing (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soleus</strong></td>
<td>24.7±1.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>15.5±1.5</td>
<td>25.4±1.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.9±1.9</td>
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<tr>
<td><strong>Red gastrocnemius</strong></td>
<td>33.8±1.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>26.3±1.5</td>
<td>35.9±1.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>23.6±1.0</td>
</tr>
</tbody>
</table>

Mean±SEM and were compared with Two-way ANOVA.

a = p<0.05 vs. Sedentary Tumor-Bearing
b = p<0.05 vs. Sedentary Control