# Effects of Prostate Cancer and Exercise Training on Left Ventricular Function and Cardiac and Skeletal Muscle Mass

by

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# Abstract

Prostate cancer is the most common type of non-skin cancer found in men and preliminary evidence suggests prostate cancer has atrophic effects on cardiac and left ventricle (LV) mass which are associated with reduced endurance exercise capacity in rats. Using a pre-clinical orthotopic model of prostate cancer, echocardiography was utilized to test 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:** Dunning R-3327 AT-1 prostate cancer cells were injected orthotopically in male Copenhagen rats aged (n=39; ~5 mo. old). Animals were randomized into four groups, exercise-trained tumor-bearing (EXTB) or control (EXCON) and sedentary tumor-bearing (SEDTB), or control (SEDCON). Exercise training was performed via a rodent treadmill set at 15m/min with a $15^{o}$ incline for 60 min/day for ~30 days. Animals underwent echocardiographic evaluation using the parasternal short axis view to examine ventricle dimensions pre-cancer or exercise (PRE) and 15 (Post 1) and 30 (Post 2) days post cancer cell injection and/or exercise training with tissues collected immediately after Post 2. **Results:** Cardiac and LV mass of SEDTB animals were significantly lower than all groups ($p<0.05$). Tumor mass was significantly negatively correlated with LV mass in EXTB (-0.75, $p<0.02$) and SEDTB animals (-0.72, $p<0.02$). EXCON group had significantly higher stroke volume Post 2 assessment compared to both sedentary groups ($p<0.05$), but not EXTB animals. **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.

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# Acknowledgements

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# Dedication

For their confidence and support in my endeavors, I would like to dedicate this thesis to my family, especially to my parents, Gary and Yvonne Baumfalk.

# Chapter 1 - Introduction

Cancer-related 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 (9, 24). 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) (18, 34). Despite up to 40% of cancer patients reporting fatigue at the time of diagnosis (24), 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 known cardiovascular parameters of exercise capacity (e.g., cardiac function).

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 (19, 57), and increased cardiovascular disease (37). All of which can contribute to fatigue and frailty of patients (33). Other adjuvant therapies, such as radiation therapy or chemotherapy, also can elicit and/or exacerbate cardiovascular dysfunction (16, 45). In the human cancer patient, it is difficult to delineate the mechanisms of fatigue from cancer versus adjuvant therapies, in *seperatum*, as it is unethical to withhold treatment to study the independent effects of cancer (11, 56). Thus, pre-clinical animal models, with and without cancer, are invaluable to investigate the tumor microenvironment and underlying mechanisms (32, 61), and cancer-related fatigue independent of treatment (15). Recent evidence suggests prostate cancer induces whole heart and left

ventricle (LV) atrophy (5) which were associated with reduced endurance exercise capacity in rats (15). Therefore, determining whether exercise training may mitigate cancer induced cardiac atrophy independent of treatment is important to potentially combat cancer-related fatigue and potentially reduce the cardiotoxicity associated with many adjuvant therapies.

Habitual exercise can decrease the morbidity and mortality of many diseases (20, 23) and is recognized as a fundamental component of cancer patient care programs (7, 22, 41) to attenuate complications, such as fatigue or loss of aerobic capacity (6, 46, 57) associated with adjuvant therapies. 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, 6,). Therefore, the purpose of the current set of investigations was to determine whether prostate cancer, independent of treatment, impacts left ventricular mechanics and/or function, and 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 forms of cancer (2, 15, 34). 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, versus sedentary counterparts, in an established pre-clinical model of prostate cancer.

# Chapter 2 - 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-hr 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 (26). 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% $CO_2$. Once cells reached ~80-90% confluence, a sample of the cells were 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 ~$10^4$ AT-1 cells**.** These methods have been used previously to induce orthotopic prostate tumors (21, 32).

In tumor-bearing (TB) rats, animals were anesthetized (2-5% isoflurane, $O_2$ balance) and a small incision of ~ 1cm 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 $10^4$ AT-1 cells were injected 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 0.05mg/kg buprenorphine (Patterson Veterinary, Boone, IA) and 0.5mg/kg S.C. acepromazine (Patterson Veterinary, Boone, IA) for analgesia and sedation, respectively, and isoflurane was withdrawn. Daily postoperative monitoring of the animals was performed until animals were placed into sedentary or exercise trained groups ~7 days post-injection.

*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 18MHz 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 was 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/$O_2$ balance, placed on a heating pad (42 °C) and maintained at 2% isoflurane/ $O_2$ balance for the duration of the study to limit anesthesia effects on heart function (43, 50). Hair was removed from the sternum using a depilatory agent 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 (14, 42). The following LV dimensions were measured: Left-ventricular end-diastolic and end-systolic dimensions (LVEDD, LVESD), and LV posterior wall thicknesses (PWT) at end-diastole and end-systole (PWS, PWD). These values were used in the calculation of volume using the Teichholz formula (53) specifically; left ventricular diastolic and systolic volumes [LVDV, LVSV= $(7/2.4+D)*D^3$), stroke volume (SV; SV= LVDV-LVSV), fractional shortening (FS; FS = [(LVEDD-LVESD)/LVEDD] × 100), and ejection fraction (EF) (EF; EF= [(LVDV-LVSV)/LVDV] × 100). Left Ventricle radial strain (deformation) and strain rate (deformation/Δ time) were derived from two-dimensional parasternal short axis using tissue Doppler imaging data as previously described (4). Myocardial function was evaluated using FS, EF, peak systolic strain, and peak systolic strain rate values with the mean values from a minimum of three cardiac cycles were used for analysis during each visit (52). A representative image collected for the non-invasive cardiac measures during systole and diastole is shown in Figure 1.

*Exercise Training*

Rats were 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. Animal exercise training began by habituation to

treadmill exercise, during which each rat walked on a motor-driven treadmill for ~ 5 min/day at 15 m/min (0° incline) for 3-5 days. After the habituation period, the incline was raised to 15° for the duration of the training period while the 15 m/min speed was maintained. During the first 2 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 ~6-week training period. This training program was adapted from McCullough et al. (32). to represent a moderate-intensity of exercise training, eliciting ~60-70% of maximal aerobic capacity response from the animal of similar age and body mass as previously described (35,36) This moderate intensity of exercise was chosen, versus a higher intensity of exercise, as the former was well-tolerated by the animals, and there is evidence that the latter may enhance tumor metastases, as suggested by Cohen (12). Both EXCON and EXTB rats underwent echocardiography and were euthanized a minimum of 24 hours after the last bout of exercise to avoid potential effects of acute exercise on reported measures. After the Post 2 ultrasound imaging, while under anesthesia (5% isoflurane, $O_2$ balance) 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.

*Citrate Synthase Activity*

To determine training efficacy, 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 (39). 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.

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# Chapter 3 - 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 ($p<0.05$, Figure 2). However, there were no significant differences between EX and SED for controls or tumor-bearing groups (Figure 2). Tumor mass was not significantly different between EXTB vs. SEDTB groups (8.6±1.7 and 6.6±1.3g, respectively). There were no differences in femur lengths across all groups (EXCON= 39.2± 0.09mm, SEDCON= 39.0± 0.11 mm, EXTB= 39.1± 0.10 mm, SEDTB=39.0± 0.12 mm, $p>0.3$).

*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 3, LVDV, LVSV, and SV were significantly higher in the EXCON rats compared to both SEDTB and SEDCON (Table 2), but not different versus that of the EXTB group ($p\leq0.08$ for all measures). Longitudinal measures in the EXTB group demonstrated a trend for an increased LVDV 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 SV (Table 2). From Pre to Post 2, there was a trend for a decreased level of LV strain in only the EXCON (75.7% vs. 59.9% $p<0.06$).

*Cardiac and Skeletal Muscle Mass and Skeletal Muscle Citrate Synthase Activity*

Cardiac and skeletal muscle mass and citrate synthase activity were all examined post exercise intervention and/or cancer in all groups. Absolute mass of the heart, LV, gastrocnemius muscle, soleus muscle, and plantaris muscle were all significantly greater ($p<0.05$) in the EXCON group versus both TB groups (Table 1). Heart and left ventricle masses were also significantly higher in the EXCON compared to the SEDCON animals (Table 2). Cardiac tissue mass was normalized to body mass and femur length (FL, Table 1) to account for possible differences in growth between groups (62). When normalized to body mass or FL, significant differences were found between groups for the heart and LV (see Figure 4). 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).

Within the EXTB bearing group, HR mass, LV mass, and body mass were all significantly correlated with tumor mass whereas only LV mass was correlated with tumor mass in the SEDTB (Figure 5). In the SEDTB group, there were no significant correlations between skeletal muscle mass and tumor mass (Figure 6). 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 6).

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).

**Table 1.** *Cardiac and muscle mass characteristics*

| | Exercise Control (n=10) | Sedentary Control (n=10) | Exercise Tumor-Bearing (n=10) | Sedentary Tumor-Bearing (n=9) |
|---|---|---|---|---|
| **Absolute Mass (g)** | | | | |
| **Heart** | 0.95±0.01[abc] | 0.81±0.01[a] | 0.79±0.02[a] | 0.64±0.01 |
| **Left Ventricle** | 0.73±0.02[abc] | 0.62±0.01[ab] | 0.58±0.01[a] | 0.47±0.01 |
| **Right Ventricle** | 0.22±0.01 | 0.20±0.02[a] | 0.21±0.01 | 0.18±0.02 |
| **Gastrocnemius** | 2.33±0.05[ac] | 2.09±0.09[a] | 1.88±0.07 | 1.78±0.07 |
| **Soleus** | 0.16±0.003[ac] | 0.15±0.004[a] | 0.14±0.004[a] | 0.12±0.003 |
| **Plantaris** | 0.28±0.004[ac] | 0.26±0.01[a] | 0.25±0.01[a] | 0.21±0.01 |
| **Tumor Mass** | - | - | 8.6±1.7 | 6.6±1.3 |
| **Muscle Mass Normalized to Body Mass (mg/g)** | | | | |
| **Heart/Body mass** | 2.69±0.04[ab] | 2.40±0.05 | 2.59±0.06[a] | 2.28±0.07 |
| **Left Ventricle/Body mass** | 2.07±0.05[abc] | 1.82±0.04[a] | 1.87±0.04[a] | 1.65±0.04 |
| **Right Ventricle/Body mass** | 0.63±0.03 | 0.58±0.05 | 0.68±0.03 | 0.63±0.06 |
| **Gastrocnemius/Body mass** | 6.7±0.13 | 6.2±0.28 | 6.3±0.21 | 6.3±0.20 |
| **Soleus/Body mass** | 0.44±0.01 | 0.43±0.01 | 0.46±0.01 | 0.43±0.02 |
| **Plantaris/ Body mass** | 0.80±0.01[e] | 0.81±0.01[a] | 0.82±0.02[a] | 0.74±0.02 |
| **Tumor/Body mass** | - | - | 30.98±5.9 | 23.62±4.7 |
| **Mass Normalized to Femur Length (FL) (mg/mm)** | | | | |
| **Heart/ FL** | 24.13±0.35[abc] | 20.96±0.32[a] | 19.69±0.57[a] | 16.63±0.53 |
| **Left Ventricle/ FL** | 18.56±0.39[abc] | 15.96±0.46[ab] | 14.25±0.29[a] | 12.00±0.28 |
| **Right Ventricle/ FL** | 5.57±0.28 | 5.01±0.39 | 5.44±0.31 | 4.64±0.46 |
| **Gastrocnemius/ FL** | 59.69±1.10[ac] | 53.78±2.49[a] | 48.33±1.65 | 45.68±1.77 |
| **Soleus/ FL** | 3.92±0.09[a] | 3.81±0.15[a] | 3.56±0.10[e] | 3.12±0.10 |
| **Plantaris/ FL** | 7.06±0.16[a] | 6.79±0.11[a] | 6.34±0.33[a] | 5.41±0.16 |

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= $p\leq0.07$ vs. Sedentary Tumor-Bearing

**Table 2.** *Echocardiographic measures*

| Left Ventricle Measures | Exercise Control (n=10) | Sedentary Control (n=10) | Exercise Tumor-Bearing (n=10) | Sedentary Tumor-Bearing (n=9) |
|---|---|---|---|---|
| **Pre-Measure** | | | | |
| **LVDV (ml)** | 0.83±0.04 | 0.85±0.07 | 0.83±0.03 | 0.79±0.05 |
| **LVSV(ml)** | 0.23±0.03 | 0.19±0.02 | 0.21±0.02 | 0.18±0.02 |
| **SV (ml)** | 0.59±0.02 | 0.66±0.06 | 0.61±0.03 | 0.60±6 |
| **FS (%)** | 37.4±2.1 | 41.8±1.5 | 39.1±1.3 | 41.2±2.0 |
| **EF (%)** | 72.5±2.3 | 77.8±1.7 | 74.8±1.5 | 76.9±2.2 |
| **Strain (%)** | 76.0±12.1 | 88.1±13.3 | 74.1±8.1 | 82±6.8 |
| **Strain Rate (v/s)** | 10.3±1.7 | 10.6±1.6 | 9.1±0.94 | 9.8±0.79 |
| **Post 1 Measure** | | | | |
| **LVDV (ml)** | 1.00±0.05 | 0.95±0.06 | 0.92±0.06 | 0.84±0.02 |
| **LVSV(ml)** | 0.25±0.03 | 0.25±0.03 | 0.23±0.02 | 0.26±0.02 |
| **SV (ml)** | 0.73±0.03 [af] | 0.70±0.03 [e] | 0.68±0.05 | 0.59±0.01 |
| **FS (%)** | 40.6±2.2 | 37.8±1.5 | 39.2±1.4 | 35.0±1.2 |
| **EF (%)** | 76.1±2.2 | 73.2±1.7 | 75.0±1.4 | 69.8±1.5 |
| **Strain (%)** | 74.4±7.6 | 70.3±9.1 | 74.6±6.1 | 76.±10.3 |
| **Strain Rate (v/s)** | 8.69±0.89 | 7.9±1.18 | 9.5±0.74 | 7.2±1.0 |
| **Post 2 Measure** | | | | |
| **LVDV (ml)** | 1.11±0.06 [abcg] | 0.85±0.07 | 0.93±0.05 | 0.79±0.03 |
| **LVSV(ml)** | 0.32±0.04 [abcg] | 0.21±0.02 | 0.24±0.02 | 0.21±0.02 |
| **SV (ml)** | 0.78±0.04 [abg] | 0.65±0.05 | 0.69±0.03 [e] | 0.58±0.03 |
| **FS (%)** | 35.4±1.3 | 40.1±1.6 | 38.5±1.4 | 38.4±2.5 |
| **EF (%)** | 70.0±1.2 | 75.9±1.9 | 74.1±1.7 | 73.7±2.5 |
| **Strain (%)** | 59.9±6.4 [d] | 93.0±6.1 | 76.5±8.1 | 76.0±10.3 |
| **Strain Rate (v/s)** | 7.1±0.7 | 10.8±0.90 | 8.8±0.85 | 10.4±1.4 |

Abbreviations: LVDV, left ventricle diastolic volume; LVSV, Left ventricle systolic volume; 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.

a= $p<0.05$ vs. Sedentary Tumor-Bearing
b= $p<0.05$ vs. Sedentary Control
c= $p\leq0.10$ vs. Exercise Tumor-Bearing
d= $p\leq0.10$ vs. Sedentary Control
e= $p\leq0.10$ 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)*

| | Exercise Control (n=9) | Sedentary Control (n=9) | Exercise Tumor-Bearing (n=9) | Sedentary Tumor-bearing (n=9) |
|---|---|---|---|---|
| **Soleus** | 24.7±1.4[ab] | 15.5±1.5 | 25.4±1.2[ab] | 16.9±1.9 |
| **Red gastrocnemius** | 33.8±1.6[ab] | 26.3±1.5 | 35.9±1.8[ab] | 23.6±1.0 |

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

a= $p<0.05$ vs. Sedentary Tumor-Bearing

b= $p<0.05$ vs. Sedentary Control

**Figure 1.** *Representative echocardiography images*

A representative rat image in both diastole (top) and systole (bottom) are presented. 2D image of LV at the level of the papillary muscle. 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.

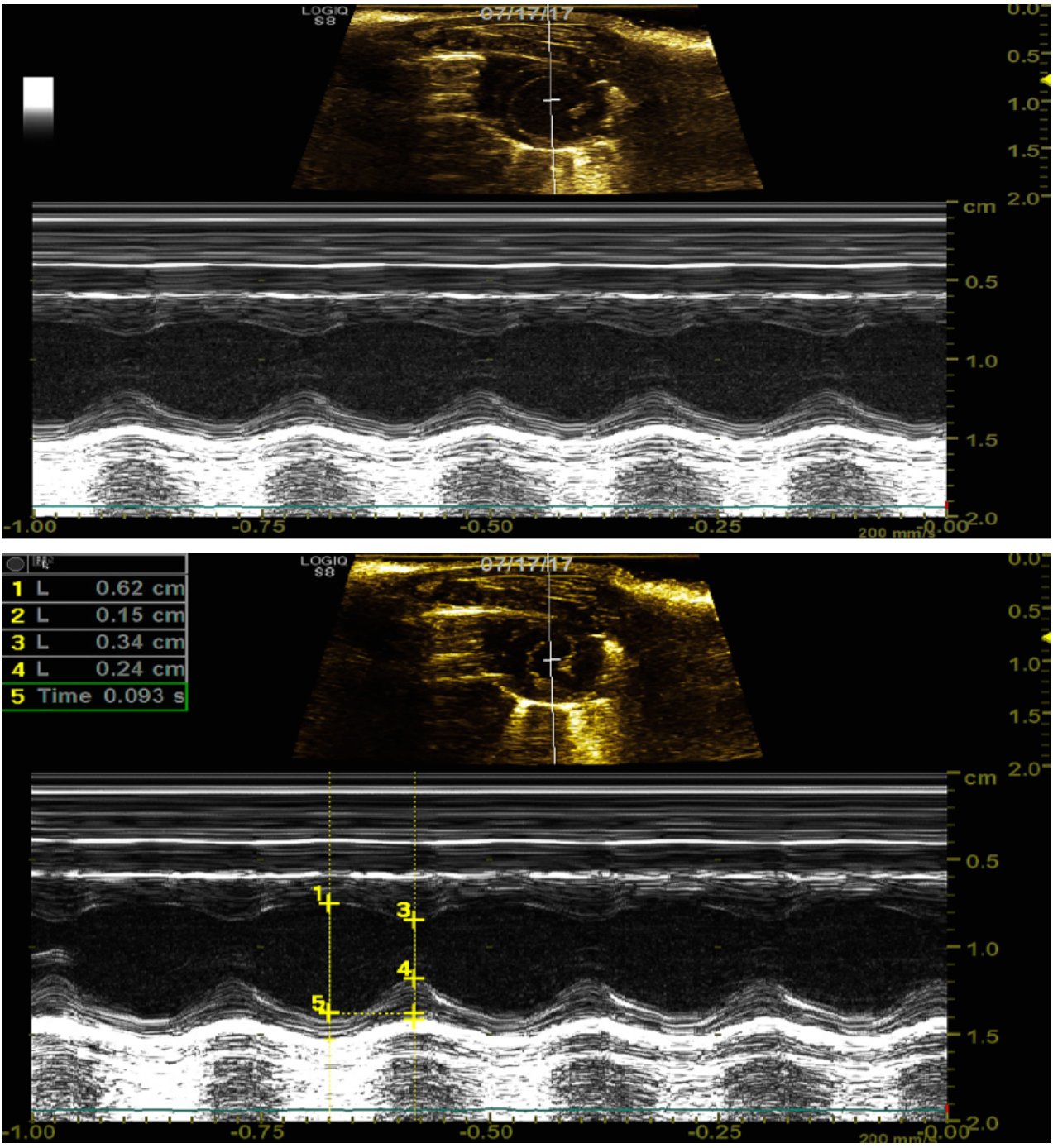


**Figure 2.** *Change in body mass*

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 (* = $p<0.05$).

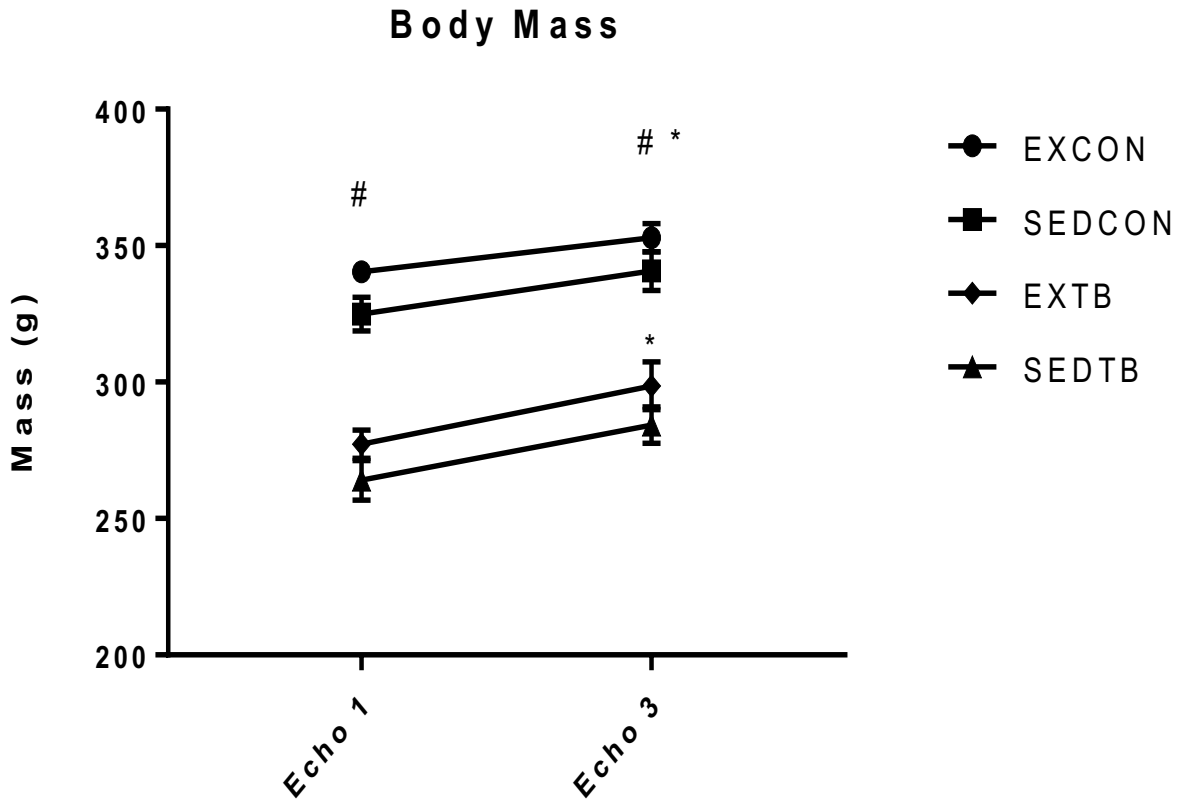


**Figure 3.** *Left ventricle change in volume*

The increase in diastolic (A), systolic (B), and stroke (C) volumes of the left ventricle from the first echocardiography measures to the final measures. The increases in these volumes were significantly higher in the exercise control animals compared to both sedentary groups(# = $p<0.05$ vs Sedentary tumor-bearing; *= $p<0.05$ vs Sedentary control, except for left ventricular systolic volume (LVSV).

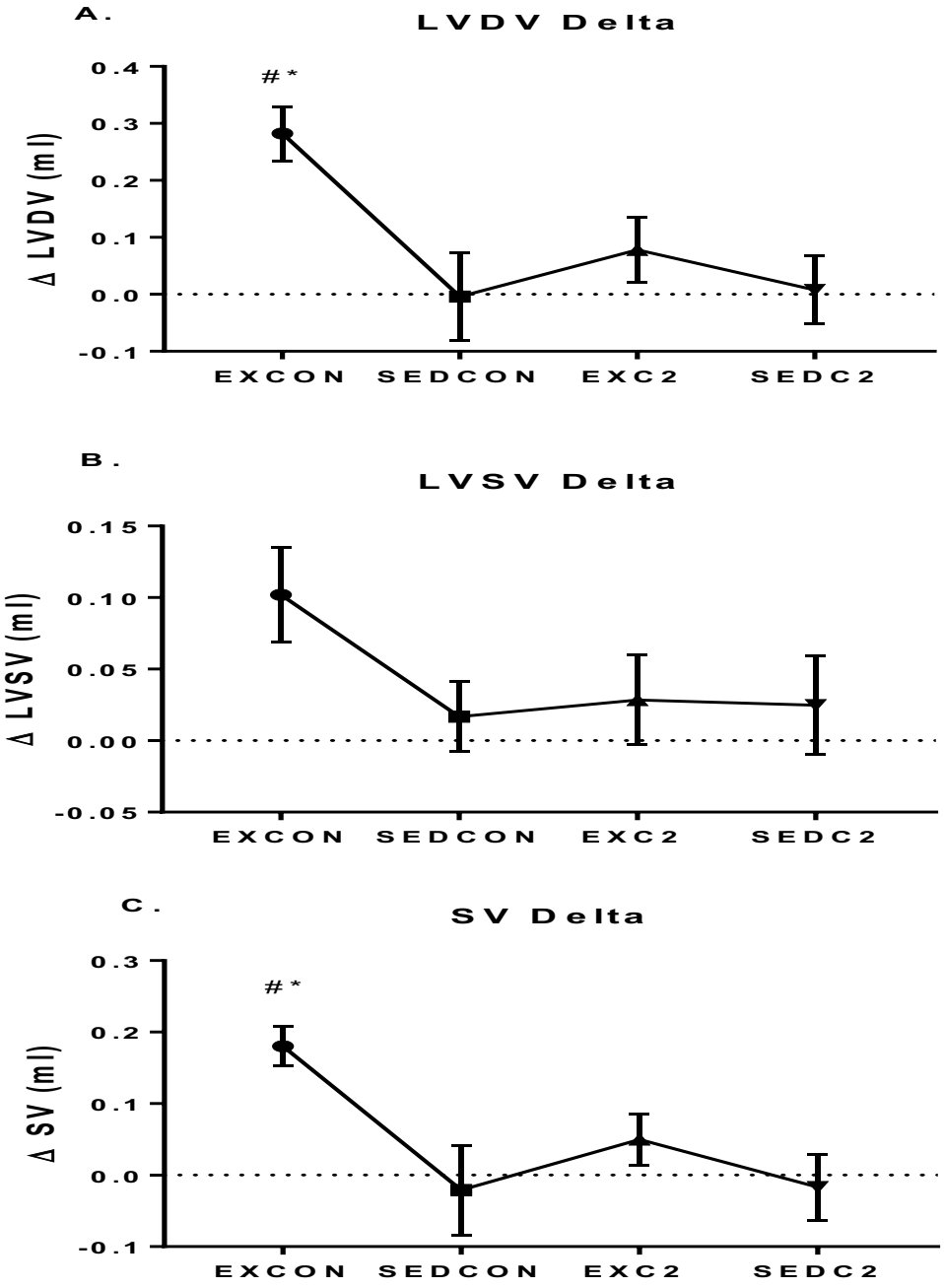


**Figure 4.** *Cardiac data*

The heart (HR), left ventricle (LV), and right ventricle (RV) wear 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.

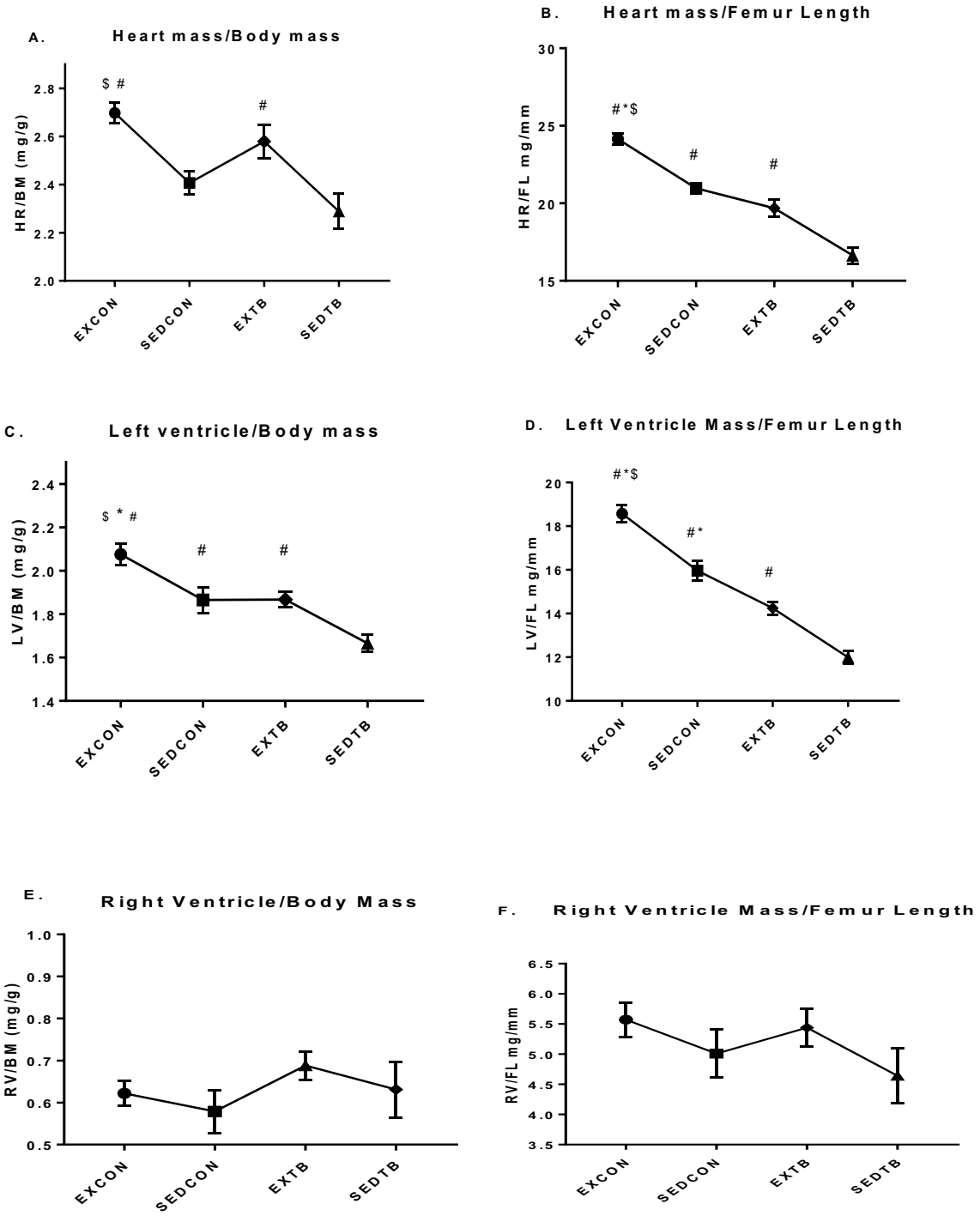


**Figure 5.** *Cardiac Correlations*

Within the tumor-bearing groups (closed circles) tumor mass was significantly 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 significantly negatively correlated with tumor mass (C). 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.

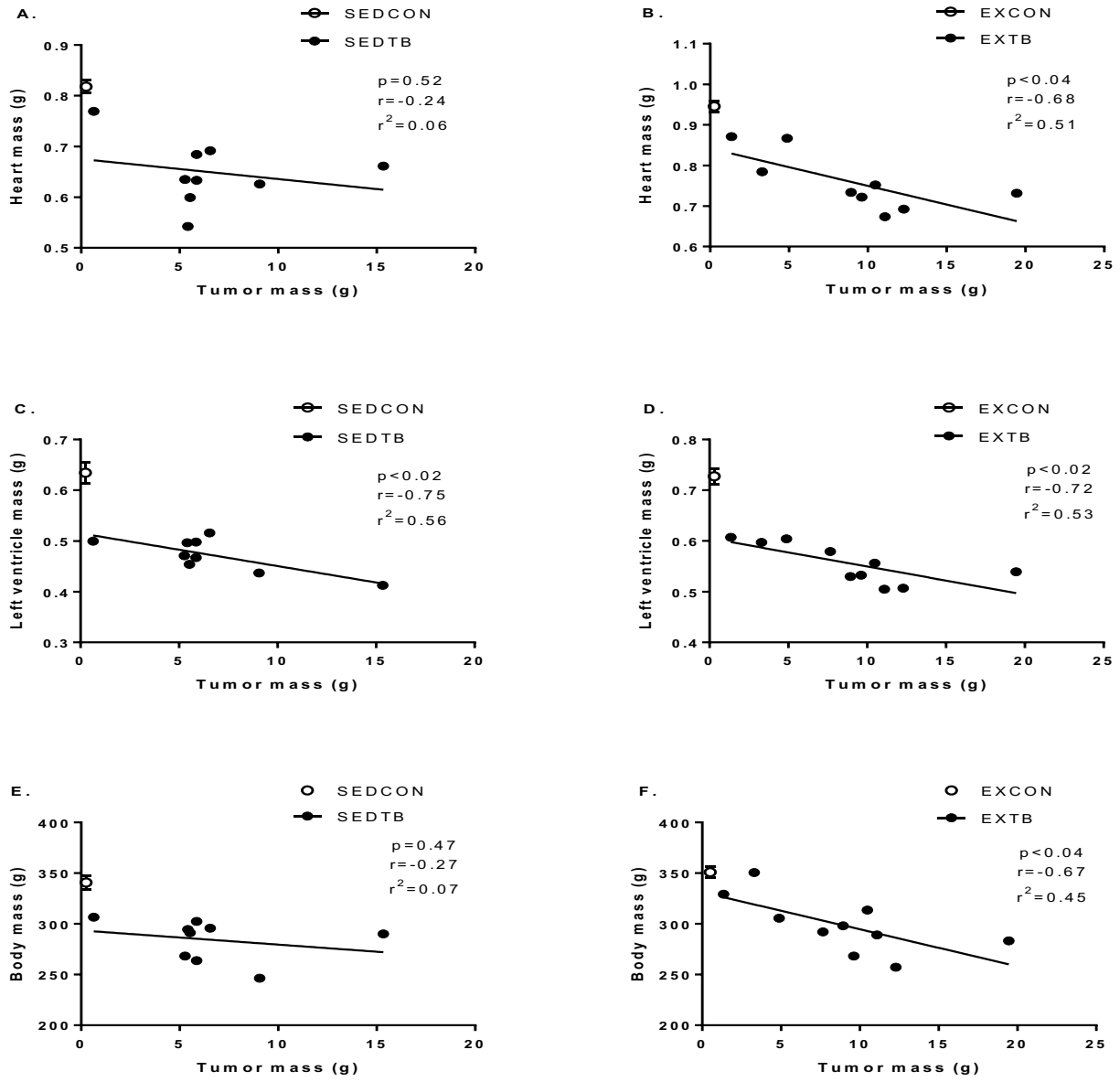
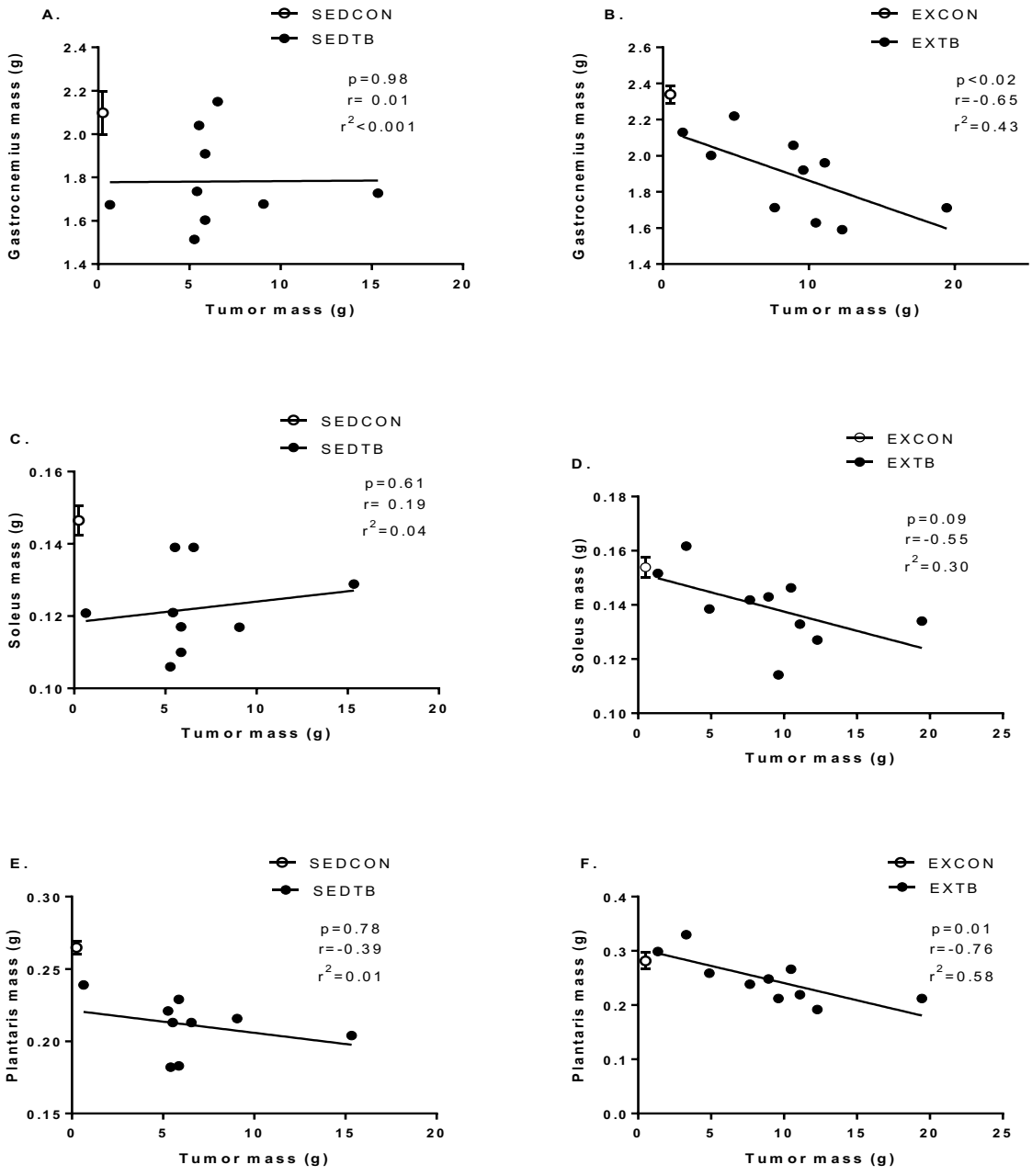


**Figure 6.** *Muscle Correlations*

Within the tumor bearing groups (closed circles) tumor mass was significantly negatively correlated with gastrocnemius mass (B), plantaris mass (F), and a trend 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.



# Chapter 4 - Discussion

Determination of the effects of prostate cancer on cardiac function and mechanics as well as skeletal muscle mass and the possible 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 significant atrophic effects of prostate cancer on the heart and skeletal muscle. Importantly, we show that exercise training can attenuate most of the atrophic effects in both heart and locomotor skeletal muscles. 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. However, the LV mass loss with cancer was attenuated with moderate intensity exercise training. These findings demonstrate the mitigating effects of aerobic exercise, on cancer-related cardiac atrophy. Hence, cancer-related cardiac and skeletal muscle atrophy are likely an underlying potential cause of fatigue which may be exacerbated by ADT or adjuvant therapies (15, 57).

*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. Previous work has shown cancer induced atrophy and cachexia in pre-clinical animal models (15, 40, 61) and humans (8, 34). Although the tumor-bearing animals in the current study were not cachexic (5% loss of body mass without signs of edema), there was significant atrophy of the heart, LV, and skeletal muscle in the sedentary group with cancer. There are several mechanisms potentially responsible for the atrophy observed herein contributing to the differences in mass, many of

which are discussed in a recent review (13). 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 (28). 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 (Table 3), indirectly suggesting gross alterations in spontaneous physical activity between sedentary groups was not present (17). Compared to skeletal muscle, much less is known regarding the effect of prostate cancer on cardiac muscle. The ubiquitin ligase system is thought to be a primary contributor in skeletal muscle, and possibly in cardiac tissue as well. However, actual mechanisms are speculative with the lysosome, calcium dependent and ubiquitin-dependent systems all as potential pathways of atrophy (1, 34). Further, 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 (1,63). Specifically, in mice with C26 colon cancer both E3 ligases were up-regulated and contributed to decreases in cardiac wall thickness and myocardium protein degradation (55, 63).

*Aerobic Exercise Training*

Exercise training is known to increase LV mass in health as well as multiple disease states, potentially increasing cardiac output (Q) and aerobic capacity and combating cardiovascular dysfunction or increasing exercise performance. Hence, the elevated cardiac masses normalized to both body weight and FL were expected in the EXCON group after training, consistent with previous research (38, 58, 60). These increases in cardiac mass seen in the EXCON group were not as prominent in the EXTB animals, which is likely due to the aforementioned mechanisms of

cardiac atrophy with cancer. The lower LV mass of the SEDTB group was likely responsible for the significantly lower LVDV and SV versus the EXCON, and a similar trend seen versus EXTB. Cardiac atrophy previously associated with time to exhaustion (15) could be attributable to modifications in the ubiquitin proteasome system. Thus, the mitigation of cardiac atrophy observed with exercise herein could be altering the effect of cancer on the ubiquitin proteasome system. Further, exercise training has been demonstrated to prevent rises in heart and skeletal muscle FoxO levels during doxorubicin treatment (28). Within the heart, exaggerated levels of MAFbx and Murph-1 levels were significantly reduced in a model of heart failure following 4 weeks of exercise training albeit of lesser volume (1). These findings are thought to be due to the chronic attenuation of inflammatory markers, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). Specifically, TNF-α is contributing to the expression of MAFbx and Murph-1 E3 ligases and was significantly correlated with higher levels of both E3 ligases mentioned previously (1), with aerobic exercise training having the potential to regulate the expression of the E3 ligases mentioned above either directly, or indirectly through mitigation of circulating TNF-α (2). In addition, Padrão and colleagues (40) demonstrated that exercise can mitigate cardiac cachexia and remodeling in a pre-clinical model of urothelial carcinoma (40) with several potential mechanisms by which exercise training may mitigate cancer-induced cachexia.

*Left Ventricular Function in Prostate Cancer and Exercise*

In contrast to our hypothesis, there were not large changes in many of 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, (10, 14, 31, 42, 51) and humans (25, 48). In the current study, we show that moderate intensity exercise training can mitigate prostate cancer induced atrophy of the LV, with exercise training resulting in a comparative heart size in the animals with cancer to that of the healthy sedentary controls. This is of higher clinical significance as heart and cardiovascular structure and function are important factors in determining the type of, and tolerance to, various anticancer therapies (1,11,45). If alterations in the heart (specifically LV) manifest with cancer prior to initiating treatment, regular physical exercise may improve heart mass, and potentially decrease both cardiac events associated with therapy (7, 57), as well as cancer-induced cardiac cachexic related deaths (34).

Non-invasively measured LVEDV as an indirect indicator of LV size, was only significantly increased in the EXCON animals over time, with a trend for an increase in the EXTB group (Table 2, Figure 3). This is in contrast to the absolute masses of the heart collected post-mortem (Table 1). Given the relative sensitivity of echocardiography it is difficult to delineate small difference in the rodent due to rapid heart rate and small chamber dimensions leading to a lack of fidelity of spatial and temporal resolution, which may explain, in part, the *in vivo* versus *in vitro* differences in measures (38). However, possible mechanisms discussed herein (E-3 ubiquitin ligases) that could be contributing to cardiac atrophy, should be investigated further as manifestation at a molecular level may delineate earlier changes than echocardiography can detect.

*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 (49, 54). 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 (47). With no differences between groups in mean tumor mass, the similar burden of the tumor between EXTB and SEDTB groups strengthens the comparisons between groups. This similar tumor-burden coupled with the orthotopic model of cancer (vs. ectopic models) also strengthens the translational development of this particular cancers effects as the tumor is matched to its host tissue (i.e., prostate tumor in prostate) (29), on the heart and skeletal muscles. 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 (21). 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 (44, 59, 60). From animal studies, the ideal length of training is typically 6-8 weeks of constant load moderate intensity exercise to induce an exercise phenotype (30). 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 ETB and SEDTB groups.

*Conclusions*

In summary, this investigation demonstrates that prostate cancer, independent of any adjuvant therapy, in a pre-clinical orthotopic prostate tumor model induces atrophy of cardiac, and select locomotor skeletal muscles. Furthermore, these reductions in muscle mass were

significantly 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 important insights into possible mechanisms of fatigue in cancer patients that could be exacerbated by concomitant treatment. Lastly, the present investigation promotes further investigation into the 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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