Am J Transl Res 2021;13(1):197-209
www.ajtr.org /ISSN:1943-8141/AJTR0115029

Original Article

Effects of high-intensity training on prostate cancer-induced cardiac atrophy

Dryden R Baumfalk[1], Alexander B Opoku-Acheampong[1], Jacob T Caldwell[1], Alec L E Butenas[1], Andrew G Horn[1], Olivia N Kunkel[1], Steven W Copp[1], Carl J Ade[1,2], Timothy I Musch[1,3], Bradley J Behnke[1,2]

[1]Department of Kinesiology, Kansas State University, Manhattan 66506, Kansas, USA; [2]Johnson Cancer Research Center, Kansas State University, Manhattan 66506, Kansas, USA; [3]Department of Anatomy and Physiology, Kansas State University, Manhattan 66506, Kansas, USA

Received May 26, 2020; Accepted November 20, 2020; Epub January 15, 2021; Published January 30, 2021

Abstract: Background: Recent evidence suggests prostate cancer independent of treatment has atrophic effects on whole heart and left ventricular (LV) masses, associated with reduced endurance exercise capacity. In a pre-clinical model, we tested the hypothesis that high-intensity training could prevent cardiac atrophy with prostate cancer and alter cardiac protein degradation mechanisms. Methods: Dunning R-3327 AT-1 prostate cancer cells (1×10^5) were injected into the ventral prostate lobe of 5-6 mo immunocompetent Copenhagen rats (n=24). These animals were randomized into two groups, tumor-bearing exercise (TBEX, n=15) or tumor bearing sedentary (TBS, n=9). Five days after surgery, TBEX animals began exercise on a treadmill (25 m/min, 15° incline) for 45-60 min/day for 18±2 days. Pre-surgery (Pre), and post-exercise training (Post) echocardiographic evaluation (Vivid S6, GE Health Care), using the parasternal short axis view, was used to examine ventricle dimensions. Markers of protein degradation (muscle atrophy F-box, Cathepsin B, Cathepsin L) in the left ventricle were semi-quantified via Western Blot. Results: There were no significant differences in tumor mass between groups (TBEX 3.4±0.7, TBS 2.8±0.6 g, P=0.3), or body mass (TBEX 317±5, TBS 333±7 g, P=0.2). Heart-to-body mass ratio was lower in TBS group compared to TBEX (2.3±0.1 vs. 2.5±0.1 mg/g, P<0.05). LV/body mass ratio was also lower in the TBS group (1.6±0.1 vs. 1.8±0.1 mg/g, P<0.05). From Pre-Post, TBEX had significant increases in SV (~20% P<0.05) whereas TBS had no significant change. There were no significant differences between groups for markers of protein degradation. Conclusion: This study suggests that high-intensity exercise can improve LV function and increase LV mass concurrent with prostate cancer development, versus sedentary counterparts. Given cardiac dysfunction often manifests with conventional anti-cancer treatments, a short-term high-intensity training program, prior to treatment, may improve cardiac function and fatigue resistance in cancer patients.

Keywords: Prostate cancer, high-intensity exercise, cardiac atrophy

Introduction

In the United States current models predict ~1.76 million people will be diagnosed with non-skin cancer in 2020. Of those, ~870,000 will be men with ~175,000 being new prostate cancer diagnoses [1]. With prostate cancer, many patients receive pharmacological or surgical androgen deprivation therapy (ADT), which is associated with increased fatigue [2], loss of muscle mass, and bone density [3, 4], as well as enhanced risk of cardiovascular disease [5, 6]. Those not undergoing ADT may receive other adjuvant therapies, such as radiation therapy or chemotherapy, which can elicit and/or exacerbate cardiovascular dysfunction [5, 7]. Approximately 40% of patients with cancer report symptoms of fatigue [8], however, elucidating mechanisms of fatigue or atrophy with cancer versus concurrent adjuvant therapies is difficult, as withholding treatment to study the independent effects of cancer would be unethical [9, 10]. Given cancer-related fatigue or cardiovascular abnormalities can compromise the completion of anti-cancer treatment regimens, it is clinically important to understand how cancer affects determinants of exercise capacity (e.g., cardiac mass and function and skeletal muscle mass).

Recent evidence suggests prostate cancer induces whole heart and left ventricle (LV) atro-

phy [11, 12]. In healthy individuals exercise training can increase both cardiac and skeletal muscle mass and, in cancer patients, may mitigate atrophy associated with cancer and anticancer therapies [13-16]. To date, the beneficial effects of exercise within the tumor microenvironment include mitigation of tumor hypoxia [9, 17], increased infiltration of immune cells [17], a more homogenous distribution of perfusion [9, 18] and a shift towards vascular normalization [19] that may contribute to increased delivery of chemotherapeutic agents [20]. However, beyond the tumor microenvironment, the effects of exercise training on cancer related cardiac atrophy and function are not well understood, even though a significant portion of cancer patients present cardiac comorbidities upon diagnosis [21].

Exercise training is efficacious in decreasing morbidity and mortality of multiple diseases [22-24], and is being recognized and recommended for cancer patient care programs to mitigate fatigue and/or a decline of aerobic capacity [4, 25, 26]. Despite the prescription of exercise programs, understanding the beneficial effects of exercise training on heart function, structure, and molecular signaling with prostate cancer, independent of therapy, is limited [21]. We have shown that long-term moderate-intensity exercise training can help mitigate tumor-induced cardiac atrophy in a pre-clinical model of prostate cancer that may be beneficial in combating cardiotoxicity associated with adjuvant therapies [13]. However, whether a shorter duration, but higher intensity program could combat cardiac atrophy associated with cancer is unknown. This is important as the period from diagnosis to treatment may not be long-enough for a patient to utilize long-duration (months) moderate-intensity exercise programs to enhance their cardiac phenotype to mitigate cancer-related atrophy. Therefore, the purpose of this investigation was to determine if high-intensity aerobic exercise training can prevent heart and skeletal muscle atrophy associated with prostate cancer, similar to that shown for moderate-intensity aerobic exercise training with other types of cancer [12, 14]. We hypothesized that: 1) prostate cancer-induced cardiac atrophy will be mitigated with high-intensity exercise training, 2) left ventricular function (assessed with 2-D echocardiography) will be preserved in the exercise-trained tumor-bearing rat compared to its sedentary counterpart, and 3) that exercise training will preserve locomotor skeletal muscle mass and oxidative capacity. To investigate potential mechanisms of protein degradation associated with atrophy/dysfunction, we measured prominent markers from different atrophy associated pathways including: muscle atrophy F-box (MAFbx) from the ubiquitin proteasome system (UPS) and cathepsins B and L (CTSB, CTSL) from the lysosomal-autophagy system. Further, cardiac Nuclear erythroid-2-p45-related factor-2 (Nrf-2) was measured as a potential target of associated oxidative-stress regulation.

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=24, ~6 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

Dunning R-3327 AT-1 strain of rat prostate adenocarcinoma cells were utilized in this study. These cells were chosen due to their similar growth characteristics (e.g. a high growth rate and low metastatic potential) as human prostate cancer [27]. AT-1 cells were cultured using 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. Upon reaching ~80-90% confluence, a sample of the cells was quantified via hemocytometer to calculate the appropriate dilution (100,000 cells/ml) of the

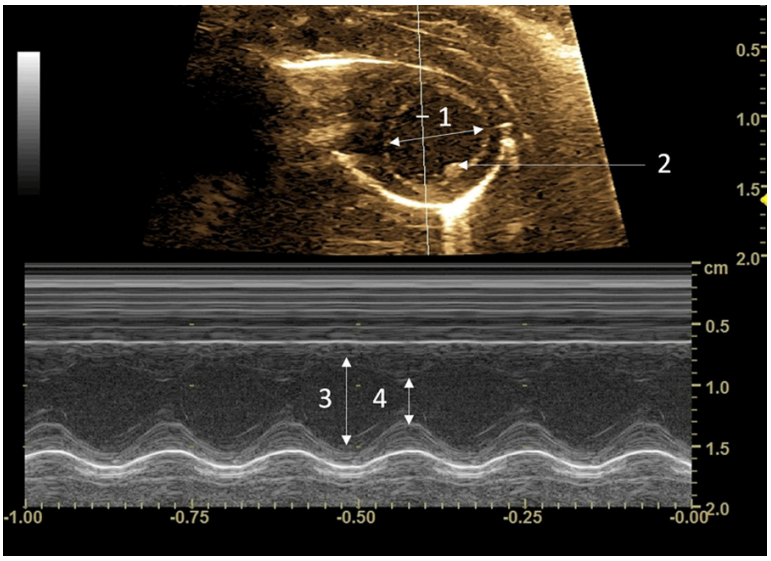

Figure 1. Representative echocardiography image. A representative 2D image (Top) and M-mode tracing (bottom) of the rat left ventricle at the level of the papillary muscle in diastole. Arrows represent: 1. the left ventricle chamber, 2. papillary muscle, 3. left ventricular diastolic dimension, and 4. left ventricular systolic dimension.

viable cells for a tumor cell stock solution placed in physiological saline solution (PSS). This solution was aliquoted such that each 0.1 ml increment contained ~1×10^5 AT-1 cells. These methods have been used previously to induce orthotopic prostate tumors [9, 11, 13].

Animals were anesthetized with 2-5% isoflurane, (O_2 balance) and after an appropriate field of anesthesia was attained, (i.e., lack of toe-pinch reflex), an abdominal incision of ~1 cm, lateral of the midline, was made. Using aseptic technique, the bladder/prostate complex was exposed. Thereafter, 10^5 AT-1 cells suspended in 0.1 ml PSS were injected into the ventral lobe of the prostate using a sterile 26 G insulin syringe. A sterile cotton tipped applicator was placed alongside the needle during removal to prevent any cell leakage. The abdominal wall was closed with sterile 3-0, polyglycolic acid coated suture (DemeTECH, Miami Lakes, FL) and the overlying skin was closed with 4-0 nylon monofilament (DemeTECH, Miami Lakes, FL) and sealed with skin adhesive (Vet-Bond 3M St. Paul, MN). Isoflurane was terminated and animals 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. Animal health and welfare were monitored daily until animals were placed into sedentary or exercise trained groups, ~5 days post-injection. For determination of loss of mass in either group versus non-tumor bearing animals, historical control/sham animal (same strain and sex, with similar age) data from our laboratory [11, 13, 28] was used to make comparisons for absolute and relative cardiac masses, as our primary comparison was within the tumor bearing animals. Five days after cell injection animals were separated into sedentary tumor-bearing (TBS; n=9) and exercise-trained tumor-bearing (TBEX; n=15) groups. During the period from injection to group placement, no difference in body mass gain was observed between groups (TBEX, 3.3±0.9; TBS, 3.7±1.4 g, P>0.05).

Echocardiographic assessment of LV function

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 ultrasound technician at two separate time points. An example of the images used for analysis is demonstrated in **Figure 1**. The first evaluation "Pre" exercise training and/or cancer was performed the day before tumor injection, and the "Post" measure was performed ~20 days after the onset of exercise training. The various system settings and parameters (i.e frame rate, depth, brightness etc.) used for echocardiographic evaluation remained unchanged throughout the experimental protocol for a given animal. Echocardiographic data was processed using the manufacturer's dedicated software for imaging analysis. Prior to echocardiographic measures, rats were subjected to 2% isolflurane anesthesia (O_2 balance), placed on a digital heating pad (42°C). Animals were maintained at 2% isoflurane/O_2 balance to limit anesthesia effects on heart function during the

imaging processes [29, 30]. Animal hair was cleared from the sternum using a depilatory agent (Nair, Johnson & Johnson, New Brunswick, NJ). 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, similar to previous studies [13, 31, 32]. The following LV dimensions were measured: left-ventricular end-diastolic (LVEDD) and end-systolic dimensions (LVESD; used to estimate volumes using the Teichholz formula [33]) and LV posterior wall thicknesses (PWT) at end-diastole (PWTD) and end-systole (PWTS). Measures of myocardial function (i.e., stroke volume, ejection fraction, fractional shortening) were evaluated using mean values from a minimum of four cardiac cycles during each visit.

Exercise training

All rats were habituated to treadmill exercise prior to Pre-measures (see below) with a motor-driven rodent treadmill for ≤5 min/day at 15 m/min (0° incline) for 3-5 days. In exercise-trained animals, after the habituation period and Pre-measures, the treadmill incline was raised to 15° for the duration of the training period and the speed was increased to 25 m/min by day 10. During the initial week of training, the duration of exercise training was increased by 10-15 min until 60-min duration was reached by the 6th day for all animals. The TBEX rats continued to exercise 5 days/week for 45-60 min/day (as tolerated) for the remainder of the 20-day training period. This training program was modified from previously used protocols [9, 13] to represent high-intensity exercise training eliciting greater than 75% of maximal aerobic capacity response from animals of similar age and body mass, as previously described [34, 35]. After a minimum of 24 hours between the last bout of exercise for the exercise group, all animals underwent Post ultrasound imaging, followed by a thoracotomy and removal of the heart while under a deep plane anesthesia (5% isoflurane/O_2 balance), and the right ventricle was detached from the left ventricle and intraventricular septum. Thereafter, tumor, prostate (when delineation from tumor was possible), soleus muscle, plantaris muscle, and gastrocnemius muscle (separated into red and white portions) were immediately excised, weighed, flash-frozen in liquid nitrogen, and stored in an ultra-low freezer (-80°C) for future analyses. The right femur was removed, cleaned of any remnant tissue, and the length was measured with a digital caliper.

Western blotting

Samples from the LV (~5 mg) were isolated into 2 mL bead mill tubes containing ~0.5 g of 1.4 mm ceramic beads, 350 µL of RP1 lysis buffer (Macherey-Nagel, Düren, Germany), and 3.5 µL of β-Mercaptoethanol, and homogenized for 1 min at 5 m/s using a Bead Mill 4 (Fisherbrand™). Total protein and RNA from tissues were prepared with the Nucleospin RNA/Protein Kit (Macherey-Nagel) according to the manufacturer's instructions. Total protein concentrations were determined using the Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY, USA). Protein samples (~30 µg) were separated on 4-12% Bis-Tris Protein Gels (Invitrogen™, Carlsbad, CA) by gel electrophoresis in MES running buffer (Invitrogen™) employing 200 V for 22 min. Gels were then transferred to mini-PVDF membranes using the iBlot 2 Dry Transfer Device (Invitrogen™). Membranes were incubated for ~3 hours with the iBind device in iBind solution (Invitrogen™) with primary antibodies anti-cathepsin B, (1:1500; Abcam, Cambridge MA.), anti-cathepsin L (1:1000; Abcam), anti-muscle atrophy F-box (MAFbx) (1:1000; Santa Cruz Biotechnologies), and anti-nuclear factor erythroid-2-related factor 2 (Nrf2) (1:1000; Santa Cruz Biotechnologies). Cathepsin B primary antibodies were incubated with a goat anti-rabbit IgG (H+L) secondary antibody conjugated with Horse Radish Peroxidase (HRP) (1:5000; Abcam). Cathepsin L, MAFbx, and Nrf-2 primary antibodies were incubated with goat anti-mouse secondary antibody IgG (H+L) conjugated with HRP (1:4000; Abcam). Membranes were then incubated for ~5 min with SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific) and imaged with C-DiGit® Blot Scanner (Li-Cor Lincoln, NE) high sensitivity method. After initial imaging, the membrane was re-incubated with anti-Beta tubulin (1:1500; Santa Cruz Biotechnologies) and Goat anti-Mouse IgG (H+L), secondary antibody conjugated with Horse Radish Peroxidase (1:5000; Abcam) for cathepsin B. The Cathepsin L, MAFbx, and Nrf-2 membranes were re-incubated with anti-Vinculin (1:2000; Abcam) and goat anti-mouse (H+L), secondary antibody conjugated with HRP (1:4000; Abcam) and imaged using the same

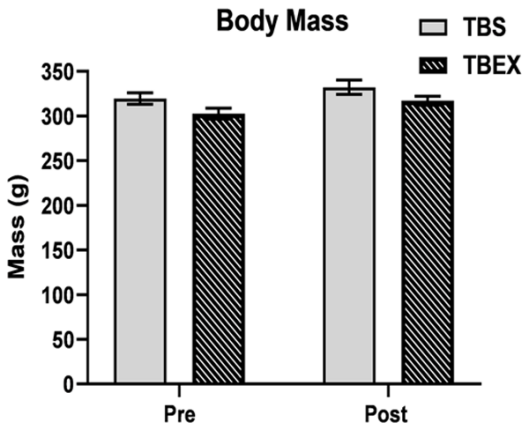

Figure 2. Change in body mass. Body mass for both sedentary (n=9) and exercise (n=15) groups were not significantly different at Pre or Post measures of echocardiography.

protocol previously described to ensure equal protein loading. The protein bands were quantified, normalized to housekeeping gene, and analyzed using the Image Studio software (Li-Cor).

Citrate synthase activity

To evaluate training efficacy, and potential effects of cancer on muscle oxidative capacity, the red portion of the gastrocnemius muscle and soleus muscle were selected for determination of citrate synthase enzymatic activity according to the original methods of Srere [36]. This mitochondrial enzyme is a marker of muscle oxidative potential associated with exercise training. In brief, skeletal muscle was homogenized in a tris buffered cocktail and diluted to a final concentration of 1:400. Next 15 µl and 30 µl samples of this 1:400 dilution were diluted further using 210 µl and 195 µl of tris buffer, respectively. In addition, 15 µl of acetyl coenzyme A (Cayman Chemical), and 30 µl of DTNB (Thermo Fisher Scientific) were added to each sample in duplicate in a 96-well plate. Samples were analyzed in a spectrophotometer (accuSkan GO; Thermo Fisher Scientific) after incubating for 5 min at 30°C before readings. Following incubation, multiple readings were collected with the spectrophotometer at 412 nm once per minute for 5 min followed by the addition of 30 µl of oxaloacetic acid (Sigma-Aldrich, St. Louis, MO) to all sample wells and analyzed again immediately. Citrate synthase enzyme activity is reported as µmol/min/g wet weight of sample tissue for all animals.

Data analysis

For all statistical analysis, the Prism (version 8.1, Graphpad software, INC., La Jolla, CA) data analysis software package was utilized. Statistical comparisons were made with either a two-sided Student's *t* test, Mann-Whitney test, and one- or two-way repeated measure analysis of variance (ANOVA) with Holm-Sidak post hoc tests as appropriate to assess statistical differences between groups for all measures. A $P<0.05$ was set for statistical significance with data reported as mean ± SEM. Given the primary outcome was the within cancer group, the initial data analysis was performed solely on the TBSED and TBEX group. A time-matched control group was not possible as the vendor (Charles River) discontinued the immunocompetent Copenhagen rat model, and comparisons to immunocompromised strains are not appropriate. Therefore, we utilized post-hoc analysis of historical control and sham values from our laboratory using one-way (ANOVA) with Holm-Sidak post hoc tests.

Results

Body mass was unchanged in both TBS and TBEX groups across the ~20-day period of experiments with no differences between groups at any time point (**Figure 2**). Tumor mass and tumor burden (tumor mass/body mass) were not different between TBEX and TBS groups (**Table 1**). The femur length was not different between TBEX and (39.0±0.1 mm) and TBS (39.2±0.2 mm) groups ($P=0.33$).

Echocardiographic assessment of LV function

Left ventricle measures Pre- to Post-intervention were used to analyze potential changes in heart function. Posterior wall thickness in diastole and systole (PWT_D, PWT_S respectively) were not different between TBEX compared to TBS group (**Table 2**). Longitudinal increases Pre-Post in volume (LVEDV, SV) for the TBEX group were significant ($P<0.05$, **Figure 3**), whereas no change occurred for the TBS group.

Cardiac and skeletal muscle mass and skeletal muscle citrate synthase activity

Absolute mass of the whole heart and LV were normalized to body mass (**Table 1**) to account for possible differences in relative size between groups. Compared to sedentary counterparts, TBEX had larger absolute heart mass (**Table 1**)

Table 1. Cardiac and skeletal muscle mass characteristics

	Sedentary Tumor-Bearing (n=9)	Exercise Tumor-Bearing (n=15)
Absolute Tissue Mass (g)		
Heart	0.74±0.02	0.80±0.02*
Left Ventricle	0.54±0.02	0.57±0.01
Right Ventricle	0.20±0.01	0.21±0.01
Gastrocnemius	1.76±0.07	1.75±0.04
Soleus	0.14±0.01	0.15±0.01
Plantaris	0.24±0.01	0.25±0.01
Tumor Mass	2.9±0.4	3.4±0.5
Tissue Mass Normalized to Body Mass (mg/g)		
Gastrocnemius/Body mass	5.3±0.2	5.6±0.1
Soleus/Body mass	0.43±0.02	0.47±0.02
Plantaris/Body mass	0.73±0.02	0.78±0.02
Tumor/Body mass (%)	1.0±0.1	1.1±0.2
Skeletal Muscle Citrate Synthase Activity (μmol/min/g)		
Soleus	18.1±2.0	24.2±1.1*
Red gastrocnemius	27.7±1.3	32.8±1.9*

Data are mean ± SEM and were compared with Student's t-tests. *P<0.05 vs. Sedentary Tumor-Bearing.

Table 2. Echocardiographic measures

Left Ventricle Measures	Sedentary Tumor-Bearing (n=9)	Exercise Tumor-Bearing (n=15)
Pre-Measures		
LVEDD (cm)	0.75±0.01	0.72±0.02
LVESD (cm)	0.46±0.02	0.44±0.02
LVEDV Teich (ml)	0.95±0.04	0.87±0.05
LVESV Teich (ml)	0.26±0.03	0.23±0.02
PWT_D (cm)	0.15±0.01	0.14±0.01
PWT_S (cm)	0.26±0.01	0.23±0.01
SV (ml)	0.68±0.02	0.63±0.04
FS (%)	38.2±1.9	38.2±1.6
EF (%)	73.5±1.2	73.6±2.0
Post-Measures		
LVEDD (cm)	0.74±0.01	0.78±0.01
LVESD (cm)	0.47±0.01	0.48±0.02
LVEDV Teich (ml)	0.96±0.04	1.08±0.08#
LVESV Teich (ml)	0.26±0.02	0.29±0.03
PWT_D (cm)	0.13±0.01	0.16±0.01
PWT_S (cm)	0.23±0.01	0.24±0.01
SV (ml)	0.67±0.03	0.77±0.05#
FS (%)	38.0±1.4	37.8±1.7
EF (%)	72.2±1.7	73.2±1.8

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; LVEDV, left ventricle end-diastolic volume; LVESV, left ventricle end-systolic volume; SV, Stroke volume; FS, fractional shortening; EF, ejection fraction; Teich, Teicholz formula. Data are mean ± SEM and were compared with Two-way ANOVA. #P<0.05 Pre vs. Post.

and relative (normalized to body mass) heart and LV masses (**Figure 4**). There was no significant difference in RV mass normalized to body mass (**Figure 4**). Skeletal muscle tissue mass was also normalized to body mass and was not different between groups for the gastrocnemius (P=0.059), soleus (P=0.23), and plantaris (P=0.21) muscles. However, skeletal muscle citrate synthase activity was greater in the TBEX group for both the soleus and red gastrocnemius muscle versus TBS (P<0.05, **Table 1**).

Left ventricle protein expression

There was no difference in CTSB or CTSL protein expression of the left ventricle from the TBEX versus TBS (**Figure 5A**, **5B**, P=0.71 and P=0.62 respectively). Further, there were no differences

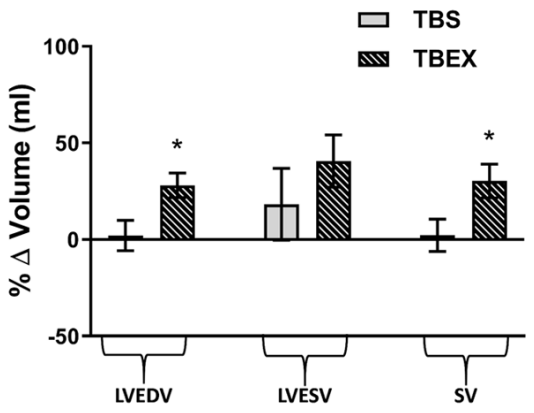

Figure 3. Percent change in volume. Pre-Post % change of cardiac measures of left ventricle diastolic volume (LVEDV), left ventricle systolic volume (LVESV), and stroke volume (SV) were compared between tumor groups (TBS; n=9 vs. TBEX; n=15). *P<0.05 vs. TBS.

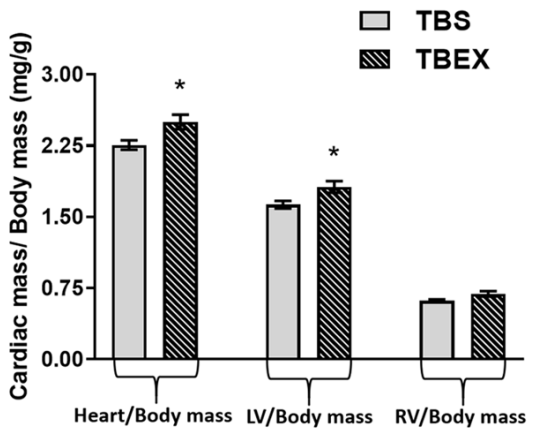

Figure 4. Cardiac muscle. The whole heart, left ventricle (LV), and right ventricle (RV) normalized to body mass were compared between tumor bearing groups (TBS; n=9 vs. TBEX; n=15). *P<0.05 vs. TBS.

in MAFbx, or Nrf-2 protein expression between groups (**Figure 5C**, **5D**, P=0.93 and P=0.44 respectively).

Discussion

Investigating the effects of prostate cancer on cardiac atrophy and function, and the ability of high-intensity continuous exercise training to mitigate the effects of cancer on these parameters, is clinically important. Many cancer therapy regimens are detrimental to cardiac function [5-7], and prior atrophy or dysfunction associated with cancer could be detrimental to patient treatment options or outcomes. The primary finding of the current investigation is that high-intensity exercise preserved heart mass in prostate tumor-bearing animals as whole heart and LV mass relative to body mass was lower in the tumor-bearing sedentary versus tumor-bearing exercise trained animals. To demonstrate the effect of cancer, when comparing the current data to age-matched control/sham animals of the same genus, species, sex, and age from Esau et al. [11], Baumfalk et al. [13], and Opoku-Acheampong et al. [28], two important findings were attained: 1) TBS had a lower relative heart and LV mass versus that found in non-tumor-bearing animals (**Figure 6**), demonstrating the underlying effects of cancer, independent of treatment, detrimentally affecting the normal cardiac phenotype, and 2) the relative heart mass of the TBEX was not different than non-tumor bearing, albeit sedentary, rats (**Figure 6**); demonstrating that even short-duration high intensity exercise programs can preserve a healthy cardiac phenotype with cancer. Collectively, there is a preservation of cardiac mass with high-intensity exercise from the insult of cancer-related cardiac atrophy, that is not observed in TBS. Thus, high-intensity exercise may help to preserve cardiac mass with cancer and potentially provide a mechanism of cardioprotection between time of diagnosis and initial treatments of potentially cardiotoxic therapies.

Aerobic exercise training and atrophy

Aerobic exercise training is efficacious in increasing LV mass in health as well as diseases such as cancer and heart failure [12, 22, 37]. Previously, we have shown moderate-intensity exercise prevented cardiac atrophy in a 5 week study using the same orthotopic prostate cancer model and cell line [13]. However, in that study the duration of training was ~40% longer and may not reflect a realistic training period to improve cardiac performance prior to treatment. Therefore, we were particularly interested in whether similar benefits on preserving cardiac mass could be achieved over a shorter time frame, albeit with a higher intensity of exercise. The current study demonstrates high-intensity exercise induced similar adaptations, in an expedited manner. This is important when patients may have a shortened time before beginning treatment to maximize cardiac adaptations. For example, across the cancer induc-

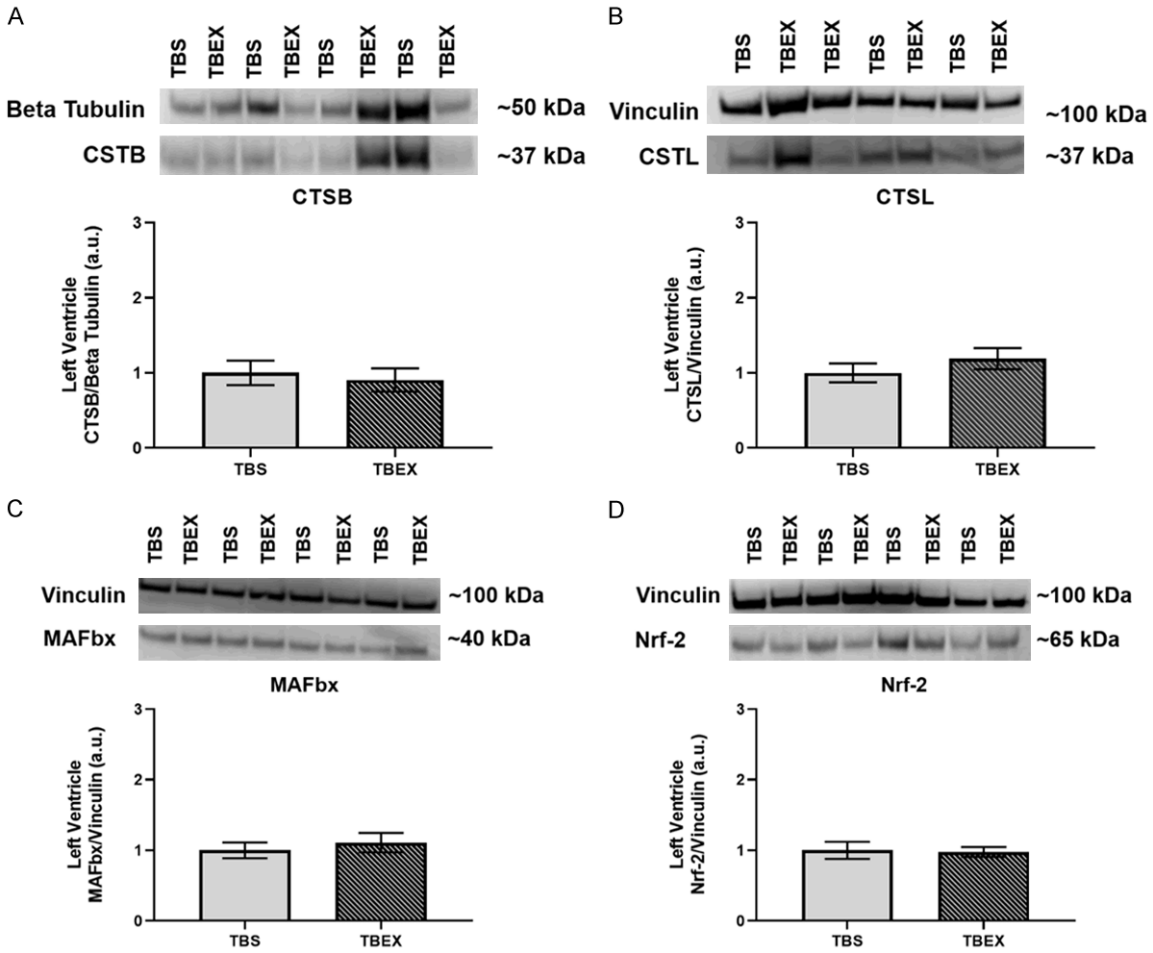

Figure 5. Left ventricle protein expression. Left Ventricle protein expression was not different between groups (TBS; n=9 vs. TBEX; n=15) for lysosomal markers Cathepsin B (CTSB; A) or Cathepsin L (CTSL; B). Both muscle atrophy F-box (MAFbx; C) and Nuclear erythroid-2-p45-related factor-2 (Nrf-2; D) protein expression were similar between groups as well (TBS; n=9 vs. TBEX; n=15).

tion through end of high-intensity training, the TBEX group demonstrated positive cardiac adaptations, compared to their sedentary counterparts, evidenced through increased SV, and LV hypertrophy from Pre-Post, not observed in TBS counterparts. Importantly, there is growing evidence that continuous moderate intensity aerobic exercise may not be as beneficial for improving heart function as higher intensity exercise [38, 39], and multiple prominent studies [40, 41] and meta-analysis [42] demonstrate the relative safety of high-intensity exercise training in disease conditions e.g., heart failure; [40, 42].

Cardiac atrophy is associated with cancer, and cardiac atrophy is known to lead to decreased time to exhaustion with endurance capacity tests [11], and loss of aerobic capacity associated with a diminished peak oxygen consumption [12, 43, 44]. Cardiac atrophy has been shown to be attenuated with exercise in models of heart failure with reduction in both MAFbx and muscle RING-finger protein-1 (MuRF-1) [45], and could be attributable to modifications in the UPS [14, 16, 21]. A chronic attenuation of inflammatory markers that are upstream of the UPS have been associated with these findings [12, 45]. Contrary to our hypothesis, there was no difference in protein expression of several proteins associated with atrophic remodeling (MAFbx, CTSB, and CTSL). Further, we did not observe an increased MAFbx in TBS vs. TBEX, contrary to findings of Adams et al. [45] of significantly lower expression of MAFbx in exercise-trained animals vs sedentary animals post myocardial infarction, which induces cardiac atrophy. The lack of a dif-

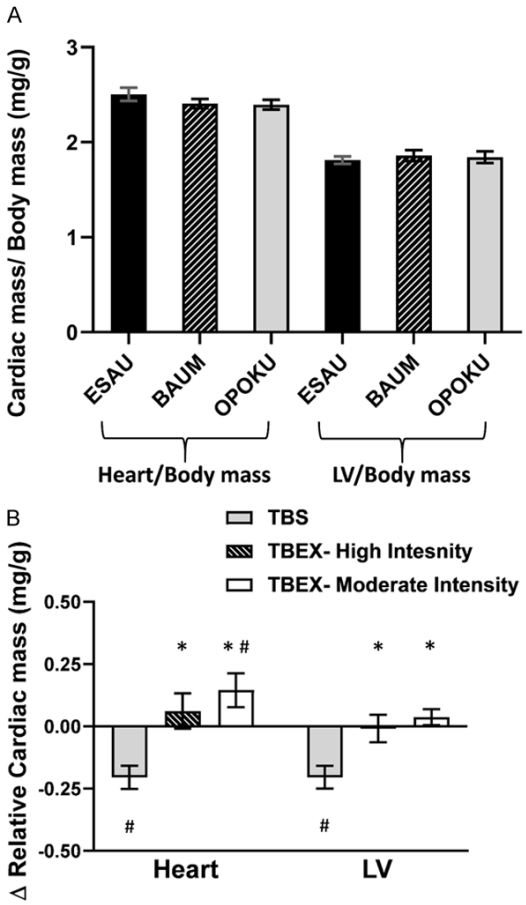

Figure 6. Comparison to non-tumor bearing groups. (A) Historical Control animal data (same age and sex) for heart and left ventricle mass normalized to body mass from [11, 13, 28] tumor bearing, n=9. (B) Changes in heart and left ventricle (LV) mass normalized to body mass, compared to historical control animals in (A). (represented by the solid x-axis line at zero) and moderate-intensity trained animal data from [11, 13, 28] tumor bearing, n=9 (TBS; n=9 TBEX-High intensity; n=15, TBEX-Moderate intensity; n=10). *P<0.05 vs. TBS, #P<0.05 vs. Historical Control.

ference between groups for MAFbx was unexpected however, there are other UPS targets, such as muscle RING-finger protein-1 (MuRF-1), that are associated with both atrophy and cachexia [46, 47].

Nrf-2 was hypothesized to be higher in the TBEX group supporting a less pro-ubiquitination environment [48] via the mutual antagonistic relationship between pro-ubiquitin nuclear factor kappa B (NF-$_{\kappa}$B) and Nrf-2 [49, 50]. With increased oxidative stress signals, Nrf-2 is activated through Kelch-like ECH-associated protein 1 (Keap1) unbinding via conformational changes in reactive cysteines [51]. NF-$_{\kappa}$B has been shown to be a pre-cursor to the increased UPS activity leading to dominant atrophy mechanisms [48, 52], and the negative regulation (via increased Nrf-2) associated with exercise training would possibly buffer this pro-ubiquitin effect. However, we did not observe a detectable increase in Nrf-2 expression in the LV of exercise-trained vs. sedentary animals with prostate cancer. This may be due to a significant increase in the NF-$_{\kappa}$B that occurs with prostate cancer, [53, 54] counteracting any differences exercise training might have upon inducing Nrf-2.

Prostate cancer and cathepsins

Cysteine cathepsins are a large group of proteases of the papain family that, among many proteases, regulate a multitude of functions (i.e., cell proliferation, migration, tissue remodeling etc.) [55]. In prostate cancer cathepsins increase invasion and risk of metastasis [56, 57]. Additionally, cathepsin L has been implicated in cardiovascular disease (i.e., heart failure, dilated cardiomyopathy, atherosclerosis) and an overexpression of cathepsin L in cardiomyocytes has been associated with degradation and dysfunction of the extracellular matrix, and a reduced expression leads to abnormal hypertrophic remodeling [55, 58]. The unaltered expression of both cathepsin B and cathepsin L in the heart with training indicate that the lysosomal abnormalities associated with the tumor microenvironment and surrounding tissue might not extend to the left ventricle.

Left ventricular function in prostate cancer and exercise

Contrary to our hypothesis, there were no changes in many parameters from non-invasive measures of cardiac function and mechanics in the TBEX versus TBS groups, despite significant differences in relative heart and LV mass between groups. Echocardiography showed longitudinal improvements in LVEDV and SV for TBEX animals not observed in their corresponding sedentary group (**Figure 3**), which was likely a function of the larger LV mass (**Figure 4**). Subsequently, the measures of LV function herein reflect a non-stressed condition, from which subclinical alterations in func-

tion may not be detected. However, there is clear evidence that there is a decrease in relative heart mass, LV mass, and endurance capacity with prostate cancer in a murine model [11, 13].

Limitations

Several limitations from this study should be addressed. 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 [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 trained phenotype [61]. With the significantly higher intensity used in this study, a training adaptation of elevated citrate synthase was obtained rapidly as evidenced by significant differences in oxidative muscle citrate synthase activity (**Table 1**). The lack of differences in markers of atrophy between groups may be due to the low tumor burden in these animals (**Table 1**). Larger tumor burdens can induce a cachectic condition and may interfere with the completion of an exercise regimen (due to altered gait, or potential pain with ambulation). Nonetheless, the lower relative heart mass in the sedentary group vs. historic non-tumor bearing animals (**Figure 6**) demonstrates even a low tumor burden can induce cardiac abnormalities. Lastly, the lack of a sham or sedentary control group is noted. The strain of animal used in the study was discontinued by the vendor before completions of the study. Therefore, we used historical control/sham of the same strain, sex, and age for comparison.

Conclusions

In summary, this investigation demonstrates that high-intensity exercise can attenuate cardiac atrophy associated with prostate cancer, independent of therapy. Specifically, high-intensity exercise mitigated relative changes in cardiac mass, LV mass, and increased overall stroke volume. Such improvements may benefit patients and supports exercise as a valuable component of cancer patient care as increasing acknowledgement that different cardiac phenotypes are associated with distinct clinical outcomes [62, 63]. Lastly, the protein expression of the atrophy markers investigated herein were not different between groups. Specifically, UPS protein MAFbx was not different between groups, which is contrary to previous findings of cachexia and atrophy [46, 58, 64]. Further, protein expression of Cathepsin B, Cathepsin L, and Nrf-2 were unaltered between TBS and TBEX animals. However, these markers are a few of the components of different atrophy-related pathways that can be promoting the cardiac atrophy associated with prostate cancer, and further investigation is required to determine the dominant mechanisms contributing to cardiac alterations occurring with prostate cancer.

Acknowledgements

We gratefully acknowledge Joe Pyle, and Taylor Rand for their support during rat training, and Tyler Hopkins for his help with assay protocols. This work was supported by the American Cancer Society (RSG-14-150-01-CCE to BJB), National Heart, Lung, and Blood Institute (R15-HL137156-01A1 to BJB), and Johnson Cancer Research Center Heart Grant (BJB, DB).

Disclosure of conflict of interest

None.

Address correspondence to: Dryden R Baumfalk, Department of Kinesiology, Kansas State University, 143E Justin Hall, Manhattan 66506, Kansas, USA. Tel: 785-443-2371; E-mail: dryden32@ksu.edu

References

[1] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7-34.

[2] Morrow GR, Andrews PL, Hickok JT, Roscoe JA and Matteson S. Fatigue associated with cancer and its treatment. Support Care Cancer 2002; 10: 389-398.

[3] Galvao DA, Taaffe DR, Spry N, Joseph D and Newton RU. Combined resistance and aerobic exercise program reverses muscle loss in men undergoing androgen suppression therapy for prostate cancer without bone metastases: a randomized controlled trial. J Clin Oncol 2010; 28: 340-347.

[4] Wall BA, Galvão DA, Fatehee N, Taaffe DR, Spry N, Joseph D and Newton RU. Reduced cardiovascular capacity and resting metabolic rate in men with prostate cancer undergoing androgen deprivation: a comprehensive cross-sectional investigation. Adv Urol 2015; 2015: 976235.

[5] Ewer MS and Ewer SM. Cardiotoxicity of anticancer treatments. Nat Rev Cardiol 2015; 7: 564.
[6] O'Farrell S, Garmo H, Holmberg L, Adolfsson J, Stattin P and Van Hemelrijck M. Risk and timing of cardiovascular disease after androgen-deprivation therapy in men with prostate cancer. J Clin Oncol 2015; 11: 1243-1251.
[7] Scott JM, Nilsen TS, Gupta D and Jones LW. Exercise therapy and cardiovascular toxicity in cancer. Circulation 2018; 137: 1176-1191.
[8] Hofman M, Ryan JL, Figueroa-Moseley CD, Jean-Pierre P and Morrow GR. Cancer-related fatigue: the scale of the problem. Oncologist 2007; 12 Suppl 1: 4-10.
[9] McCullough DJ, Nguyen LM, Siemann DW and Behnke BJ. Effects of exercise training on tumor hypoxia and vascular function in the rodent preclinical orthotopic prostate cancer model. J Appl Physiol 2013; 115: 1846-1854.
[10] Segal RJ, Reid RD, Courneya KS, Sigal RJ, Kenny GP, Prud'Homme DG, Malone SC, Wells GA, Scott CG and Slovinec D'Angelo ME. Randomized controlled trial of resistance or aerobic exercise in men receiving radiation therapy for prostate cancer. J Clin Oncol 2009; 27: 344-351.
[11] Esau PJ, Gittemeier EM, Opoku-Acheampong AB, Rollins KS, Baumfalk DR, Poole DC, Musch TI, Behnke BJ and Copp SW. Prostate cancer reduces endurance exercise capacity in association with reductions in cardiac and skeletal muscle mass in the rat. Am J Cancer Res 2017; 7: 2566.
[12] Alves CR, da Cunha TF, da Paixão NA and Brum PC. Aerobic exercise training as therapy for cardiac and cancer cachexia. Life Sci 2015; 125: 9-14.
[13] Baumfalk DR, Opoku-Acheampong AB, Caldwell JT, Ade CJ, Copp SW, Musch TI and Behnke BJ. Effects of prostate cancer and exercise training on left ventricular function and cardiac and skeletal muscle mass. J Appl Physiol 2018; 126: 668-680.
[14] Murphy KT. The pathogenesis and treatment of cardiac atrophy in cancer cachexia. Am J Physiol Circ Physiol 2016; 310: 466.
[15] Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, Nieman DC and Swain DP. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc 2011; 43: 1334-1359.
[16] Padrão AI, Nogueira-Ferreira R, Vitorino R, Carvalho D, Correia C, Neuparth MJ, Pires MJ, Faustino-Rocha AI, Santos LL, Oliveira PA, Duarte JA, Moreira-Gonçalves D and Ferreira R. Exercise training protects against cancer-induced cardiac remodeling in an animal model of urothelial carcinoma. Arch Biochem Biophys 2018; 645: 12-18.
[17] Wiggins JM, Opoku-Acheampong AB, Baumfalk DR, Siemann DW and Behnke BJ. Exercise and the tumor microenvironment: potential therapeutic implications. Exerc Sport Sci Rev 2018; 46: 56-64.
[18] Jones LW, Viglianti BL, Tashjian JA, Kothadia SM, Keir ST, Freedland SJ, Potter MQ, Moon EJ, Schroeder T, Herndon JE and Dewhirst MW. Effect of aerobic exercise on tumor physiology in an animal model of human breast cancer. J Appl Physiol 2010; 108: 343-348.
[19] Schadler KL, Thomas NJ, Galie PA, Bhang DH, Roby KC, Addai P, Till JE, Sturgeon K, Zaslavsky A, Chen CS and Ryeom S. Tumor vessel normalization after aerobic exercise enhances chemotherapeutic efficacy. Oncotarget 2016; 7: 65429.
[20] Morrell MBG, Alvarez-Florez C, Zhang A, Kleinerman ES, Savage H, Marmonti E, Park M, Shaw A and Schadler KL. Vascular modulation through exercise improves chemotherapy efficacy in Ewing sarcoma. Pediatr Blood Cancer 2019; 66: e27835.
[21] Antunes JMM, Ferreira RMP and Moreira-Gonçalves D. Exercise training as therapy for cancer-induced cardiac cachexia. Trends Mol Med 2018; 24: 709-727.
[22] Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, Nieman DC and Swain DP; American College of Sports Medicine. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc 2011; 43: 1334-1359.
[23] Harber MP, Kaminsky LA, Arena R, Blair SN, Franklin BA, Myers J and Ross R. Impact of cardiorespiratory fitness on all-cause and disease-specific mortality: advances since 2009. Prog Cardiovasc Dis 2017; 60: 11-20.
[24] Pedersen BK and Saltin B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. Scand J Med Sci Sports 2015; 25: 1-72.
[25] Gardner JR, Livingston PM and Fraser SF. Effects of exercise on treatment-related adverse effects for patients with prostate cancer receiving androgen-deprivation therapy: a systematic review. J Clin Oncol 2014; 32: 335-46.
[26] Parsons JK. Prostate cancer and the therapeutic benefits of structured exercise. J Clin Oncol 2014; 32: 271-2.
[27] Isaacs JT, Heston WD, Weissman RM and Coffey DS. Animal models of the hormone-sen-

sitive and-insensitive prostatic adenocarcinomas, dunning R-332T-H, R-3327-HI, and R-3327-AT. Cancer Res 1978; 38: 4353-4359.
[28] Opoku-Acheampong AB, Baumfalk DR, Horn AG, Kunkel ON, Ganta CK, McCullough DJ, Siemann DW, Muller-Delp J and Behnke BJ. Prostate cancer cell growth characteristics in serum and prostate-conditioned media from moderate-intensity exercise-trained healthy and tumor-bearing rats. Am J Cancer Res 2019; 9: 650.
[29] Sano Y, Ito S, Yoneda M, Nagasawa K, Matsuura N, Yamada Y, Uchinaka A, Bando YK, Murohara T and Nagata K. Effects of various types of anesthesia on hemodynamics, cardiac function, and glucose and lipid metabolism in rats. Am J Physiol Circ Physiol 2016; 311: 1360-1366.
[30] Stein AB, Tiwari S, Thomas P, Hunt G, Levent C, Stoddard MF, Tang XL, Bolli R and Dawn B. Effects of anesthesia on echocardiographic assessment of left ventricular structure and function in rats. Basic Res Cardiol 2007; 102: 28-41.
[31] Pawlush DG, Moore RL, Musch TI and Davidson WR Jr. Echocardiographic evaluation of size, function, and mass of normal and hypertrophied rat ventricles. J Appl Physiol (1985) 1993; 74: 2598-2605.
[32] Craig JC, Colburn TD, Caldwell JT, Hirai DM, Tabuchi A, Baumfalk DR, Behnke BJ, Ade CJ, Musch TI and Poole DC. Central and peripheral factors mechanistically linked to exercise intolerance in heart failure with reduced ejection fraction. Am J Physiol Circ Physiol 2019; 317: 434-444.
[33] Teichholz LE, Kreulen T, Herman MV and Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence or absence of asynergy. Am J Cardiol 1976; 37: 7-11.
[34] Musch TI, Eklund KE, Hageman KS and Poole DC. Altered regional blood flow responses to submaximal exercise in older rats. J Appl Physiol 2004; 96: 81-88.
[35] Musch TI, Bruno A, Bradford GE, Vayonis A and Moore RL. Measurements of metabolic rate in rats: a comparison of techniques. J Appl J Appl Physiol (1985) 1988; 65: 964-970.
[36] Srere PA. Citrate Synthase. Methods Enzymol 1969; 13: 3-11.
[37] Moreira JB, Bechara LR, Bozi LH, Jannig PR, Monteiro AW, Dourado PM, Wisløff U and Brum PC. High-versus moderate-intensity aerobic exercise training effects on skeletal muscle of infarcted rats. J Appl Physiol 2013; 114: 1029-1041.
[38] Wisløff U, Støylen A, Loennechen JP, Bruvold M, Rognmo Ø, Haram PM, Tjønna AE, Helgerud J, Slørdahl SA, Lee SJ, Videm V, Bye A, Smith GL, Najjar SM, Ellingsen Ø and Skjærpe T. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. Circulation 2007; 115: 3086-3094.
[39] Hannan A, Hing W, Simas V, Climstein M, Coombes J, Jayasinghe R, Byrnes J and Furness J. High-intensity interval training versus moderate-intensity continuous training within cardiac rehabilitation: a systematic review and meta-analysis. Open Access J Sport Med 2018; 9: 1-17.
[40] Rognmo O, Moholdt T, Bakken H, Hole T, Mølstad P, Myhr NE, Grimsmo J and Wisløff U. Cardiovascular risk of high-versus moderate-intensity aerobic exercise in coronary heart disease patients. Circulation 2012; 126: 1436-1440.
[41] Conraads VM, Pattyn N, De Maeyer C, Beckers PJ, Coeckelberghs E, Cornelissen VA, Denollet J, Frederix G, Goetschalckx K, Hoymans VY, Possemiers N, Schepers D, Shivalkar B, Voigt JU, Van Craenenbroeck EM and Vanhees L. Aerobic interval training and continuous training equally improve aerobic exercise capacity in patients with coronary artery disease: the SAINTEX-CAD study. Int J Cardiol 2015; 179: 203-210.
[42] Wewege MA, Ahn D, Yu J, Liou K and Keech A. High-intensity interval training for patients with cardiovascular disease-is it safe? A systematic review. J Am Heart Assoc 2018; 7: 21.
[43] Alberga AS, Segal RJ, Reid RD, Scott CG, Sigal RJ, Khandwala F, Jaffey J, Wells GA and Kenny GP. Age and androgen-deprivation therapy on exercise outcomes in men with prostate cancer. Support Care Cancer 2012; 20: 971-981.
[44] Dolan LB, Gelmon K, Courneya KS, Mackey JR, Segal RJ, Lane K, Reid RD and McKenzie DC. Hemoglobin and aerobic fitness changes with supervised exercise training in breast cancer patients receiving chemotherapy. Cancer Epidemiol Biomarkers Prev 2010; 19: 2826-2832.
[45] Adams V, Linke A, Gielen S, Erbs S, Hambrecht R and Schuler G. Modulation of Murf-1 and MAFbx expression in the myocardium by physical exercise training. Eur J Prev Cardiol 2008; 15: 293-299.
[46] Bodine SC and Baehr LM. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. Am J Physiol Endocrinol Metab 2014; 307: E469-84.
[47] Judge SM, Wu CL, Beharry AW, Roberts BM, Ferreira LF, Kandarian SC and Judge AR. Genome-wide identification of FoxO-dependent gene networks in skeletal muscle during C26 cancer cachexia. BMC Cancer 2014; 14: 997.

[48] Muthusamy VR, Kannan S, Sadhaasivam K, Gounder SS, Davidson CJ, Boeheme C, Hoidal JR, Wang L and Rajasekaran NS. Acute exercise stress activates Nrf2/ARE signaling and promotes antioxidant mechanisms in the myocardium. Free Radic Biol Med 2012; 52: 366-376.
[49] Wardyn JD, Ponsford AH and Sanderson CM. Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways. Biochem Soc Trans 2015; 43: 621-626.
[50] Reed SA, Senf SM, Cornwell EW, Kandarian SC and Judge AR. Inhibition of IkappaB kinase alpha (IKKα) or IKKbeta (IKKβ) plus forkhead box O (Foxo) abolishes skeletal muscle atrophy. Biochem Biophys Res Commun 2011; 405: 491-496.
[51] Baldelli S, Aquilano K and Ciriolo MR. Punctum on two different transcription factors regulated by PGC-1α: Nuclear factor erythroid-derived 2-like 2 and nuclear respiratory factor 2. Biochim Biophys Acta 2013; 1830: 4137-4146.
[52] Smuder AJ, Hudson MB, Nelson WB, Kavazis AN and Powers SK. Nuclear factor-κB signaling contributes to mechanical ventilation-induced diaphragm weakness*. Crit Care Med 2012; 40: 927-934.
[53] Palayoor ST, Youmell MY, Calderwood SK, Coleman CN and Price BD. Constitutive activation of IκB kinase α and NF-κB in prostate cancer cells is inhibited by ibuprofen. Oncogene 1999; 18: 7389-7394.
[54] Staudt LM. Oncogenic activation of NF-kappaB. Cold Spring Harb Perspect Biol 2010; 2: a000109.
[55] Fonović M and Turk B. Cysteine cathepsins and their potential in clinical therapy and biomarker discovery. Proteomics Clin Appl 2014; 8: 416-426.
[56] Löser R and Pietzsch J. Cysteine cathepsins: their role in tumor progression and recent trends in the development of imaging probes. Front Chem 2015; 3: 37.
[57] Sudhan DR, Pampo C, Rice L and Siemann DW. Cathepsin L inactivation leads to multimodal inhibition of prostate cancer cell dissemination in a preclinical bone metastasis model. Int J Cancer 2016; 138: 2665-2677.
[58] Tian M, Asp ML, Nishijima Y and Belury MA. Evidence for cardiac atrophic remodeling in cancer-induced cachexia in mice. Int J Oncol 2011; 39: 5.
[59] la Garza MS, Rubies C, Batlle M, Bijnens BH, Mont L, Sitges M and Guasch E. Severity of structural and functional right ventricular remodeling depends on training load in an experimental model of endurance exercise. Am J Physiol Circ Physiol 2017; 313: H468.
[60] Weiner RB and Baggish AL. Exercise-induced cardiac remodeling. Prog Cardiovasc Dis 2012; 54: 380-386.
[61] Kregel KC, Allen DL, Booth FW, Fleshner MR, Henriksen EJ, Musch TI, O'Leary DS, Parks CM, Poole DC and Ra'anan AW. Resource book for the design of animal exercise protocols. Am Physiol Soc 2006; 152: 23.
[62] Ernande L, Audureau E, Jellis CL, Bergerot C, Henegar C, Sawaki D, Czibik G, Volpi C, Canoui-Poitrine F, Thibault H, Ternacle J, Moulin P, Marwick TH and Derumeaux G. Clinical implications of echocardiographic phenotypes of patients with diabetes mellitus. J Am Coll Cardiol 2017; 70: 1704-1716.
[63] Sweeney M, Yiu A and Lyon AR. Cardiac atrophy and heart failure in cancer. Card Fail Rev 2017; 3: 62.
[64] Springer J, Tschirner A, Haghikia A, von Haehling S, Lal H, Grzesiak A, Kaschina E, Palus S, Potsch M, von Websky K, Hocher B, Latouche C, Jaisser F, Morawietz L, Coats AJ, Beadle J, Argiles JM, Thum T, Foldes G, Doehner W, Hilfiker-Kleiner D, Force T and Anker SD. Prevention of liver cancer cachexia-induced cardiac wasting and heart failure. Eur Heart J 2014; 35: 932-941.