Prostate cancer, exercise, and the heart: therapeutic implications & cardiovascular complications

by

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B.S., Kansas State University, 2016
B.S., Kansas State University, 2016
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AN ABSTRACT OF A DISSERTATION

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Abstract

Current projections for 2021 indicate ~1.8 million individuals will be diagnosed with non-skin cancer in the United States. Of those, over 800,000 will be men with over 20% of them being new prostate cancer diagnosis. Prostate cancer is often slow growing and can go undetected for years and may not be medically treated when found if low disease progression is exhibited. Specific to the tumor, these solid tumor's ability to adapt and resist therapy cornerstones such as radiation, that over 75% of all cancer patients receive, makes them difficult to treat in advanced disease. Prostate cancer specifically is a solid tumor that is known for the staples of hypoxia, necrosis, aberrant unfunctional vasculature, that lead to treatment resistance. In the following experiments presented in my dissertation, we investigate these challenges to treatment using an orthotopic model of prostate cancer in rats utilizing multiple cell lines that have similar characteristics as human prostate cancer. Due to preliminary data indicating the ability of chronic aerobic exercise to mitigate regions of hypoxia, in chapter one we sought to determine the distinct changes in tumor hypoxia and cell death to both moderate, high-intensity exercise training regimens in both the core and periphery of tumors. Further, we sought to test the possibility of improved radiosensitivity following both a moderate exercise training regimen, and a single acute bout of exercise in (Chapter 2). We found that both moderate, high intensity exercise training protocols can mitigate tumor hypoxia and improve markers of cell death. Subsequently, when exposed to a single 2gy dose of radiation, both moderate exercise training, and a single acute bout of exercise were able to increase radiosensitivity in tumor bearing rats. Outside of tumor specific responses to exercise, approximately 40% of patients with cancer report symptoms of fatigue, 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. Given cancer-related fatigue or cardiovascular abnormalities can compromise the completion of anti-cancer treatment regimes, it is clinically important to understand how cancer affects determinants of exercise capacity (e.g., cardiac mass and function and skeletal muscle mass). Prior work has shown that prostate cancer in an orthotopic model, can induce cardiac atrophy in the absence of anti-cancer therapies. The ability of moderate intensity exercise training was able to prevent this, however, this led to the hypothesis for the third investigation that high-intensity aerobic exercise training can prevent heart and skeletal muscle atrophy associated with prostate cancer. We found that that high intensity exercise training regime was able to prevent cardiac atrophy associated with prostate cancer, in a shorter time frame than moderate intensity exercise. The product of these three investigations provide insights into the impact of exercise on both treatment, and prevention of cardiac dysfunction in a rat orthotopic model of prostate cancer.

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Abstract

Current projections for 2021 indicate ~1.8 million individuals will be diagnosed with non-skin cancer in the United States. Of those, over 800,000 will be men with over 20% of them being new prostate cancer diagnosis. Prostate cancer is often slow growing and can go undetected for years and may not be medically treated when found if low disease progression is exhibited. Specific to the tumor, these solid tumor's ability to adapt and resist therapy cornerstones such as radiation, that over 75% of all cancer patients receive, makes them difficult to treat in advanced disease. Prostate cancer specifically is a solid tumor that is known for the staples of hypoxia, necrosis, aberrant unfunctional vasculature, that lead to treatment resistance. In the following experiments presented in my dissertation, we investigate these challenges to treatment using an orthotopic model of prostate cancer in rats utilizing multiple cell lines that have similar characteristics as human prostate cancer. Due to preliminary data indicating the ability of chronic aerobic exercise to mitigate regions of hypoxia, in chapter two we sought to determine the distinct changes in tumor hypoxia and cell death to both moderate, high-intensity exercise training regimens in both the core and periphery of tumors. Further, we sought to test the possibility of improved radiosensitivity following both a moderate exercise training regimen, and a single acute bout of exercise in (Chapter 3). We found that both moderate, high intensity exercise training protocols can mitigate tumor hypoxia and improve markers of cell death. Subsequently, when exposed to a single 2gy dose of radiation, both moderate exercise training, and a single acute bout of exercise were able to increase radiosensitivity in tumor bearing rats. Outside of tumor specific responses to exercise, approximately 40% of patients with cancer report symptoms of fatigue, however, elucidating mechanisms of fatigue or atrophy with cancer versus concurrent adjuvant therapies is difficult, as withholding treatment to study the

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Preface

Chapter 1 is a brief background into this body of work. Chapter 2 represents a study that is in the final stages of data analysis and being prepared for submission. Chapter 3 represents a completed study, that is in preparation to be submitted for peer review. Chapter 4 of my dissertation is an original research article that has been published following the peer-review process and is reproduced here with the permission from the publisher.

Chapter 1 - Background

Even amid a global pandemic, cancer is one of the most studied diseases in the world today. Deservedly so, as it accounts for the second most deaths (~10 million) in the world annually only to cardiovascular disease [1,2]. The study and treatment of tumors dates to The Edwin Smith Papyrus of ancient Egypt, and the Father of Medicine (Hippocrates) describing tumors as karkinos, in which the term carcinoma was born [3,4]. Although found throughout the body, little was known about the etiology of the disease, how to treat it, and many even believed it to be contagious through the 18th century [4]. This century also became a well-known time of epidemiological improvement through observance, specifically in two instances, one of which was of high rates of both breast and cervical cancer occurrence among nuns thought to be due to some factor associated with celibacy. Secondly, the observations of Percival Pott of scrotal cancers being linked to men working as chimney sweeps was seminal in the association of cancer with environmental factors. Both instances pushed the etiology of tumors a little further and helped pioneer new ideas of treatment and prevention outside of Galenic type surgery and mystery tonics [4]. Although, still often used in treatment of primary tumors, surgery, even of a radical nature as presented by William Halstead, was unable to halt many forms of this disease. Soon many forms of additional treatments arose (radiation, chemotherapy, etc.) in the late 19th and 20th century [4,5]. As the individual use or combination of surgery, chemotherapy, and radiotherapy approaches to treat cancer began to help cure some instances, upwards of a 70% overall failure rate led President Nixon to declare a war on cancer in 1971 [5], and 50 years later nearly $200 billion dollars has been spent on fighting this lethal disease. Following this short history, the rest of this review will be directed at prostate cancer, with the goal to expand on some nuances of the disease, and the impact exercise has had in both potential treatment outcomes, and quality of life in cancer patients that has become a marked area interest.

Currently, in the United States there has been ~1.8 million individuals diagnosed with non-skin cancer. Of those 800,000 will be men with ~20% of them being new prostate cancer diagnosis [6]. Prostate cancer is often slow growing and can go undetected for years and may not be medically treated when found if low disease progression is exhibited. If localized to the prostate, the disease can often be cured, however, if it has spread to the pelvic bones or elsewhere outside the prostate, 5-year survival drops over 60% [6,7]. The primary risk factors for

prostate cancer are age, obesity, ethnicity, and family history, with African American's having the highest prevalence of this disease. Prostate cancer is often defined as androgen sensitive, or insensitive, which can dictate additional treatment in the form of either physical or chemical androgen deprivation therapy (ADT) outside the usual surgical interventions and radiotherapy [7]. Early detection is key for this disease, and diagnosis is primarily based on prostate-specific antigen (PSA) testing and transrectal ultrasound-guided imaging [8]. With most patients having a high rate (98%) of 5-year disease free survival with early detection, it often does not receive the same "death sentence" other cancers carry such as pancreatic or lung cancers [7]. This is an overall positive outlook for those with prostate cancer, however, prostate cancer is a solid tumor forming adenocarcinoma that often cause therapeutic hurdles in its treatment, that occur at the level of the tumor microenvironment. Due to ethical constraints in humans, pre-clinical models have been developed *in-vivo* to study the tumor microenvironment that recapitulate the human condition.

The model used herein and throughout this dissertation is an orthotopic model that resembles human prostate cancer progression and disease state first introduced in the 1970's [9,10]. This model is not the predominant model in the field, as subcutaneous models are often used for their ease and ability to generate tumors with some similarities to human cancer [11], but these ectopic models of cancer have many concerns including regional and hormonal signaling, blood flow, vascularization, and growth that differ from orthotopic tumors [12–14]. Various animal models and cancer cell lines expressing different characteristics [9,15] have been used throughout the years to study the tumor microenvironment either subcutaneously, or orthotopically across multiple cancer types. This model of prostate cancer remains a well-established and valuable medium for studying solid prostate tumors [9,12]. The tumor microenvironment of solid tumors is often a large part of why anti-cancer therapies fail [16]. Tumors grown beyond $2mm^3$ require their own blood vessel network to continue to grow, and develop vasculature that is aberrant, and often porous that when combined with high interstitial pressure of tumors can result in poor blood flow [17,18]. One critical nutrient tumors are often lacking is oxygen, that often leads to areas of hypoxic cells either transiently or chronically, that can both lead to treatment resistance to conventional anticancer therapies and prevent control of the disease [14,19]. Tumor hypoxia often leads to a more aggressive phenotype (regarding growth and metastasis) and is thought to be due to the release of a hypoxia-inducible factors

(HIF) [20,21]. HIF is a heterodimer split into several subunits (α-1,2,3 & β1) that sense changes in O_2 levels, and upon low oxygen conditions, will translocate to the nucleus [22,23]. Of the different subunits, HIF-1α and HIF-2α are typically associated with acute, and chronic hypoxia respectively, which can give insights into the types of modifications occurring in the tumor microenvironment. These areas of hypoxia in solid tumors present major challenges to radiotherapy strategies, as oxygen is a powerful radiosensitizer, with anoxic cells requiring 3x the dose of radiation of that of a normal cell [16,24,25]. The upregulation of HIF in solid tumors is evident in the disease treatment and progression and is an area of clinical concern. A variety of methods have been used to mitigate hypoxia of solid tumors from vascular growth inhibitors [26], erythropoietin infusion[27], and hyperthermia [28] with mixed results [25]. Recently, McCullough et al demonstrated that due in part to the lack of functional resistance vasculature of prostate tumors, an increase in the perfusion pressure from exercise could increase blood flow to a tumor acutely, enhancing oxygenation of the tumor and reducing hypoxia [18]. Further, this decrease in hypoxia was also found in chronic aerobic exercise training as tumor vasculature was less aberrant and possibly provided a more homogenous distribution of blood flow following aerobic exercise training [29,30]. This provided the first evidence that exercise, and chronic exercise training could manipulate the tumor microenvironment favorably (in respect to blood flow and oxygenation) to possibly improve radiation therapy and was the groundwork for this dissertation project.

Complications outside of the tumor itself often arise with cancer and present major challenges to patients. With the increased metabolic demand and protein turnover of the aberrant tumor microenvironment [30], patients often exhibit an alarming amount of weight loss before or during treatment of various forms of cancer [31–34]. This weight loss and atrophy can be severe (cachexia) which is defined as a loss of 5% of body mass (with or without fat loss) without the presence of edema within 12 months due to an underlying cause of disease [34,35]. Anywhere between 40-80% of cancer patients are afflicted with cachexia and 30% of all cancer deaths are directly related to this cachectic condition [33,36]. These reductions in cardiac and skeletal muscle mass have been implicated in the cause of fatigue and loss of quality of life in patients, with mechanisms of atrophy thought to be due to a loss of balance in protein turnover and synthesis [33,35,37]. Several molecular pathways have been investigated regarding reductions in

both skeletal and cardiac masses during cachectic states [33,38,39]. However, a particular set of markers required for skeletal muscle atrophy is Forkhead Box O (FoxO) that upregulate the effects of ubiquitin E3 ligases of atrogin-1/f-box1 (MAFbx) and muscle ring figner-1 (Murph-1) [38,39]. This pathway is often associated with inflammatory conditions and has been associated with upregulation of inflammatory markers tumor necrosis factor alpha (TNF-α), interleukin six (IL-6), and increased ROS [33,40,41]. These effects are often seen in conjunction with therapy [33,35,40], and not separated from the disease itself. Prostate cancer patients often experience loss of quality of life, fatigue, and a myriad of health alterations throughout disease progression and treatment [36,42]. Indeed, in a pre-clinical model Esau and colleagues made the discovery that there was a decrease in cardiac and skeletal muscle mass associated with prostate cancer, in lieu of loss of body mass or anti-cancer treatments that are often associated with fatigue or atrophy [43,46]. This cardiac atrophy was associated with a decreased time to exhaustion in tumor bearing animals compared to sham operated controls [46], indicating a role in early symptoms of fatigue seen in with cancer. This led to further investigations by our laboratory into the reductions in cardiac mass associated with prostate cancer, and the ability of exercise to possibly prevent this [47]. Chapter 4 of this dissertation expands upon these findings and directly investigates the roles of E3 ligases and lysosomal markers of atrophy.

Exercise, like cancer, comes back to ancient roots where daily exercise was first recommended by Sushruta of India. Hippocrates was one of the first to study exercise (as he was with cancer) in disease treatment and advocated for exercise use in alleviating or preventing disease [47]. Exercise has undergone its own evolution since then, and today is a major industry and is also severely lacking from most Americans routines with less than 40% of the population meeting exercise guidelines [48,49]. This poses a major health problem as regular physical activity and exercise have a plethora of health benefits in terms of both increasing normal quality of life and cognitive function, decreasing the risk of cardiovascular disease, some forms of cancer, and minimizing the risk of dementia [49,50]. With the benefits of habitual exercise and physical activity being well established, the type, intensity, and duration of exercise and physical activity for health benefits has been debated over the years with a consensus being met of 150 minutes of moderate aerobic physical or exercise per week, or 75 minutes of intense aerobic exercise. This should also be combined with at least 2 resistance exercise sessions to maintain skeletal muscle mass [49,51]. Cancer is a very challenging disease as it presents complexities in

so many locations and can cause various symptoms that make it difficult to reach consensus exercise recommendations, as many also had safety concerns of implementing exercise for those undergoing treatment (surgery, radiation, chemotherapy etc.) [53]. Although the safety and use of exercise in cancer patient's standard of care is becoming more evident, it remains elusive in its use for cancer treatment efficacy [52–55]. In prostate cancer patients specifically, exercise has been deemed safe, and efficacious in its use for adjuvant therapy at normal recommended levels as allowed with medical treatment [53]. Indeed, exercise (both aerobic and resistance training) has been recently used to address the loss of skeletal muscle mass and fatigue associated with cancer and its treatments (specifically ADT) [43,57].

Although certainly not new in its use for prostate cancer adjuvant therapy, the combination of exercise possibly being effective at both preventing atrophy of cardiac and skeletal muscle, as well as improve radiotherapy outcomes, is the basis for this dissertation. A total of 3 separate investigations will be used to demonstrate the impacts exercise has on the therapeutic outcomes, and the mitigation of cardiac atrophy in a pre-clinical model of prostate cancer. Further, aerobic exercise specifically will be evaluated regarding the intensity, and timing of aerobic exercise interventions used.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians 2021; 71:209–249.
2. Abbafati C, …., Wiangkham T. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet 2020; 396:1204–1222.
3. Karpozilos A, Pavlidis N. The treatment of cancer in Greek antiquity. European Journal of Cancer 2004; 40:2033–2040.
4. Sudhakar A. History of Cancer, Ancient and Modern Treatment Methods. Journal of Cancer Science & Therapy 2009; 01:i–iv.
5. DeVita VT, Rosenberg SA. Two Hundred Years of Cancer Research. New England Journal of Medicine 2012; 366:2207–2214.

6. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA: A Cancer Journal for Clinicians 2021; 71:7–33.
7. Stephen W. Leslie, Taylor L. Soon-Sutton;, Hussain Sajjad;, Larry E Siref. Prostate cancer. Elsevier; 2020.
8. Chang Y, Kim JH, Noh JW, Cho YS, Park HJ, Joo KJ, Ryu S. Prostate-specific antigen within the reference range, subclinical coronary atherosclerosis, and cardiovascular mortality. Circulation Research 2019; 124:1492–1504.
9. Isaacs JT, Heston WDW, Weissman RM, Coffey DS. Animal Models of the Hormone-sensitive and-insensitive Prostatic Adenocarcinomas, Dunning R-332T-H, R-3327-HI, and R-3327-AT. Cancer Research 1978; 38:4353–4359.
10. Lubaroff DM, Culp DA. Experience with an animal model for the study of prostatic carcinoma. Transactions of the American Association of Genito-Urinary Surgeons 1977; 69:72–77.
11. Ramanath Rao B, Nakeff A, Eaton C, Heston WDW. Establishment and Characterization of an in Vitro Clonogenic Cell Assay for the R-3327-At Copenhagen Rat Prostatic Tumor. Cancer Research 1978; 38:.
12. Killion JJ, Radinsky R, Fidler IJ. Orthotopic models are necessary to predict therapy of transplantable tumors in mice. Cancer and Metastasis Reviews 1998; .
13. Garcia E, Becker VGC, McCullough DJ, Stabley JN, Gittemeier EM, Opoku-Acheampong AB, Sieman DW, Behnke BJ. Blood flow responses to mild-intensity exercise in ectopic vs. orthotopic prostate tumors; dependence upon host tissue hemodynamics and vascular reactivity. Journal of Applied Physiology 2016; .
14. Wiggins JM, Opoku-Acheampong AB, Baumfalk DR, Siemann DW, Behnke BJ. Exercise and the Tumor Microenvironment: Potential Therapeutic Implications. Exercise and Sport Sciences Reviews 2018; 46:56–64.
15. Quarmby VE, Beckman WC, Cooke DB, Lubahn DB, Joseph DR, Wilson EM, French FS. Expression and localization of androgen receptor in the R-3327 Dunning rat prostatic adenocarcinoma. Cancer Research 1990; 50:735–9.
16. Vaupel P. Tumor microenvironmental physiology and its implications for radiation oncology. Seminars in Radiation Oncology; Tumor Physiology 2004; 14:198–206.

17. Blood CH, Zetter BR. Tumor interactions with the vasculature: angiogenesis and tumor metastasis. BBA - Reviews on Cancer 1990; 1032:89–118.
18. McCullough DJ, Stabley JN, Siemann DW, Behnke BJ. Modulation of blood flow, hypoxia, and vascular function in orthotopic prostate tumors during exercise. Journal of the National Cancer Institute 2014; .
19. Vaupel P. Hypoxia and Aggressive Tumor Phenotype: Implications for Therapy and Prognosis. The Oncologist 2008; 13:21–26.
20. Vaupel P, Harrison L. Tumor Hypoxia: Causative Factors, Compensatory Mechanisms, and Cellular Response. The Oncologist 2004; 9:4–9.
21. Milosevic M, Chung P, Parker C, Bristow R, Toi A, Panzarella T, Warde P, Catton C, Menard C, Bayley A, Gospodarowicz M, Hill R. Androgen withdrawal in patients reduces prostate cancer hypoxia: Implications for disease progression and radiation response. Cancer Research 2007; 67:6022–6025.
22. Eltzschig HK, Carmeliet P. Hypoxia and inflammation. The New England Journal of Medicine 2011; 364:656–665.
23. LaGory EL, Giaccia AJ. The ever-expanding role of HIF in tumour and stromal biology. Nature Cell Biology 2016; 18:356–365.
24. Gray LH, Conger AD, Ebert M, Hornsey S, Scott OC. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. The British Journal of Radiology 1953; 26:638–648.
25. Cairns R, Papandreou I, Denko N. Overcoming Physiologic Barriers to Cancer Treatment by Molecularly Targeting the Tumor Microenvironment Unique Microenvironment of Solid Tumors. Mol Cancer Res 2006; 4:61–70.
26. Winkler F, Kozin S v., Tong RT, Chae SS, Booth MF, Garkavtsev I, Xu L, Hicklin DJ, Fukumura D, di Tomaso E, Munn LL, Jain RK. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: Role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell 2004; 6:553–563.
27. Henke M, Laszig R, Rübe C, Schäfer U, Haase KD, Schilcher B, Mose S, Beer KT, Burger U, Dougherty C, Frommhold H. Erythropoietin to treat head and neck cancer patients with anaemia undergoing radiotherapy: Randomised, double-blind, placebo-controlled trial. Lancet 2003; 362:1255–1260.

28. Dewhirst MW, Vujaskovic Z, Jones E, Thrall D. Re-setting the biologic rationale for thermal therapy. International Journal of Hyperthermia 2005; 21:779–790.
29. McCullough DJ, Nguyen LM-D, Siemann DW, Behnke BJ. Effects of exercise training on tumor hypoxia and vascular function in the rodent preclinical orthotopic prostate cancer model. Journal of Applied Physiology 2013; 115:1846–1854.
30. Schadler KL, Thomas NJ, Galie PA, Bhang DH, Roby KC, Addai P, Till JE, Sturgeon K, Zaslavsky A, Chen CS, Ryeom S. Tumor vessel normalization after aerobic exercise enhances chemotherapeutic efficacy. Oncotarget 2016; 7:65429.
31. Deuster PA, Morrison SD, Ahrens RA. Endurance exercise modifies cachexia of tumor growth in rats. Medicine and Science in Sports and Exercise 1985; 17:385–392.
32. Barkhudaryan A, Scherbakov N, Springer J, Doehner W. Cardiac muscle wasting in individuals with cancer cachexia. ESC Heart Failure 2017; .
33. Judge SM, Wu CL, Beharry AW, Roberts BM, Ferreira LF, Kandarian SC, Judge AR. Genome-wide identification of FoxO-dependent gene networks in skeletal muscle during C26 cancer cachexia. BMC Cancer 2014; 14:997.
34. Murphy KT. The pathogenesis and treatment of cardiac atrophy in cancer cachexia. American Journal of PhysiologyHeart and Circulatory Physiology 2016; 310:466.
35. Fox KM, Brooks JM, Gandra SR, Markus R, Chiou CF. Estimation of cachexia among cancer patients based on four definitions. Journal of Oncology 2009; .
36. Tisdale MJ. Cachexia in cancer patients. Nature Reviews Cancer 2002; 2:862–871.
37. Charalambous A, Kouta C. Cancer Related Fatigue and Quality of Life in Patients with Advanced Prostate Cancer Undergoing Chemotherapy. BioMed Research International 2016; .
38. Rom O, Reznick AZ. The role of E3 ubiquitin-ligases MuRF-1 and MAFbx in loss of skeletal muscle mass. Free Radical Biology and Medicine 2016; .
39. Bodine SC, Latres E, Baumhueter S, Lai VKM, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Erqian Na, Dharmarajan K, Pan ZQ, Valenzuela DM, Dechiara TM, Stitt TN, Yancopoulos GD, Glass DJ. Identification of ubiquitin ligases required for skeletal Muscle Atrophy. Science, 2001
40. Bodine SC, Baehr LM. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. American Journal of Physiology Endocrinology and Metabolism 2014; 307:E469-84.

41. Kavazis AN, Smuder AJ, Powers SK. Effects of short-term endurance exercise training on acute doxorubicin-induced FoxO transcription in cardiac and skeletal muscle. Journal of Applied Physiology 2014

42. 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, Anker SD. Prevention of liver cancer cachexia-induced cardiac wasting and heart failure. European Heart Journal 2014; 35:932–941.

43. Bourke L, Gilbert S, Hooper R, Steed LA, Joshi M, Catto JW, Saxton JM, Rosario DJ. Lifestyle changes for improving disease-specific quality of life in sedentary men on long-term androgen-deprivation therapy for advanced prostate cancer: a randomised controlled trial. European Urology 2014; 65:865–872.

44. Powers SK, Lynch GS, Murphy KT, Reid MB, Zijdewind I. Disease-induced skeletal muscle atrophy and fatigue. Medicine and Science in Sports and Exercise 2016; 48:2307.

45. Wall BA, Galvão DA, Fatehee N, Taaffe DR, Spry N, Joseph D, Newton RU. Reduced Cardiovascular Capacity and Resting Metabolic Rate in Men with Prostate Cancer Undergoing Androgen Deprivation: A Comprehensive Cross-Sectional Investigation. Advances in Urology 2015

46. Esau PJ, Gittemeier EM, Opoku-Acheampong AB, Rollins KS, Baumfalk DR, Poole DC, Musch TI, Behnke BJ, Copp SW. Prostate cancer reduces endurance exercise capacity in association with reductions in cardiac and skeletal muscle mass in the rat. American Journal of Cancer Research 2017; 7:2566.

47. Baumfalk DR, Opoku-Acheampong AB, Caldwell JT, Ade CJ, Copp SW, Musch TI, Behnke BJ. Effects of prostate cancer and exercise training on left ventricular function and cardiac and skeletal muscle mass. Journal of Applied Physiology 2018; .

48. Tipton CM. The history of “Exercise Is Medicine” in ancient civilizations. Advances in Physiology Education 2015; 38:109–117.

49. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, Nieman DC, Swain DP. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: Guidance for prescribing exercise. Medicine and Science in Sports and Exercise 2011; 1334–1359.

50. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee I-M, Nieman DC, Swain DP. 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. Medicine and Science in Sports and Exercise 2011; 43:1334–1359.
51. Lee DC, Sui X, Ortega FB, Kim YS, Church TS, Winett RA, Ekelund U, Katzmarzyk PT, Blair SN. Comparisons of leisure-time physical activity and cardiorespiratory fitness as predictors of all-cause mortality in men and women. British Journal of Sports Medicine 2011; 45:504–510.
52. Pedersen BK, Saltin B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. Scandinavian Journal of Medicine & Science in Sports 2015; 25:1–72.
53. Wolin KY, Schwartz AL, Matthews CE, Courneya KS, Schmitz KH. Implementing the Exercise Guidelines for Cancer Survivors. Journal of Supportive Oncology 2012; 10:171–177.
54. Physical Activity and Cancer Fact Sheet - National Cancer Institute. Accessed 2021.
55. Ashcraft KA, Peace RM, Betof AS, Dewhirst MW, Jones LW. Efficacy and Mechanisms of Aerobic Exercise on Cancer Initiation, Progression, and Metastasis: A Critical Systematic Review of In Vivo Preclinical Data. Cancer Research 2016; 76:4032–4050.
56. Yang L, Morielli AR, Heer E, Kirkham AA, Cheung WY, Usmani N, Friedenreich CM, Courneya KS. Effects of exercise on cancer treatment efficacy: A systematic review of preclinical and clinical studies. Cancer Research 2021; 81:4889–4895.
57. Galvao DA, Taaffe DR, Spry N, Joseph D, 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. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology 2010; 28:340–347.

Chapter 2 - Effect of moderate and high intensity exercise on hypoxia and radiosensitivity in prostate tumor bearing rats

Abstract

Background: Prostate tumors typically have hypoxic regions that are resistant to traditional radiation therapy. Prior evidence from our laboratory has demonstrated improved perfusion and reduced hypoxia of prostate tumors with moderate intensity exercise training; such changes have potential to enhance radiation therapy. However, there is a dearth of evidence for the magnitude of alterations of intra-tumor hypoxia markers with exercise, as well as the ideal intensity of exercise for combating hypoxia in solid tumors. Using a pre-clinical orthotopic model of prostate cancer, we tested the hypothesis that exercise training at both moderate and high intensities would result in a decrease in hypoxia assessed via immunohistochemical analysis of hypoxic markers. Further, that enhanced radiotherapy responses would occur in exercise trained tumor bearing animals compared to sedentary counterparts. **Methods:** Immunocompetent male Copenhagen rats aged 6 months (n=50) were injected orthotopically (ventral lobe of prostate) with $1x10^5$ Dunning R-3327 AT-1 prostate adenocarcinoma cells. All animals were first acclimated to treadmill exercise and randomized into three groups: tumor bearing sedentary (TBS), moderate intensity training, (TBMIT), or high intensity training (TBHIT). After 7 days of recovery from surgery, both exercise groups began progressive exercise training on a motorized treadmill at either 20/min with a 5% incline, or 30m/min 15% incline for 10 minutes a day progressing to 60 min/day for TBMIT and TBHIT, respectively, for approximately 5 weeks. Immunohistochemical analysis and whole-body ionizing radiation (2Gy) were performed at the end of the 5-week period of training followed by clonogenic cell survival assay to assess survival fraction. **Results:** There were no significant differences (p>0.05) in tumor mass between groups (TBS 7.6±0.92; TBMIT 7.2±1.7; TBHIT 6.6±1.1 g). Overall expression levels of hypoxia inducible factor 1-alpha (HIF-1α, n=42) were significantly greater in the TBS group vs both the TBMIT and TBHIT (TBS 37.9±7.4; TBMIT 25.5±6.1; TBHIT 14.4±5.7%, p<0.05), with a positive correlation of tumor mass and HIF-1α expression TBMIT vs TBHIT (p<0.01). The survival fraction (n=8) was also significantly higher in TBS when compared to both TBMIT and TBHIT (TBS 39.9±5.3; TBMIT 23.2±3.2; TBHIT 20.7±4.9%, p<0.05). **Conclusion:** This study

suggests that compared to sedentary counterparts, both chronic moderate and high intensity exercise training modify the tumor microenvironment (decreased levels of HIF-1α) favorably. Given exercise therapy is becoming increasingly recommended to mitigate fatigue and increase quality of life in patients, a potential therapeutic component could add to the efficacy of exercise prescription in a clinical setting.

Introduction

Prostate cancer is a solid tumor that will account for roughly ~20% of all non-skin cancer diagnoses predicted among men in 2021, accounting for the 2nd most deaths among men annually only to lung cancer [1]. One problematic characterization of solid tumors is hypoxia, which can lead to therapy complications. Hypoxia is often associated with poor tumor vascularization that permits both perfusive and diffusive impairments in oxygenation [2,3], which can be damaging in many cells and is commonly associated with increased expression of hypoxia-inducible factors (HIF) [20,21]. HIF is a heterodimer with several subunits that respond to changes in O2 levels, and upon low oxygen conditions, will translocate to the nucleus [22,23]. Of the different subunits, HIF-1α and HIF-2α are typically associated with acute, and chronic hypoxia respectively which can give insights into the types of modifications occurring in the tumor microenvironment [4]. Tumors possess adapted cells (through expression of factors such as HIF-1α) that lead to resistance to many anti-cancer therapies such as radiation therapy that is employed in ~50% of prostate cancer patients [5]. This is often associated with poor prognosis of cancer patient outcomes [3,6], and elevated levels of HIF-1α is often associated with increased expression of Ki-67, a marker of cellular proliferation that is associated with poor treatment outcomes [7,8]. Both markers are associated with increased aggressiveness and incidences of cancer metastasis[9,10], which could lead to recurrence of the disease [5,10–12]. Increased amounts or fractions of radiation therapy needed in hypoxic tumors can also be detrimental to surrounding tissue that can lead to side effects ranging from fatigue, sexual dysfunction, sore skin/irritation, depression, and overall decline in quality of life in prostate cancer patients [13,14]. This is not a new concern, and a need to improve radiotherapy has existed for many years. As radiotherapy is dependent on sufficient oxygen levels to provide adequate damage to

tumor cells [15], a multitude of methods to mitigate hypoxia of solid tumors from vascular growth inhibitors [16], erythropoietin infusion [17], and/or hyperthermia [18], have led to mixed results in improvements of oxygenation of the tumor [19].

Exercise may provide a novel means to improve cancer treatment outcomes. Although exercise has been shown to be a mainstay of most health associated routines to decrease morbidity and mortality of many diseases [20,21], it is still not a mainstay in the daily or weekly routine of most Americans [20]. Further, the lack of physical activity in men has been shown to increase the risk of prostate cancer [22]. In large clinical studies, prostate cancer patients who engaged in physical activity and/or exercise training either before or after diagnosis tend to have improved treatment outcomes [23]. Animal models have demonstrated that exercise has the potential to attenuate the level of tumor hypoxia [2,24], generate a more homogenous distribution of perfusion [2,25], and a shift towards vascular normalization with and without radiation therapy [25,26]. These modifications to the tumor microenvironment may contribute to increased delivery of chemotherapeutic agents [27] or potentially improve radiosensitization of tumor cells [2]. The relative intensity of exercise for these changes has been debated [2,28], but there is a paucity of data on the intensity of exercise necessary to improve hypoxia or diminish malignant markers of disease progression in tumors, and if different exercise intensities have different efficacy/potential to radiosensitize solid tumors. Therefore, the purpose of this investigation was to determine the efficacy of both moderate- (MIT) and high- (HIT) intensity training protocols to manipulate the tumor microenvironment to favorably radiosensitize prostate tumors in vivo. Specifically, the hypothesis that exercise of both intensities will diminish the expression of hypoxic, proliferative, and apoptotic markers (HIF-1α, HIF-2α, Ki-67, and Caspase-3 respectively) quantified with immunohistochemical (IHC) analysis via a semi-

automated software program (HALO, Indica Labs), while radiosensitization will be assessed via clonogenic colony formation assays.

Methods

Animals

All 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 immune-competent Copenhagen rats (n=50, ~4-6 mo. old; CR/CRL: 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.

Cell culture

Dunning R-3327 AT-1; rat prostate adenocarcinoma cells were brought up from frozen storage and used at passages 4-6. Briefly, AT-1 cells were cultured in 75cm2 cell culture treated flasks (Corning, Durham, NC) using RPMI-1640 media (GE Healthcare Life Sciences, Marlborough, MA) supplemented with 10% fetal bovine serum (FBS; RMBIO, Missoula, MT), 1% PenStrep (100 U/ml Penicillin and 100 µg/ml Streptomycin; Thermo Fisher Scientific, Waltham, MA), 100 mM sodium pyruvate (Thermo Fisher Scientific, Waltham, MA) and 0.025 mM dexamethasone (Cayman Chemical, Ann Arbor, MI) and incubated at 37°C with 5% CO2.

Orthotopic Model of Cancer

Upon reaching ~80% confluence, cells were quantified via hemocytometer to calculate appropriate dilutions (1,000,000 cells/ml) of 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 ~1x10^5 AT-1 cells to induce solid prostate tumors as previously demonstrated [29–31]. Animals were anesthetized quickly with 5%, and then maintained on 2% isoflurane (O_2 balance) Once a deep plane of anesthesia was obtained, (i.e., lack of toe-pinch reflex), with aseptic technique, an abdominal incision of ~1 cm, lateral of the midline, was made exposing the bladder/prostate complex. After the prostate was identified, ~1x105 AT-1 cells suspended in 0.1 ml PSS were injected into the ventral lobe of the prostate using a sterile 26G insulin syringe. During injection a sterile cotton tipped applicator was used to prevent any cell leakage from the prostate. 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).

After final suture placement, isoflurane was terminated and animals were administered 0.05 mg/kg buprenorphine (Patterson Veterinary) and 0.5 mg/kg acepromazine (Patterson Veterinary) S.C. for analgesia and sedation, respectively. Animal health and welfare were monitored ~5 days post-injection twice daily until radomization into their respective groups. Animals (n=50) were separated into sedentary tumor-bearing (TBS; n=21) and moderate intensity exercise training tumor-bearing (TBMIT n=13) and high intensity exercise training tumor-bearing (TBHIT n=16) groups. For IHC, a total of 42 tumors were analyzed for changes in the tumor microenvironment. The remaining 8 animals utilized for clonogenic cell survival assays were as follows, TBS; n=3 TBMIT; n=3 TBHIT n=2, due to the unexpected discontinuation of the animal model, no further animals were available for clonogenic cell survival assays, and data at a single dose of 2gy of radiation is reported here.

Treadmill Exercise

All rats were habituated to treadmill exercise prior to cancer cell injection with a motor-driven rodent treadmill for ≤5 min/day at speeds between 15-25 m/min (0° incline) for 3-5 days. In exercise-trained animals, after the habituation period, both exercise groups began progressive exercise training on a motorized treadmill at either 20/min with a 5% incline, or 30m/min 15% incline for 10 minutes a day progressing to 60 min/day for TBMIT and TBHIT, respectively, for approximately 5 weeks. 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 10th day for all animals. Both exercise groups continued to exercise 5 days/week for 60 min/day for the remainder of the 5-week training period. This training program was modified from previously used protocols [30,31] to represent moderate- and high intensity exercise training eliciting greater than 60%, and 75% of maximal aerobic capacity respectively.

Radiation

For in vivo studies, all animals were subjected to the same environment for radiation before collection of tissues, including being housed in a small plastic cage during whole body irradiation. Radiated and non-radiated animals (for establishment of plating efficiency pre-liminary data collection) were subjected to 2 Gray and 0 Gray (Gy) radiation, respectively, with a linear accelerator (Clinac 2100 CD,Varian Medical Systems, Palo Alto, CA). Low-dose radiation was selected as 2Gy is the dosage of a single fraction that would be administered clinically [5]. Either 24hr after the final bout of exercise, or immediately following radiation,

tissue collections began by arterial blood collection from the abdominal aorta (via puncture) for serum separations in serum separator tubes (Becton, Dickinson and Company, Franklin Lakes, NJ), followed by removal of the heart while the animals were under a deep plane anesthesia (5% isoflurane/O2 balance). Thereafter, tumor, prostate (when delineation from tumor was possible), soleus muscle, and gastrocnemius muscle (separated into red and white portions) were immediately excised, weighed, and either flash-frozen in liquid nitrogen and stored in a freezer (-80°C) for future analyses, or preserved via fixation overnight in 10% neutral-buffered formalin.

Clonogenic Survival

Tumors were harvested within 1 hour after the completion of radiation for determination of clonogenic survival according to the methods of Franken, et al., [32] and Brix, et al., [33]. Tumors were mechanically dissociated in PBS, and then further digested with an enzyme cocktail (in-house, trypsin and collagenase mixture) to a single cell suspension. Cells were then re-suspended in normal media, and counted to determine appropriate dilutions, followed by seeding at varying densities of cells in 60mm and 100mm tissue culture dishes for optimization, and for main experimental conditions, 100 and 500 cells per plate were utilized. Five replicates per plating density that were performed, for a total of 10 plates per animal experiment. Plates were placed in a humidified incubator maintained at 37°C and 5% CO2 for ~10 days for development of cell colonies. Following colony formation, cells were fixed and stained with 20% methanol and 0.5% crystal violet in water. Using a stereomicroscope at 10-40x magnification, individual colonies containing >50 cells from each plate were counted as a single colony. Plating efficiency (PE) was determined from the fraction of control cells (non-radiated animal tumors) initially plated that formed colonies. Survival fraction for each group was determined by dividing the number of colonies formed by the number of cells seeded multiplied by the plating efficiency determined from the control condition. The survival fractions were expressed as an average of the two plating densities for each condition.

Immunohistochemistry and Analysis.

The following IHC processing was carried out Kansas State University Pathology Core. Paraffin-embedded tissues were sectioned into 4-μm-thick sections before being dewaxed in xylene, rehydrated, and rinsed in graded ethanol solutions. Following antigen retrieval via pressure cooker for 10 min at 100°C, sections were then immersed in a 0.3% hydrogen peroxide solution for 30 min to block endogenous peroxidase activity, rinsed in phosphate-buffered saline

(PBS) for 5 min, and incubated with the primary antibody rabbit anti-human HIF-1α (Abcam, ab51608), HIF-2α (Abcam, ab109616), Androgen Receptor (AR, Abcam, ab133273,), and nuclear protein Ki67 (Ki-67, Abcam, ab15580) at 4°C overnight. The sections were then incubated with a horseradish goat-anti-rabbit IgG secondary antibody (Vector Labs Burlingame, CA, BA-1000; 1:200 dilution) and VECTASTAIN Elite ABC-HRP Kit (Vector Labs, PK-6100). Bound antibodies were visualized by use of Nova Red (Vector Labs) or 3, 3'-diaminobenzidine tetrahydrochloride (DAB, Vector Labs). and counterstained with hematoxylin. Rabbit Flex Universal negative control (Dako, Glostrup, Denmark, IS60061-2) was used for negative control.

The National Institute of Environmental Health Sciences (NIEHS) carried out the following IHC procedures for all tumors for Cleaved Caspase-3 staining. Using the HRP-polymer technique, Formalin-fixed, paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated through graded ethanol. Heat-induced epitope retrieval was performed using EDTA buffer (pH8.5, Biocare Medical) in the Decloaker Pressure chamber for 15 minutes at 110°C; after which endogenous peroxidase blocking was done by immersing the sections in 3% H2O2 for 15 minutes. Non-specific sites were blocked with Rodent Block R (Biocare Medical) for 20 minutes. The tissue sections were then incubated in rabbit monoclonal Caspase-3 antibody (cell Signaling Technology, #9664) at a 1:1000 dilution for 1h at room temperature. Rabbit IgG, monoclonal (Abcam, ab125938) was applied to the negative control at the equivalent dilution. The antigen-antibody complex was detected using Rabbit on Rodent HRP polymer (Biocare Medical, Pacheco, CA) and 3,3-diaminobenzidine (Dako). Slides were then counterstained with hematoxylin, dehydrated, and coverslipped.

Quantification of positive staining was supervised by a board-certified pathologist, upon which areas of positive staining for hypoxia markers in prostate tumors was determined, as well as non-specific staining patterns. Following this inspection, tumor sections were scanned using a Pannoramic Midi scanner (3D Histech, Budapest, Hungary) with a 20× objective, giving up to 2-megapixel resolution for analysis. Scanned images of slides were then then annotated for these regions, using HALO software (Indica Labs, Albuquerque, NM) and quantified using the HALO module for CytoNuclear semi-automated analysis (version 1.4), which was set to identify antibody binding based on DAB (brown) staining pattern of the nucleus or cytoplasm, and identify total positive nuclei based on hematoxylin stain for total cell count. HALO used color deconvolution from RGB optical density to isolate signals from the chromogen and counterstains

to define a "positive" cell. Initial positive stains were done by averaging values from 10 random slides to establish minimum stain OD, and then subsequently checked through manual observation to ensure proper detection see (Figure 2.1). Next, various parameters of identification were used to help prevent over-segmentation and contrast threshold issues using a built in real-time tuning window that allows for observation of settings before analyzing an image. Other image detection parameters for each specific antibody used were unchanged throughout the analysis process to prevent bias, and tissue edge distance was set to 50μm to eliminate edge effects often seen with IHC. Algorithms were developed on slides stained simultaneously in the same batch. To verify analysis parameters, we performed a secondary analysis evaluating 10 equal random fields from annotated areas of adjacent sections from 5 tumors from each group. Cells with positive staining (see Figure 2.1) were normalized to total cell count, for both areas (each quantified separately) of the periphery and the core of the tumor, with data represented as total. Three independent observers scored 4 random tiles from each stain pattern to determine the systems quantification to the gold standard of manual counts, with no significant differences between HALO analysis and the 3-independent observers (Data not shown).

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 Srere [34]. 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 in a 96-well plate. Samples were incubated in a spectrophotometer (accuSkan GO; Thermo Fisher Scientific, Waltham, MA) 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 oxaloacetic 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 9.3, Graphpad software, INC., La Jolla, CA) data analysis software was used for all statistical analyses. Statistical comparisons were made with one-way analysis of variance (ANOVA) with Holm-Sidak post hoc tests as appropriate to assess statistical differences between groups for all measures. When non-equal standard deviations or normality failure occurred, Welch's or Kruskal-Wallis corrections were made as needed. Pearson correlations and linear regression analyses were performed to quantify relationships between tumor mass and levels of hypoxia of tumors. For analysis of survival curves, standard PE-based analysis as previously described [33] was used to calculate survival fractions. A $p<0.05$ was set for statistical significance for all experiments with data reported as mean±SEM.

Results

Body mass was not different between groups across the period of experiments ($P>0.05$), and post-mortem tumor mass was not statistically significant between any group ($P>0.05$, Table 1.). Both TBMIT and TBHIT groups had greater whole heart mass normalized to body mass ($P<0.05$, Table 2.1) Skeletal muscle citrate synthase activity was not different between training intensities for either the soleus or the red portion of gastrocnemius ($p>0.05$ Table 2.1). However, TBMIT and TBHIT had greater citrate synthase activity in both the soleus and red gastrocnemius muscle versus TBS. ($p<0.05$, Table 2.1).

Hypoxia quantification

There was a significant reduction in percentage of total cells stained for HIF-1α in both the TBMIT, and TBHIT groups vs TBS when core and periphery measures were combined ($p<0.05$ Figure 2.3). There was also less HIF-1α staining in the periphery of tumor alone for TBMIT, and TBHIT groups vs TBS ($p<0.05$ Figure 2.3), but only TBHIT had a reduction in the core vs TBS ($p<0.05$ Figure 2.3). HIF-2α staining had no changes with exercise of either intensity when compared to TBS in the periphery, ($p>0.05$, Figure 2.3) but was significantly lower in the core in the TBMIT group vs both TBS and TBHIT ($p<0.05$, Figure 2.3). This was coupled with a lower core and periphery combined staining of HIF-2α in TBMIT vs TBS (<0.05, Figure 2.3). There was also a positive correlation of tumor mass associated with increased expression of HIF-1α, but not HIF-2α (Figure 2.3).

Ki-67 and Caspase-3 Quantification

For the proliferative marker Ki-67, there was a reduced expression in both the TBMIT and TBHIT vs. TBS ($p<0.05$, Figure 2.4) for combined core and periphery measures. However, there was only a significant difference found between TBS and TBHIT in the periphery of the tumor when analyzed separately, with no difference in core measures ($p>0.05$, Figure 2.4). Cleaved Caspase-3 expression was increased in only in the TBMIT vs. TBS when assessing the tumor periphery, but when combined, core and tumor percentage of cells stained were significantly higher only in the TBHIT vs TBS ($p<0.05$, Figure 2.4).

Clonogenic Response

PE were not significantly different between groups (TBS= 44±8, TBMIT= 40±5, and TBHIT= 42±2%, $p>0.05$). The PE from each group was used to calculate appropriate densities

for all experiments. There was a significant difference in survival fraction (SF) at 2Gy of radiation for both TBMIT and TBHIT vs TBS ($p<0.05$, Figure 2.5), albeit in a small sample size, was ~15% and ~17% reduction in survival for respectively.

Discussion

This study sought to identify if aerobic exercise of differing intensities (moderate and high) would modify the tumor microenvironment favorably through alleviation of markers of hypoxia and proliferation assessed using semi-quantitative IHC, that would potentially enhance radiosensitivity of prostate tumors. This is clinically important as the use of exercise for adjuvant therapy has been a burgeoning area of research [35–37]. One area that has garnered interest, is the dosing and timing of exercise for therapeutic benefits or improvement of patient's quality of life [37–39]. There are several novel findings from these studies indicating potential for differing levels of exercise being effective in improving therapeutic outcomes. Specifically, we show for the first time, in vivo, that both moderate- and high intensity exercise can sensitize solid tumors to radiotherapy treatment, assessed via clonogenic survival assay, that is associated with decreased levels of hypoxia in the tumor microenvironment. Both exercise training intensities decreased whole HIF-1α expression vs sedentary counterparts, as well as a significant reduction in HIF-2α in TBMIT vs TBS, but not seen in TBHIT (Figure 2.3) indicating a possible reduction in hypoxia acutely and chronically being different between the exercise modalities. Lastly, both exercise intensities were able to decrease expression levels of proliferation marker Ki-67, which is often associated with poor prognostic outcomes [8]. Collectively, this suggests the potential of exercise to be used as adjuvant therapy to help improve tumor therapeutic outcomes through enhanced radiosensitivity in solid tumors, in combination with the proposed direct positive impact on the tumor microenvironment.

There is an abundance of evidence indicating regions of hypoxia exist in solid tumors due to perfusive and diffusive limitations [9,40]. This hypoxia is a known indicator for poor radiotherapy outcomes, and often is a target of therapy [9,41]. It is oft thought that as the tumor

grows, increased areas of necrosis form near the core of the tumor as the tumor's growth outstrips its vasculature, preventing proper nutrient delivery [3]. Thus, our separation of the tumor into both peripheral and core sections for analysis, to investigate the area in which exercise may impact the tumor microenvironment. Incidentally, there was increased expression of HIF-1α in the core vs the periphery, and a positive correlation of increased expression with tumor size (Figure 2.3.). However, this was not evident with HIF-2α expression, with the TBMIT group even having a decreased expression in the core vs peripheral assessments (Figure 3.) This finding was unexpected as the long-held belief of HIF-2α as an indicator of chronic hypoxia rather than acute that is associated with HIF-1α [4]. Certainty HIF factors can be altered by more than oxygenation alone [22], and many mechanisms could be leading to this finding that will require further investigation. The overall reduction of both HIF-1α and HIF-2α was only observed with MIT, which could be due to increased stress leading to a more aggressive tumor phenotype in HIT animals that has been associated with increased metastasis [42]. This was not evident in this study, as all animals had no visible macro-metastasis at time of tissue collection. Nonetheless, future studies should investigate peripheral markers of inflammation to examine whole body modification of exercise that may differ between tumor bearing and non-tumor bearing animals.

Previous studies have examined the radiosensitization of cancer via hyperthermia [41], or pharmaceutical agents [43] that have often been unable to target the tumor specifically, and thus tend to have mixed results [2]. Aerobic exercise has been studied for its specific effects on tumor growth and proliferation with mixed results both in vivo and in vitro [28,29,35,44,45]. Recently, exercise has been investigated not only for its ability to modify whole tumor growth, but to alter the tumor microenvironment favorably for enhancement of both chemotherapy [25,35,46] and

radiation [2,26]. In our study, neither exercise intensity impacted tumor mass directly (Table 1.), which is debated in the literature [26,28,35,42], but has been consistent in this model of prostate cancer previously [24]. This incidentally allows us to assume that all groups would have a similar area of hypoxia given the HIF-1α relationship to tumor mass in this study, unless altered by exercise interventions. This is the first investigation in an orthotopic rat model to specifically determine the impact of chronic exercise training intensity on tumor hypoxia and radiosensitivity. Prior studies from McCullough et al. in a similar model established the efficacy of moderate intensity exercise to improve tumor oxygenation via increased blood flow during exercise [24] and decrease levels of tissue hypoxia via moderate aerobic exercise training [31], indicating potential for exercise both acutely and chronically over time. Following chronic exercise training interventions solid tumors have been shown to undergo vascular remodeling that manifests in improved oxygenation, thought to be through improved vascular function, and more homogenous blood flow [2,35,39]. Indeed McCullough et al. showed no difference in the number of perfused vessels but indicated that reductions in hypoxia could be due to greater blood flow or increased capillary hematocrit in existing vasculature. However, this isn't always the case in other models of cancer (breast) were hypoxia associated with HIF-1α has increased following aerobic exercise training [44]. This however was using a different exercise stimulus, (free wheel running) and could be due to regional (i.e. ectopic) differences of the tumor itself. Although blood flow or oxygenation was not directly measured, these are some of the postulated mechanisms behind the decreased expression of HIF-1α and HIF-2α, and the enhanced radiotherapy response seen in this investigation [2]. Indeed, in a recent study in mice, using an ectopic model, by Dufresne et al, a sensitization to fractionated radiation therapy following exercise training was able to decrease areas of necrosis, and decrease tumor volume in mice [26].

These findings although encouraging, and support systemic changes impacting the radiotherapy, would likely be due to different mechanisms in ectopic and orthotopic models of cancer for tumor microenvironment modification. Specifically, the hypothesis of this study rests on the foundation of work previously reported that orthotopic and subcutaneous tumor models of the same cell line, do not have the same perfusion response to exercise, with the latter having a decrease in blood flow, and the former seeing a nearly ~200% increase in blood flow during exercise [47]. The use of an orthotopic model in this study provides an increased level of translatability, that is often lost in subcutaneous models [48]. Thus, the exercise specific changes in hypoxia seemed to enhance the radiosensitivity of prostate tumors, to a similar degree for both intensities of exercise.

The programming and feasibility of initiating exercise after the diagnosis of prostate cancer has been a topic of consideration for many years [38,49]. Although exercise in general (both resistance- and aerobic training) is recommended for most healthy individuals [50], it is particularly advocated for in cancer patients due to multi-dimensional (cardioprotection, skeletal muscle sparing etc.) benefits throughout treatment [38,51]. However, the ability to study the effects of exercise on the prostate cancer tumor microenvironment in this pre-clinical model without treatment, gives insights into what could be occurring in this slow-growing disease, as it would be unethical to withhold treatment from patients. From time of diagnosis of prostate cancer, to surgery/treatment is typically 5-10 weeks in humans [52]. This fits a similar time frame that this study showed efficacy to improve radiotherapy outcomes and could possibly translate to the clinical setting.

Limitations

Although this study provides many novel findings, several limitations need addressed. From animal studies, the ideal length of training is typically 6-8 weeks of constant load moderate intensity exercise to induce an exercise phenotype [53]. 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. However, the increase in citrate synthase activity seen in both TBMIT and TBHIT groups indicated a clear training response. Further, this time frame fits with a normal timeline to first treatment after prostate cancer diagnosis [52]. The use of hypoxic markers as indicators for reduction in hypoxia can be misleading, and do not always reflect true oxygenation of the tumor [10,54]. However, these have been used before in conjunction with physical PO_2 measure via electrode, that have shown inverse relationship between oxygenation and HIF expression or protein content [12,40,55]. Lack of animals used for radiotherapy outcomes is a concern and ideally multiple levels or fractionated doses would have been utilized, however, this animal line was discontinued before the completion of all studies and limited the animals that could be used for radiation responses. In line with this, the histological investigation of the tumor microenvironment when exposed to the combination of both exercise, and radiation would also give more insights into what would typically be seen in cancer patients undergoing radiation treatments, but was unable to be completed due to previously mentioned animal discontinuation. Lastly, further analysis of H&E staining pattern for necrotic differences between groups is currently underway and will be included in the manuscript prepared for submission.

Conclusions

This investigative study into the impact of aerobic exercise training of both moderate- and high intensity exercise indicates the therapeutic benefits of exercise in this pre-clinical model of prostate cancer. Specifically, exercise of both intensities was able to decrease markers associated with tumor hypoxia and decrease markers of proliferation associated with poor prognostic outcomes. These modifications of the tumor microenvironment are thought to be associated with the increased radiosensitivity to a single 2Gy dose of radiation also observed in both intensities of exercise. These findings provide optimism for the efficacy of exercise use in as adjuvant therapy for prostate cancer patients. Specifically, this has clinical relevance, as patients who have not begun treatment could utilize exercise to "prime" the tumor from time of diagnosis, to potentially enhance treatment. However, additional studies are needed investigating outcomes of tumors in vivo, over prolonged periods of time to establish the level of radiation enhancement exercise potentially provides over increased levels of radiation in either single or fractionated doses.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA: A Cancer Journal for Clinicians 2019; 69:7–34.
2. Wiggins JM, Opoku-Acheampong AB, Baumfalk DR, Siemann DW, Behnke BJ. Exercise and the Tumor Microenvironment: Potential Therapeutic Implications. Exercise and Sport Sciences Reviews 2018; 46:56–64.
3. Vaupel P, Kelleher DK. Blood Flow and Oxygenation Status of Prostate Cancers. Oxygen Transport to Tissue XXXIV 2013; 299–305.
4. Muz B, Puente P de la, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. Hypoxia 2015; 3:83.
5. Stephen W. Leslie, Taylor L. Soon-Sutton;, Hussain Sajjad;, Larry E Siref. Prostate cancer. Elsevier; 2020.
6. Hamdy FC, Donovan JL, Lane JA, Mason M, Metcalfe C, Holding P, Davis M, Peters TJ, Turner EL, Martin RM, Oxley J, Robinson M, Staffurth J, Walsh E, Bollina P, Catto J, Doble A, Doherty A, Gillatt D, Kockelbergh R, Kynaston H, Paul A, Powell P, Prescott S, Rosario DJ, Rowe E, Neal DE. 10-Year Outcomes after Monitoring, Surgery, or Radiotherapy for Localized Prostate Cancer. New England Journal of Medicine 2016; 375:1415–1424.
7. Bos R, van Diest PJ, van der Groep P, Shvarts A, Greijer AE, van der Wall E. Expression of hypoxia-inducible factor-1alpha and cell cycle proteins in invasive breast cancer are estrogen receptor related. Breast Cancer Research : BCR 2004; 6:1–10.
8. Verma R, Gupta V, Singh J, Verma M, Gupta G, Gupta S, Sen R, Ralli M. Significance of p53 and ki-67 expression in prostate cancer. Urology Annals 2015; 7:488.
9. Vaupel P. Tumor microenvironmental physiology and its implications for radiation oncology. Seminars in Radiation Oncology; Tumor Physiology 2004; 14:198–206.
10. Vaupel P. Hypoxia and Aggressive Tumor Phenotype: Implications for Therapy and Prognosis. The Oncologist 2008; 13:21–26.
11. Milosevic M, Warde P, Meńard C, Chung P, Toi A, Ishkanian A, McLean M, Pintilie M, Sykes J, Gospodarowicz M, Catton C, Hill RP, Bristow R. Tumor Hypoxia Predicts Biochemical Failure following Radiotherapy for Clinically Localized Prostate Cancer. Clinical Cancer Research 2012; 18:2108–2114.

12. Turaka A, Buyyounouski MK, Hanlon AL, Horwitz EM, Greenberg RE, Movsas B. Hypoxic Prostate/Muscle Po2 Ratio Predicts for Outcome in Patients With Localized Prostate Cancer: Long-Term Results. International Journal of Radiation Oncology Biology Physics 2012; 82:e433–e439.
13. Eisemann N, Nolte S, Schnoor M, Katalinic A, Rohde V, Waldmann A. The ProCaSP study: quality of life outcomes of prostate cancer patients after radiotherapy or radical prostatectomy in a cohort study. BMC Urology 2015; 15.
14. Smith DP, King MT, Egger S, Berry MP, Stricker PD, Cozzi P, Ward J, O'Connell DL, Armstrong BK. Quality of life three years after diagnosis of localised prostate cancer: population based cohort study. The BMJ 2009; 339:195.
15. Rockwell S, Dobrucki IT, Kim EY, Marrison ST, Vu VT. Hypoxia and radiation therapy: Past history, ongoing research, and future promise. Current Molecular Medicine 2009; 9:442.
16. Winkler F, Kozin S v., Tong RT, Chae SS, Booth MF, Garkavtsev I, Xu L, Hicklin DJ, Fukumura D, di Tomaso E, Munn LL, Jain RK. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: Role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell 2004; 6:553–563.
17. Henke M, Laszig R, Rübe C, Schäfer U, Haase KD, Schilcher B, Mose S, Beer KT, Burger U, Dougherty C, Frommhold H. Erythropoietin to treat head and neck cancer patients with anaemia undergoing radiotherapy: Randomised, double-blind, placebo-controlled trial. Lancet 2003; 362:1255–1260.
18. Dewhirst MW, Vujaskovic Z, Jones E, Thrall D. Re-setting the biologic rationale for thermal therapy. International Journal of Hyperthermia 2005; 21:779–790.
19. Cairns R, Papandreou I, Denko N. Overcoming Physiologic Barriers to Cancer Treatment by Molecularly Targeting the Tumor Microenvironment Unique Microenvironment of Solid Tumors. Mol Cancer Res 2006; 4:61–70.
20. Harber MP, Kaminsky LA, Arena R, Blair SN, Franklin BA, Myers J, Ross R. Impact of Cardiorespiratory Fitness on All-Cause and Disease-Specific Mortality: Advances Since 2009. Progress in Cardiovascular Diseases 2017; 60:11–20.
21. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee I-M, Nieman DC, Swain DP. 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. Medicine and Science in Sports and Exercise 2011; 43:1334–1359.

22. Wang Y, Jacobs EJ, Gapstur SM, Maliniak ML, Gansler T, McCullough ML, Stevens VL, Patel A v. Recreational Physical Activity in Relation to Prostate Cancer-specific Mortality Among Men with Nonmetastatic Prostate Cancer. European Urology 2017; 72:931–939.

23. Kenfield SA, Stampfer MJ, Giovannucci E, Chan JM. Physical activity and survival after prostate cancer diagnosis in the health professionals follow-up study. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology 2011; 29:726–732.

24. McCullough DJ, Stabley JN, Siemann DW, Behnke BJ. Modulation of blood flow, hypoxia, and vascular function in orthotopic prostate tumors during exercise. Journal of the National Cancer Institute 2014.

25. Schadler KL, Thomas NJ, Galie PA, Bhang DH, Roby KC, Addai P, Till JE, Sturgeon K, Zaslavsky A, Chen CS, Ryeom S. Tumor vessel normalization after aerobic exercise enhances chemotherapeutic efficacy. Oncotarget 2016; 7:65429.

26. Dufresne S, Guéritat J, Chiavassa S, Noblet C, Assi M, Rioux-Leclercq N, Rannou-Bekono F, Lefeuvre-Orfila L, Paris F, Rébillard A. Exercise training improves radiotherapy efficiency in a murine model of prostate cancer. FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology 2020; 34:4984–4996.

27. Morrell MBG, Alvarez-Florez C, Zhang A, Kleinerman ES, Savage H, Marmonti E, Park M, Shaw A, Schadler KL. Vascular Modulation Through Exercise Improves Chemotherapy Efficacy in Ewing Sarcoma. Pediatric Blood & Cancer 2019; 66:e27835.

28. Dufresne S, Richard C, Dieumegard A, Orfila L, Delpon G, Chiavassa S, Martin B, Rouvière L, Escoffre J-M, Oujagir E, Denis de Senneville B, Bouakaz A, Rioux-Leclercq N, Potiron V, Rébillard A. Voluntary Wheel Running Does Not Enhance Radiotherapy Efficiency in a Preclinical Model of Prostate Cancer: The Importance of Physical Activity Modalities? Cancers 2021; 13:5402.

29. Opoku-Acheampong AB, Baumfalk DR, Horn AG, Kunkel ON, Ganta CK, McCullough DJ, Siemann DW, Muller-Delp J, Behnke BJ. Prostate cancer cell growth characteristics in serum and prostate-conditioned media from moderate-intensity exercise-trained healthy and tumor-bearing rats. American Journal of Cancer Research 2019; 9:650.

30. Baumfalk DR, Opoku-Acheampong AB, Caldwell JT, Ade CJ, Copp SW, Musch TI, Behnke BJ. Effects of prostate cancer and exercise training on left ventricular function and cardiac and skeletal muscle mass. Journal of Applied Physiology 2018; 126:668–680.
31. McCullough DJ, Nguyen LM-D, Siemann DW, Behnke BJ. Effects of exercise training on tumor hypoxia and vascular function in the rodent preclinical orthotopic prostate cancer model. Journal of Applied Physiology 2013; 115:1846–1854.
32. Franken NAP, Rodermond HM, Stap J, Haveman J, van Bree C. Clonogenic assay of cells in vitro. Nature Protocols 2006 1:5 2006; 1:2315–2319.
33. Brix N, Samaga D, Belka C, Zitzelsberger H, Lauber K. Analysis of clonogenic growth in vitro. Nature Protocols 2021 16:11 2021; 16:4963–4991.
34. PA., Srere. Citrate synthase: [EC 4.1. 3.7. Citrate oxaloacetate-lyase (CoA-acetylating)]. Methods Enzymol 1969; 3–11.
35. Ashcraft KA, Peace RM, Betof AS, Dewhirst MW, Jones LW. Efficacy and Mechanisms of Aerobic Exercise on Cancer Initiation, Progression, and Metastasis: A Critical Systematic Review of In Vivo Preclinical Data. Cancer Research 2016; 76:4032–4050.
36. Tsuji K, Matsuoka YJ, Ochi E. High-intensity interval training in breast cancer survivors: a systematic review. BMC Cancer 2021; 21:1–11.
37. Yang L, Morielli AR, Heer E, Kirkham AA, Cheung WY, Usmani N, Friedenreich CM, Courneya KS. Effects of exercise on cancer treatment efficacy: A systematic review of preclinical and clinical studies. Cancer Research 2021; 81:4889–4895.
38. Hart NH, Galvão DA, Newton RU. Exercise medicine for advanced prostate cancer. Current Opinion in Supportive and Palliative Care 2017; 11:247–257.
39. Schumacher O, Galvão DA, Taaffe DR, Chee R, Spry N, Newton RU. Exercise modulation of tumour perfusion and hypoxia to improve radiotherapy response in prostate cancer. Prostate Cancer and Prostatic Diseases 2021; 24.
40. Vaupel P, Kallinowski F, Okunieff P. Blood Flow, Oxygen and Nutrient Supply, and Metabolic Microenvironment of Human Tumors: A Review. Cancer Res 1989; 49:6449.
41. Elming PB, Sørensen BS, Oei AL, Franken NAP, Crezee J, Overgaard J, Horsman MR. Hyperthermia: The Optimal Treatment to Overcome Radiation Resistant Hypoxia. Cancers 2019, Vol 11, Page 60 2019; 11:60.

42. Cohen LA, Boylan E, Epstein M, Zang E. Voluntary exercise and experimental mammary cancer. Advances in Experimental Medicine and Biology 1992.
43. Siemann DW, Rojiani AM. Enhancement of radiation therapy by the novel vascular targeting agent ZD6126. International Journal of Radiation Oncology Biology Physics 2002; 53:164–171.
44. Jones LW, Viglianti BL, Tashjian JA, Kothadia SM, Keir ST, Freedland SJ, Potter MQ, Moon EJ, Schroeder T, Herndon JE, Dewhirst MW. Effect of aerobic exercise on tumor physiology in an animal model of human breast cancer. Journal of Applied Physiology 2010; 108:343–348.
45. Orange ST, Jordan AR, Saxton JM. The serological responses to acute exercise in humans reduce cancer cell growth in vitro: A systematic review and meta-analysis. Physiological Reports 2020; 8-12
46. Morrell MBG, Alvarez-Florez C, Zhang A, Kleinerman ES, Savage H, Marmonti E, Park M, Shaw A, Schadler KL. Vascular modulation through exercise improves chemotherapy efficacy in Ewing sarcoma. Pediatric Blood & Cancer 2019; e27835.
47. Garcia E, Becker VGC, McCullough DJ, Stabley JN, Gittemeier EM, Opoku-Acheampong AB, Sieman DW, Behnke BJ. Blood flow responses to mild-intensity exercise in ectopic vs. orthotopic prostate tumors; dependence upon host tissue hemodynamics and vascular reactivity. Journal of Applied Physiology 2016; 12:15-24.
48. Killion JJ, Radinsky R, Fidler IJ. Orthotopic models are necessary to predict therapy of transplantable tumors in mice. Cancer and Metastasis Reviews 1998; 17:279-284
49. Parsons JK. Prostate cancer and the therapeutic benefits of structured exercise. Journal of Clinical Oncology 2014; 32:271–2.
50. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. Medicine and Science in Sports and Exercise 2009; 41:687.
51. Galvao DA, Taaffe DR, Spry N, Joseph D, 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. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology 2010; 28:340–347.
52. Redaniel MT, Martin RM, Gillatt D, Wade J, Jeffreys M. Time from diagnosis to surgery and prostate cancer survival: A retrospective cohort study. BMC Cancer 2013; 13:1–6.

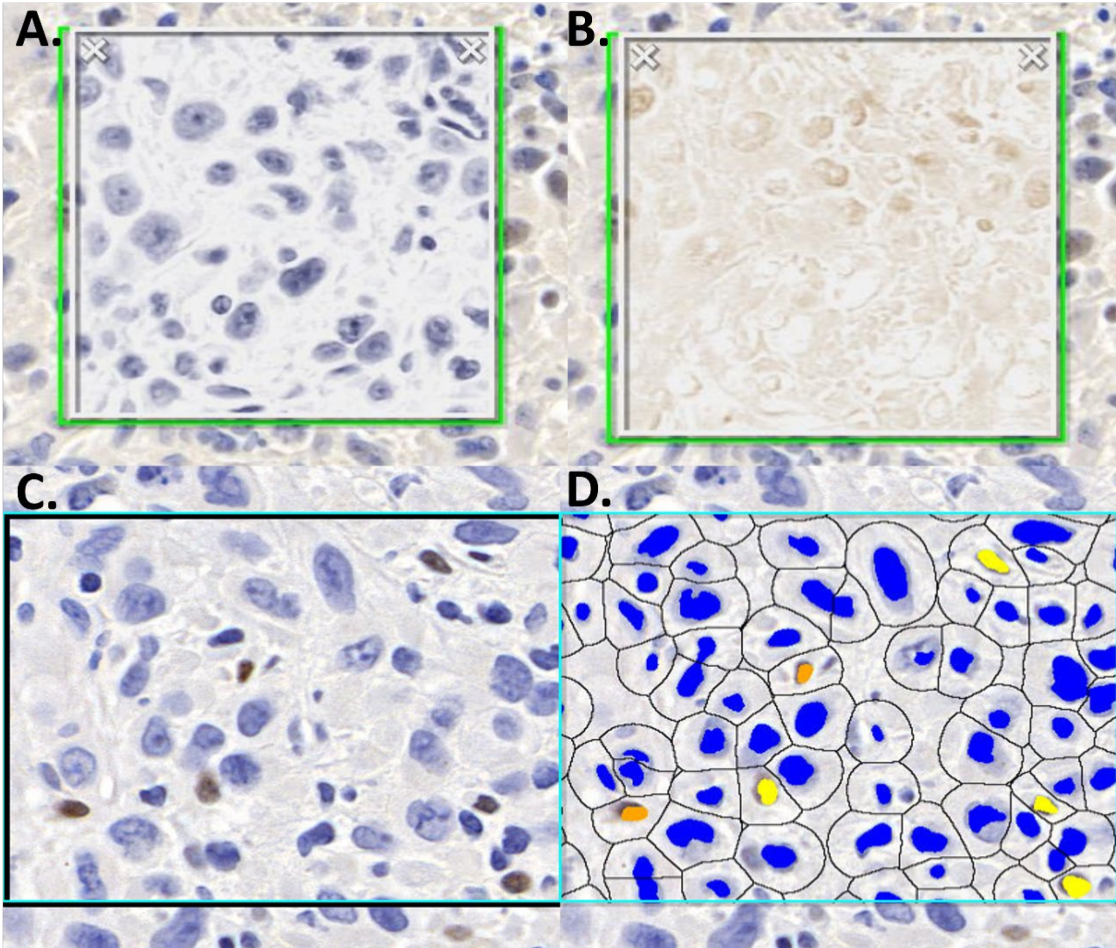
53. Kregel KC, Allen DL, Booth FW, Fleshner MR, Henriksen EJ, Musch TI, O'Leary DS, Parks CM, Poole DC, Ra'anan AW. Resource book for the design of animal exercise protocols. American Physiological Society 2006.
54. LaGory EL, Giaccia AJ. The ever-expanding role of HIF in tumour and stromal biology. Nature Cell Biology 2016; 18:356–365.
55. Moulder JE, Rockwell S. Hypoxic fractions of solid tumors: Experimental techniques, methods of analysis, and a survey of existing data. International Journal of Radiation Oncology*Biology*Physics 1984; 10:695–712.

Table 2.1. Animal Characteristics

Animal Characteristics	TBS (n=10)	TBMIT (n=10)	TBHIT (n=14)
Body and Tumor Mass (g)			
Body Mass	324±5.7	317±7.8	324±7.1
Tumor Mass	7.6±0.92	7.2±1.7	6.6±1.1
Tissue Mass Normalized to Body Mass (mg/g)			
Heart/Body mass	2.40±0.12	2.67±0.11*	2.69±0.15*
Gastrocnemius/Body mass	6.14±0.61	6.27±0.82	6.43±0.69
Soleus/Body mass	0.47±0.10	0.49±0.09	0.51±0.07
Tumor/Body mass (%)	2.89±0.39	2.64±0.43	1.93±0.34
Skeletal Muscle Citrate Synthase Activity (µmol/min/g)	n=9	n=7	n=8
Soleus	15.1±1.6	21.9±1.1*	23.1±1.3*
Red gastrocnemius	20.7±1.7	27.8±1.9*	29.6±1.8*

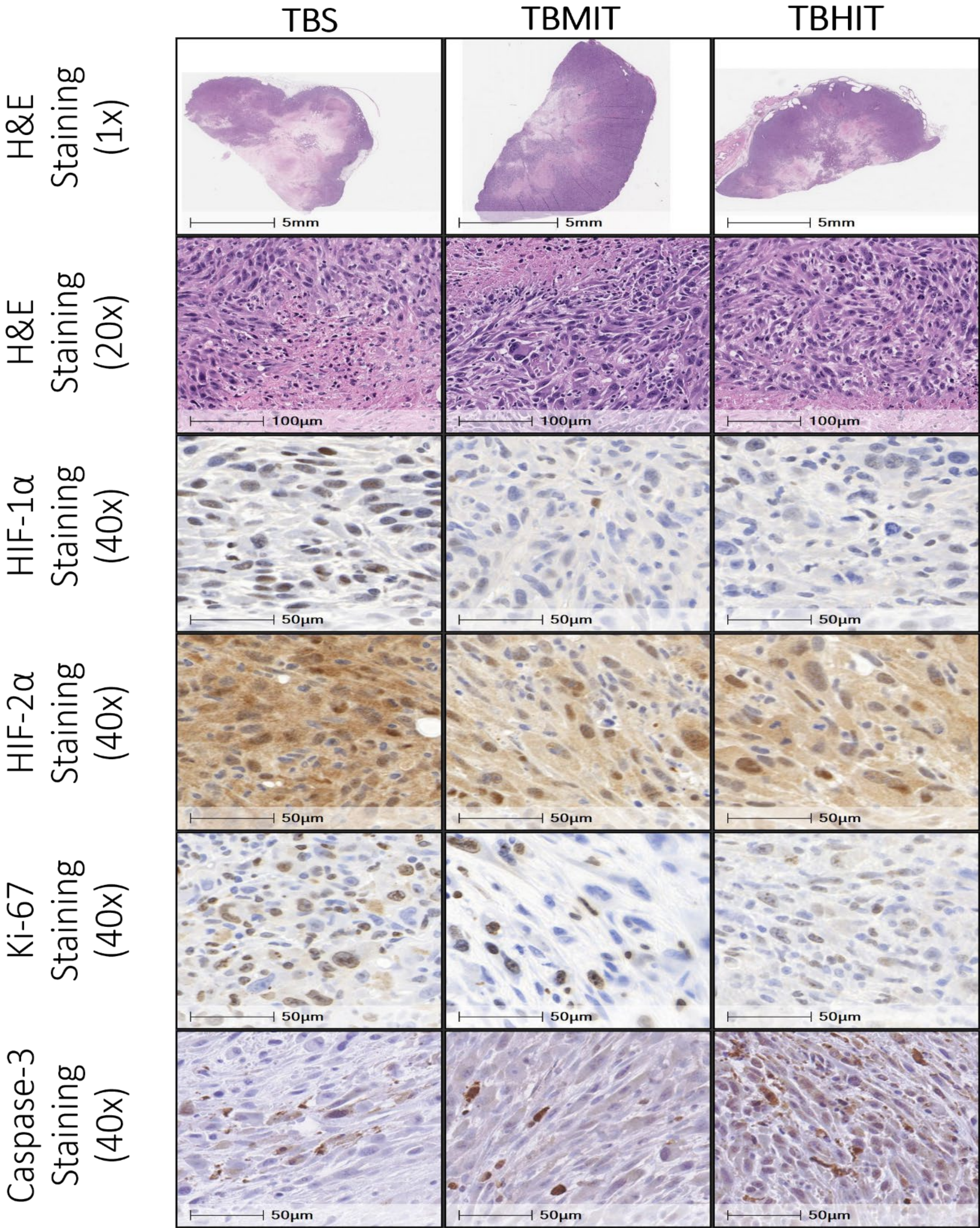
Data are mean±SEM *= $p<0.05$ vs. Sedentary Tumor-Bearing

Figure 2.1. Representative HALO Analysis



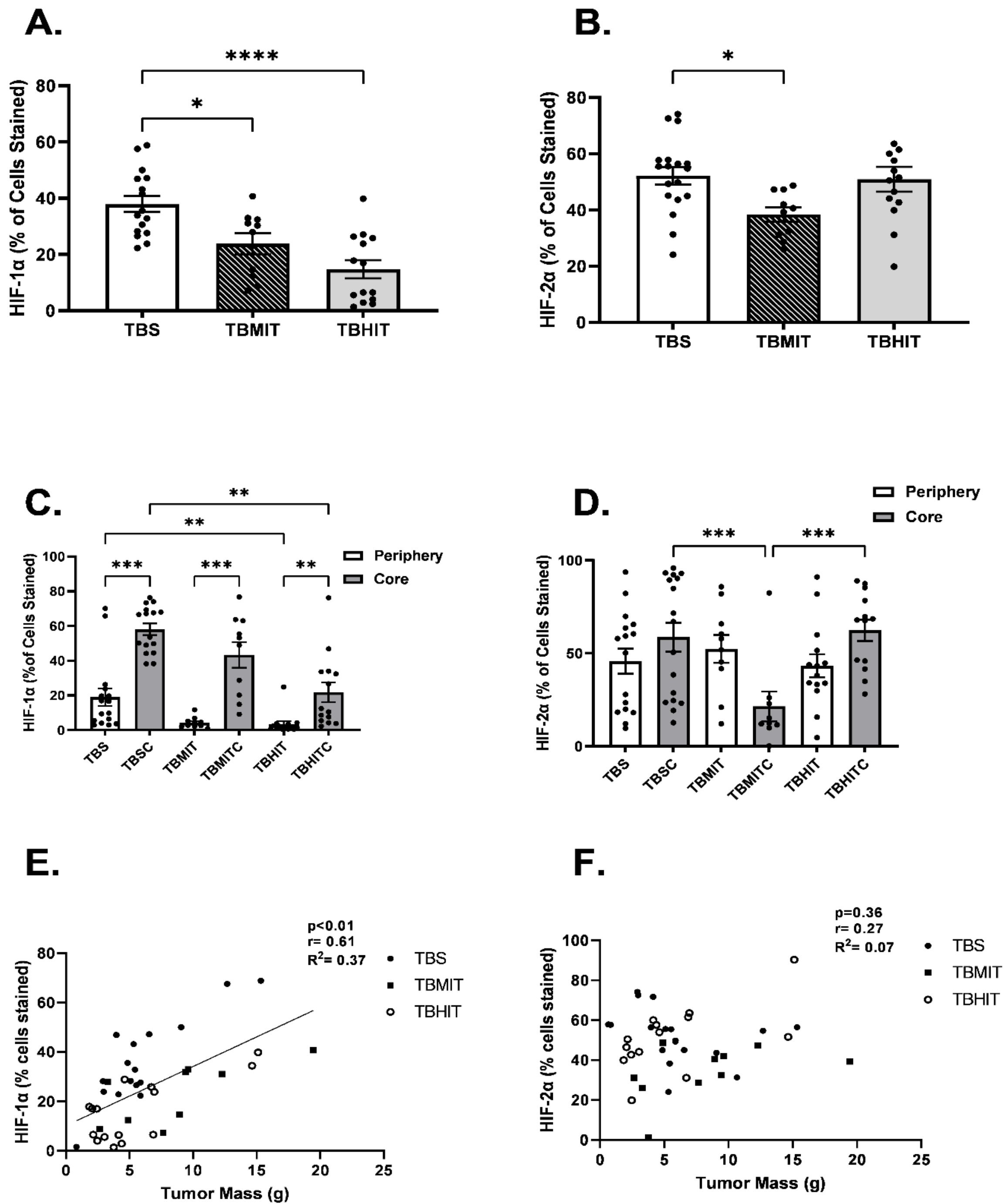
The process of deconvolution to identify nucleus of cells for hematoxylin in panel A. and DAB (brown) positive stain in B. Panel C is a representation of a normal grid before cell quantification with staining intensity in panel D with yellow-colored cells being the weakest, and orange being a moderate stain.

Figure 2.2. Representative Staining



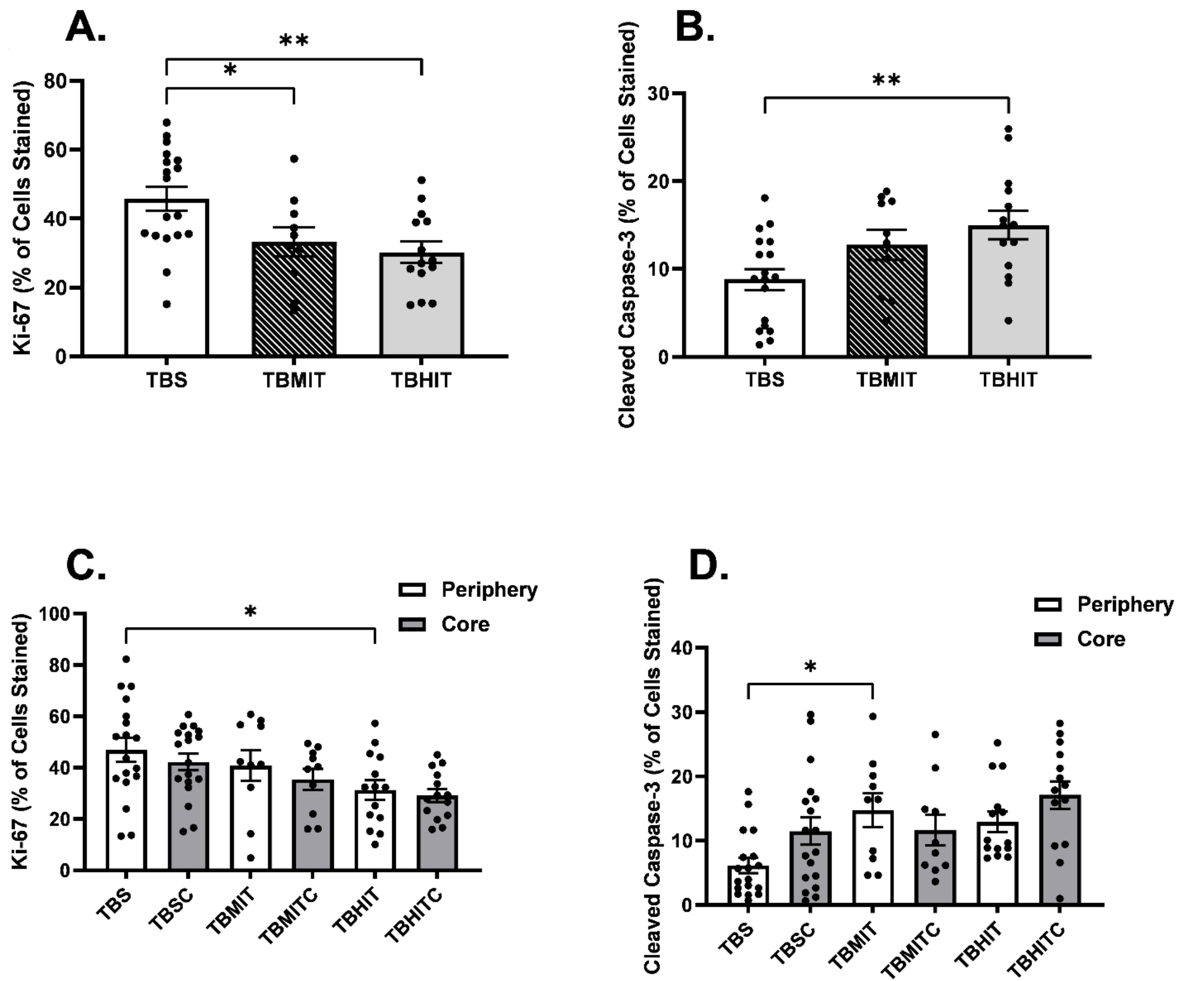
Representative tumor sections for all antibodies (40x) and H&E (1x, 20x) stains from each group of animals.

Figure 2.3. Hypoxic Marker Analysis



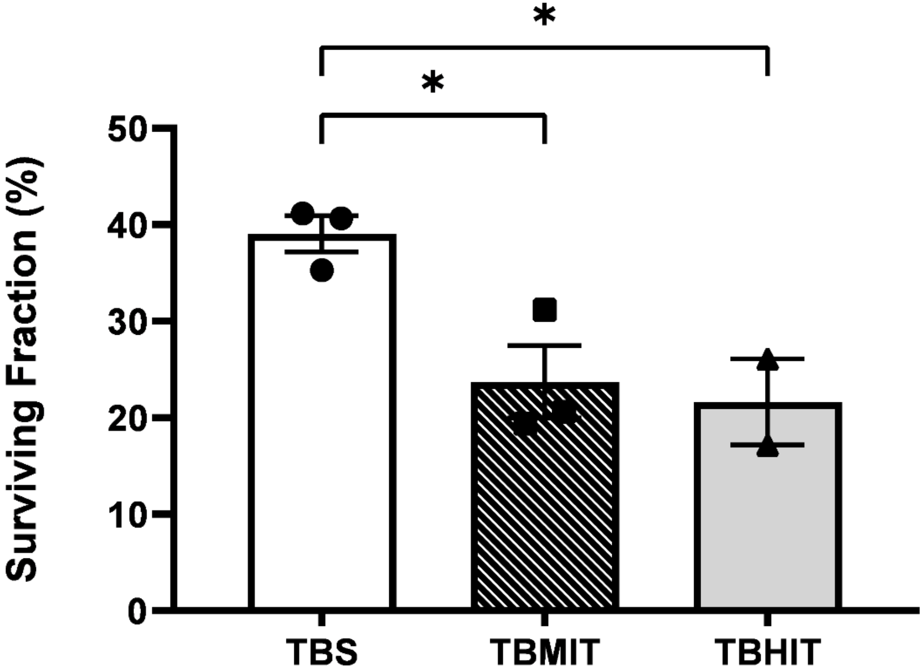
Percentage of total cells stained (A.) for HIF-1α, and HIF-2α (B.) and sections of the core and periphery of tumor for HIF-1α (C.) and HIF-2α (D.) were assessed with a One-way ANOVA and Holm-Sidak post hoc tests) between groups. *, $p<0.05$, ** $p<0.01$ *** $p<0.001$, **** $p<0.0001$. Tumor mass was positively correlated with the percentage of HIF-1α stained cells (E.) but not with HIF-2α (F.).

Figure 2.4. Proliferation and Apoptosis Analysis



Percentage of total cells stained of combined, and core and periphery separately of sectioned tumors stained for Ki-67 (A, C), and Cleaved Caspase-3 (B, D) respectively, were assessed with a One-way ANOVA using the Kruskal Wallace test due to failure of normality tests. *, $p<0.05$, ** $p<0.01$ *** $p<0.001$, **** $p<0.0001$.

Figure 2.5. Clonogenic Survival Results



Clonogenic survival *in vivo* to a single 2Gy dose of radiation was assessed with a One-way ANOVA and Holm-Sidak post hoc tests) between groups. *, $p<0.05$.

Chapter 3 - Effect of acute and chronic exercise on radiosensitivity in prostate tumor-bearing rats

Abstract

Background: Solid tumors contain hypoxic regions that have long been known to be resistant to radiation therapy. Increasing the efficacy of the primary treatment (radiotherapy) of prostate cancer is of clinical importance and has typically been pursued pharmacologically. Prior evidence from our lab has shown that exercise training can improve the oxygenation of a tumor, thereby decreasing regions of hypoxia. Using a pre-clinical orthotopic model of prostate cancer, we tested the hypothesis that exercise training would result in an enhanced radiotherapy response of prostate tumors due to previous results demonstrating exercise training's ability to alter the tumor microenvironment. Further, we tested the hypothesis that a single bout of exercise would also enhance radiosensitivity of prostate tumors. **Methods:** Dunning R-3327 MATLyLu prostate adenocarcinoma cells ($2x10^5$) were injected into the ventral lobe of 5-month-old male RNU (NIH nude) rats (n=40, 33 completed studies). These animals were acclimated to treadmill exercise, and then randomized into three groups, Tumor Bearing Sedentary (TBS, n=12), acute exercise (TBAEX, (n=12), or Chronic Exercise (TBEX, n=9). After ~5 days of recovery from surgery, TBEX animals began progressive exercise training on a motorized treadmill at 25 m/min with a 5% incline for 10 minutes a day progressing to 60 min/day for a ~5-week period, whereas TBAEX were exercised for one 30-minute bout at the same intensity 15-20 minutes before irradiation. Whole body ionizing radiation was performed on all animals at 2Gy at the end of the 5-week period of training followed by clonogenic cell survival assay to assess survival fraction. Pre-injection (Pre-measure) and post-exercise training (Post-measures) a subset of animals also performed $\dot{V}_{O2peak}$ testing for assessment of aerobic capacity changes. In vitro studies to assess radiosensitivity and viability were performed in both MATLyLu, and PC-3 cell lines utilizing serum supplemented media from each group of animals to examine systemic effects of exercise. **Results:** There were no significant differences (all $p>0.05$) in tumor mass between groups (TBS 7.4±1.2; TBAEX 7.6±1.1; TBEX 6.2±1.6 g). The survival fraction was significantly different in TBS and both acute and chronic exercise (TBS 50.7±4.2; TBAEX 41.2±2.2; TBEX 42.7±3.9%, $p<0.05$). Following training, TBEX increased $\dot{V}_{O2peak}$ by 6.4±1.3

$ml \cdot min^{-1} \cdot kg^{-1}$ ($p<0.05$) whereas TBS and TBAEX both had a decrease of 5.9±1.2, and 5.4±1.2 $ml \cdot min^{-1} \cdot kg^{-1}$ at Post-measures ($p<0.05$). Both MATLyLu and PC-3 cancer cell had decreased viability when grown in TBEX animals' serum following 2Gy of radiation (TBAEX 15.9±4.2; TBEX 18.1±3.7%, $p<0.05$), and a decreased survival fraction at the same dose of radiation in the TBAEX and TBEX vs TBS ($P<0.05$) **Conclusion:** This study suggests that both acute and chronic exercise have the potential to augment the tumor microenvironment favorably to enhance radiotherapy compared to sedentary counterparts. Further, prostate cancer independent of treatment, significantly diminishes maximal aerobic capacity, but was mitigated with moderate intensity exercise training. Given prostate cancer patients often present fatigue and loss of quality of life, moderate-intensity exercise training may be useful to improve both therapeutic response of the tumor, and quality of life of patients via maintaining or improving aerobic capacity.

Introduction

Prostate Cancer is the most diagnosed non-skin cancer in men, with over 160,000 diagnoses predicted in 2021 [57]. This cancer is a solid tumor which accounts for the second most cancer related deaths among men only to lung cancer [58]. Two mainstays of prostate cancer treatment are androgen deprivation therapy, and radiation therapy (RT), with both being utilized as standards of care for localized or advanced prostate cancer with over 50% of cases receiving either one or both forms of treatment in combination with surgical resection for curative intent [7,59]. However, radiation therapy specifically is coming at a cost of a plethora of side effects ranging from fatigue, sexual dysfunction, sore skin, depression, and overall decline in quality of life [60,61]. Further, solid tumors commonly contain areas of hypoxia (related to poorly regulated vasculature) that can lead to increased aggressiveness and incidences of cancer metastasis and radiotherapy resistance [16,19], which could lead to recurrence of the disease [7,19,62,63]. Radiotherapy is dependent on adequate oxygen levels as it is an electron-affinic molecule that will play a role in the mechanisms following energy absorption to damage DNA [64]. A variety of methods have been used to mitigate hypoxia of solid tumors from vascular growth inhibitors [26], erythropoietin infusion[27], or hyperthermia [28], to mixed results [25]. The hypoxic resistance of solid tumors is a long-known issue, and is of major medical concern [64,65].

Physically inactive men often have an increased risk for prostate cancer [66], a lifestyle factor that is associated with a myriad of metabolic and chronic disease [67]. Men diagnosed with prostate cancer and engaged in physical activity and exercise training tend to have a favorable prognosis [68]. Exercise training is efficacious in decreasing morbidity and mortality of multiple diseases [49,67], and is currently being used for cancer patient care programs as adjuvant therapy for prevention of fatigue or decline of aerobic capacity [44,56,69]. In terms of benefits for prostate cancer patients, exercise can be a potent means to improve multiple areas at once [70–72]. To date, pre-clinical models have shown that exercise has potential to mitigate tumor hypoxia [14,18], generate a more homogenous distribution of perfusion [14,73], and a shift towards vascular normalization [73]. These adaptations may contribute to increased delivery of chemotherapeutic agents [74], or improved radiosensitization [14] in reference to specific tumor microenvironment adaptations. 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 [75]. Pre-clinical studies from our lab have shown benefits in prevention of cardiac and skeletal muscle atrophy associated with increased fatigue with aerobic exercise training [45,46,76]. There is a paucity of data on the combination of exercise being used as both a potential therapeutic enhancer, and adjuvant therapy. Therefore, this investigation sought to utilize an orthotopic model of prostate cancer to address three main purposes: 1) to study the efficacy of both acute and chronic exercise training to manipulate the tumor microenvironment favorably and radiosensitize prostate tumors *in vivo*, and tumor cells *in vitro* through systemic modifications, 2) Evaluate the level of cardiac and skeletal muscle atrophy associated with prostate cancer, and if loss of aerobic capacity is associated with this atrophy, 3) distinguish the benefits between a single acute bout of exercise and chronic aerobic exercise training, that can impact tumor radiosensitization.

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 athymic nude rats (n=40, ~5-7 mo. old; RNU/316: 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.

Cell culture

Dunning R-3327 MATLyLu; rat dorsal prostate adenocarcinoma, and PC-3 human prostate adenocarcinoma cells were utilized in this study. Cells were purchased from American Type Culture Collection (ATCC), and cultured following their instructions. Briefly, MATLyLu cells were cultured in 75cm^2 cell culture treated flasks (Corning, Durham, NC) using RPMI-1640 media (GE Healthcare Life Sciences, Marlborough, MA) supplemented with 10% FBS (RMBIO, Missoula, MT), 1% PenStrep (100 U/ml Penicillin and 100 µg/ml Streptomycin; Thermo Fisher Scientific, Waltham, MA), 100 mM sodium pyruvate (Thermo Fisher Scientific, Waltham, MA) and 0.025 mM dexamethasone (Cayman Chemical, Ann Arbor, MI) and incubated at 37°C with 5% CO2. PC-3 cells were cultured using the same flasks in RPMI-1640 media (ATCC) supplemented with 10% FBS and 2 mM L-glutamine and incubated at 37°C with 5% CO2. Growth medium for serum conditioned media studies was optimized as previously shown [24].

Orthotopic Model of Cancer

Upon reaching ~80% confluence, cells were quantified via hemocytometer to calculate appropriate dilutions (1,000,000 cells/ml) of 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 ~2x10^5 MATLyLu cells. These methods have been used previously to induce orthotopic prostate tumors [24,32,35].

Animals were anesthetized quickly with 5%, and then maintained on 2% isoflurane (O2 balance) Once a deep plane of anesthesia was obtained, (i.e., lack of toe-pinch reflex), with aseptic technique, an abdominal incision of ~1 cm, lateral of the midline, was made exposing the bladder/prostate complex. After the prostate was identified, ~2x105 MATLyLu cells suspended

in 0.1 ml PSS were injected into the ventral lobe of the prostate using a sterile 26G insulin syringe. During injection a sterile cotton tipped applicator was used to prevent any cell leakage from the prostate. 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). After final suture placement, 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 ~5 days post-injection twice daily until animals were randomized into their respective groups five days post cell injection. Animals were separated into sedentary tumor-bearing (TBS; n=11) and Acute Exercise tumor-bearing (TBAEX n=11) and (TBEX n=10) groups. The remaining 8 animals were used to determine plating efficiencies of each group before radiation as follows (TBS; n=3) and Acute Exercise tumor-bearing (TBAEX n=2) and (TBEX n=3). In total, 7 animals either did not complete, or were excluded from final analysis in these studies: 2 to metastasis and 5 from tumor generation failure.

Treadmill Exercise

All rats were habituated to treadmill (Harvard Apparatus, Holliston, MA) exercise prior to cancer cell injection 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 the treadmill incline was raised to 5% 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 60 min/day for the remainder of the 5-week training period. This training program was modified from previously used protocols [32,35] to represent moderate-intensity exercise training eliciting greater than 60% of maximal aerobic capacity ($\dot{V}o_2$peak) response, calculated from subset of animals that underwent $\dot{V}o_2$peak Post-Test testing. Both TBS and TBAEX groups where weekly habituated to the treadmill during tumor development.

Aerobic Capacity Testing

Testing of animal's aerobic capacity was performed as previously described and firmly established by my collaborators [36,37]. Briefly, this method employed the use of a small

metabolic chamber on one lane of a custom motor driven treadmill. Gas analysis was taken in real time within series CO_2 and O_2 analyzers (models CD-3A and S-3A/I, respectively, AEI Technologies). Calibration was performed before and after each testing session with calibration gasses that span the expected range of values based on previous investigations. The aerobic capacity test began by first weighing each animal, and then placing them on the treadmill for 2-3 minutes to obtain baseline values. Each test began at 15m/min up a 5% grade for 3 min as a familiarization and warm-up. During the second stage of the test, the treadmill speed was increased to 25m/min with grade held constant for the remainder of the test. Speed was held constant for 2 minutes at this speed, and then increased by 5-10m/min every minute until the rat was no longer willing/able (~4-7min) to keep pace which resulted in immediate termination of the test to avoid injury, and the animal was re-weighed. To evaluate successful tests, criteria of change in gait immediately before test termination and/or no further increases in $\dot{V}O_2$ despite increases in treadmill speed were implemented. If neither of these criteria were met tests were repeated following a 24hr period. Tests were completed both before cell injections (Pre-measures), and after ~5-6 weeks from cell injections (Post-measures).

Radiation Treatment

For in vivo studies, all animals were subjected to the same environment for radiation before collection of tissues. Radiated and non-radiated animals were subjected to 2 Gray and 0 Gray (Gy) radiation, respectively, with a linear accelerator (Clinac 2100 CD, Varian Medical Systems). Low-dose radiation was selected as 2Gy is the dosage of a single fraction that would be administered clinically. Acute animals performed one 30-minute bout of exercise and then radiated within 10–15-minute window after the completion of the exercise bout. Chronic exercise animals were radiated 24 hours after the last bout of exercise to avoid any acute effects of exercise. After radiation, tissue collections began by arterial blood collection from the abdominal aorta (via puncture) for serum separations in serum separator tubes (Becton, Dickinson and Company, Franklin Lakes, NJ), followed by removal of the heart (right ventricle was detached from the left ventricle and intraventricular septum post-mortem) while the animals were under a deep plane anesthesia (5% isoflurane/O2 balance).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 frozen for future analysis. For in vitro studies, all cells were radiated at 0,2,4,6,8Gy of radiation (RadSource RS-2000 Biological Research Irradiator, Buford, GA) at a dose rate of 100cGy/min.

Clonogenic Survival

For in vivo studies, tumors were harvested within 30 mins after the completion of radiation for determination of clonogenic survival according to the methods of Franken, et al., [38] and Brix, et al., [39]. After tumor digestion with enzyme cocktail (in-house, trypsin and collagenase mixture) to a single cell suspension, cells were re-suspended in normal media, and were counted to determine appropriate dilutions, followed by seeding varying densities of cells in 100mm and 60mmtissue culture dishes for optimization, and for main experimental conditions, 100-500 cells per plate were utilized. Five replicates per plating density were performed, for a total of 10 plates per animal experiment. Plates were placed in a humidified incubator maintained at 37°C and 5% CO_2 for ~10 days for development of cell colonies. Following colony formation, cells were fixed and stained with 20% methanol and 0.5% crystal violet in water. Using a stereomicroscope at 10-40x magnification, individual colonies containing >50 cells from each plate were counted as a single colony (see Figure 3.1). Plating efficiency (PE) was determined from the fraction of control cells initially plated that formed colonies. Survival fraction for each group was determined by dividing the number of colonies formed by the number of cells seeded multiplied by the plating efficiency determined from the control condition. The survival fractions were expressed as an average of the two plating densities for each condition. For in vitro studies, cells were plated in 12-well plates after being passaged treated by pooled conditioned serum from each group of animals as previously described by our group [24]. These plates were used (25-100 cells/well) to accommodate 4 biological replicates at all levels of radiation and treatment groups, with 2 separate experiments performed (8 total biological replicates) and the mean values used to report results.

Viability of Serum Supplemented Cells

Both MATLyLu and PC-3 cells were seeded in 96-well culture plates at a density of $2x10^4$ cells with serum supplemented media from either TBS, AEXTB, or TBEX animals in triplicate for 24, 48, 72hrs of incubation time both with, and without a treatment of radiation at 2 Gy. Viable cell number was quantified using the Cell Titer 96 Non-Radioactive Cell Proliferation Assay (MTT, Promega) with a spectrophotometer (accuSkan GO). All plates were

read at a wavelength of 570nm, and viable cell numbers are expressed as a percentage of the cell number (absorbance of the assay) of the TBS serum supplemented group. The assay was performed in replicates of 3 using pooled serum from each group, from 6 independent experiments being reported here.

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 Srere [40]. 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 in a 96-well plate. Samples were incubated in a spectrophotometer (accuSkan GO; Thermo Fisher Scientific, Waltham, MA) 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 oxaloacetic 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 9.3, Graphpad software, INC., La Jolla, CA) data analysis software was used for all statistical analyses. Statistical comparisons were made with one-or two-way or repeated measure analysis of variance (ANOVA) with Holm-Sidak post hoc tests as appropriate to assess statistical differences between groups for all measures. When non-equal standard deviations or normality failure occurred, Welch's or Kruskal-Wallis corrections were made as needed. Pearson correlations and linear regression analyses were performed to quantify relationships between tumor mass, $\dot{V}O_2$peak, and cardiac tissues. For analysis of survival curves, the linear-quadratic fit as applied to all conditions, as well as standard PE-based analysis as previously described [39], a two-way ANOVA was also utilized to evaluate differences at each level of radiation. A $p<0.05$ was set for statistical significance for all experiments with data reported as mean±SEM.

Results

Body mass was not different between groups across the period of experiments with (no differences between groups at any time point). Post-mortem tumor mass was lower, but not statistically significant between any group (Figure 3.2).

Cardiac and Skeletal Muscle Mass and Skeletal Muscle Citrate Synthase Activity

Absolute mass of the whole heart and left ventricle (LV) were normalized to body mass after measurement of Femur length was not different between groups (data not shown) to account for possible differences in relative size between groups. Compared to sedentary counterparts, TBEX did not have a significantly larger heart ($p = 0.08$ vs TBS, $p=0.11$ vs TBAEX Table 3.1) or left ventricle mass vs TBS or TBAEX ($p = 0.12$ vs TBS, $p=0.09$ vs TBAEX Table 3.1). However, when normalized to body mass, TBEX had greater heart (Figure 3.3) and LV masses compared to both groups ($p<0.05$, Table 3.1). Skeletal muscle mass was also normalized to body mass and was not different between groups for the gastrocnemius ($p > 0.05$), soleus ($p > 0.05$), or plantaris ($p > 0.05$), although there was a trend for a difference between TBS and TBEX ($p = 0.07$) muscles. However, skeletal muscle citrate synthase activity was greater in the TBEX group for both the soleus and red gastrocnemius muscle versus TBS and TBAEX ($p<0.05$, Table 3.1). Cardiac mass normalized to body mass was negatively correlated with tumor mass across all groups ($p<0.01$, Figure 3.3).

Aerobic Exercise Capacity

There was no difference in Pre-measures of $\dot{V}_{O2}$ between groups (Figure 3.4). The impact of exercise training on aerobic capacity was evident as only the TBEX animals who had increase in $\dot{V}_{O2peak}$ from Pre-measures to Post-measures ($p<0.05$, Figure 3.4). However, there was a decrease ($p<0.05$, Figure 3.4) in $\dot{V}_{O2peak}$ indicating a negative impact of prostate cancer on both the TBS and TBAEX groups. At Post-measures, the TBEX had a significantly higher $\dot{V}_{O2peak}$ ($p<0.05$, Figure 3.4) than both TBS and TBAEX groups. Post-measure $\dot{V}_{O2peak}$ had a significant positive correlation ($p<0.05$ Figure 3.4) with heart to body mass ratios in all animals, with EXTB having the largest heart to body mass ratio of the three groups (Figure 3.3)

Clonogenic Response

For *in vivo* studies, PE were not significantly different between groups (TBS= 32±6, TBAEX= 33±5, and TBEX= 29±3%, $p>0.05$) The PE from each group was used to calculate appropriate densities for all experiments. There was a significant difference in survival fraction

(SF) at 2Gy of radiation for both TBAEX and TBEX vs TBS ($p<0.05$, Figure 3.5) For *in vitro* measures, cell survival curves were generated (Figure 3.5) using PE of 37.2% and 43.7% for MATLyLu and PC-3 cells respectively, that were calculated using normal growth media and used for all plating densities. MATLyLu cells cultured and maintained in serum supplemented media from TBAEX and EXTB animals' lower survivability of cells than TBS animals at both 2Gy, and 4Gy doses of radiation when analyzed via two-way ANOVA. ($p<0.05$, Figure 3.5) This was not the case with PC-3 cells, where the curves did differ slightly, the only significant difference was detected at 2Gy of radiation between the TBS and EXTB groups ($p<0.05$, Figure 3.5) The survival curve radiological parameters for *in vitro* experiments can be found in Table 3.2. There was a rejected null hypothesis of the same curve fit for all groups, and different curves were suggested for each group after a comparison of fits only for the MATLyLu cells.

Serum supplemented Viability Assessment

In the series of serum supplemented MTT assays, a common theme emerged for reductions in viable cell number in the TBAEX and TBEX groups compared to the TBS (normalized to 100%). In the MATLyLu cells plated without irradiation, there was significant reductions in viable cell number at 24, and 48 hours in the TBEX animals compared to the TBS ($p<0.05$, Figure 3.6) In the single 2Gy irradiated MATLyLu studies, there was a decreased viability in both the TBAEX and TBEX group vs TBS only at 24 h ($p<0.05$, Figure 3.6). TBS had significantly greater viable cell numbers at 24 h hours vs TBAEX ($p<0.05$, Figure 3.7) in the non-irradiated PC-3 cells as well. Following irradiation at 2Gy, like MATLyLu, there was a decrease in the viable PC-3 cell number in both the TBAEX and TBEX groups vs TBS only at 24hr ($p<0.05$, Figure 3.7).

Discussion

Investigating the ability of aerobic exercise both acutely, and chronically to enhance radiosensitivity of prostate tumors prostate cancer is clinically important. There are several novel findings from this series of studies investigating the impact of exercise on prostate cancer atrophy mitigation, and radiosensitization of solid tumors. Specifically, we show for the first time, in vivo, that aerobic exercise both acutely, and over a period of training, can sensitize solid tumors to radiotherapy treatment, that resulted in a decrease in the survivability of tumor cell. Further, that serum from both acute, and chronically exercised animals can both increase radiosensitivity of prostate cancer cells, and decrease the viability of prostate tumor cells with, and without radiation treatments. Lastly, both sedentary groups (TBS TBAEX) of animals had a diminished $\dot{V}_{o2peak}$, that was associated with a lower Heart/Body mass ratio, that was prevented with exercise training, although not novel, contributes to the benefits seen herein of exercise training across the progression of cancer seen in clinical studies [21,26]. Collectively, this suggests the potential of systemic influences on both overall improved cardiovascular function, and improved radiosensitivity in solid tumors in combination with the proposed direct effects on the tumor microenvironment These findings combined with the exercises ability to improve aerobic capacity and potentially quality of life of cancer patients is an important step to improving patients' quality and outcomes of care.

Previous studies have examined the radiosensitization of cancer via hyperthermia [41], or pharmaceutical agents [42] that have often been unable to target the tumor specifically, and thus tend to have mixed results [45]. Aerobic exercise has been studied for its specific effects on tumor growth and proliferation with mixed results [43,44,55]. Recently, exercise has been investigated not only for its ability to modify whole tumor growth, but to alter the tumor microenvironment favorably for enhancement of both chemotherapy [29,46] and radiation [45,47]. To our knowledge this is the first investigation in an orthotopic rat model to specifically show tumor radiotherapy responses following to both a single acute bout, and chronic exercise training. In our study, chronic exercise alone did not impact tumor mass (Figure 3.2), which has been consistent in this model of prostate cancer [18]. Both chronic exercise training and an acute bout of exercise were able to significantly decrease the survivability of tumor cells when compared to sedentary counterparts *in vivo* and *in vitro* assessed via clonogenic survival assays (Figure 3.5). This comes following studies from McCullough et al. showed possible efficacy to

improve tumor oxygenation via increased blood flow during exercise [27]. This increase in oxygenation during exercise could partially explain the acute enhanced radiotherapy response in the TBAEX animals through mitigation of hypoxic radioresistant regions within the tumor [14]. However, we did not directly measure hypoxia of these solid tumors, and transient increases in oxygenation would presumably fade within minutes of the end of exercise [48,49]. While animals were radiated as quickly as possible following the acute bout of exercise, it is recognized this window could have been missed. Therefore, systemic changes via acute exercise that could be altering the tumor microenvironment are likely playing a role (although not measured in this study) in an enhanced radiotherapy response seen in this investigation. This was supported by the *in vitro* findings of an enhanced radiosensitization (Figure 3.5) in the MATLyLu cell line as well as a decreased cell viability following radiation at 2Gy (Figure 3.6). Following the chronic exercise training intervention solid tumors have been shown to undergo vascular remodeling that is implicated in improved oxygenation, more homogenous blood flow, and decreased areas of necrosis [35,47,]. Although none of these parameters were measured in this study, these are some of the postulated mechanisms behind the enhanced radiotherapy response seen in this investigation [28]. The likelihood of systemic effects of chronic aerobic exercise training likely also played a role in this response (much like acute exercise) as decrease in both survival fraction and viability during the *in vitro* serological studies in MATLyLu cells exposed to the same amount of radiation, as well as in lieu of therapy. Indeed, in a recent study in mice, using an ectopic model, by Dufresne et al, a sensitization to fractionated radiation therapy was able to decrease areas of necrosis, and decrease tumor volume [47]. These findings although encouraging, likely support systemic changes impacting the radiotherapy responses in this investigation, as different mechanisms in ectopic and orthotopic models of cancer have been shown to have extreme difference in perfusion response to exercise and proposed modulation of the tumor microenvironment [28].

We investigated the anti-cancer/therapeutic effects of acute and chronic aerobic exercise in these studies in both *in vivo and in vitro* models. This can be challenging, as the various timing, training intensities, and/or durations (acute or chronic exercise) can elicit differential responses in the levels of many circulating factors (tumor necrosis factor alpha (TNF-α), insulin-like growth factor-1 (IGF-1), interleukin 6 (IL-6), and insulin-like growth factor-binding protein-1 (IGFBP-1)), both growth or cytokine factors, that play significant roles in tumor cell growth

[44,55-56, 59]. Although not quantified, these various factors are thought to have different systemic responses to both acute and chronic exercise training [44,59]. Therefore, results in this study represent how moderate exercise training and acute exercise may impact prostate cancer cell viability immediately and chronically to suppress tumor progression over both the disease's long latency period [7]. The use of androgen-insensitive prostate cancer cell lines [58] to avoid potential effects of altered testosterone concentrations between the two exercise states were also used [58]. Specifically, long-term aerobic exercise can decrease serum testosterone [56], while acute exercise can transiently increase serum testosterone [57]. Thus, we attempted to prevent changes in cell viability found herein to be due to altered serum androgen concentrations.

Prostate cancer patients often lose aerobic capacity, experience fatigue, and loss of quality of life [20,25] The study of prostate cancer in pre-clinical models is important in understanding if these mechanisms are resulting from the cancer, or the anti-cancers deployed to treat the disease. Both atrophy and cachexia from various forms of cancer may occur in pre-clinical animal models and humans [61] that could contribute to losses in aerobic capacity independent of therapy [23,33]. Although the tumor-bearing animals in the current study were not cachexic, there was significant atrophy of the heart and LV when normalized to body mass in both the TBS and TBAEX animals' vs TBEX (Figure 3.3), that also had a inverse relationship with tumor mass (Figure 3.3) This relationship was particularly interesting considering the lack of such an inverse relationship between tumor mass and select hindlimb skeletal muscle mass (data not shown) that may reflect distinct molecular pathways regulate cardiac atrophy compared to skeletal muscle atrophy in pre-clinical models [34,39,41,61]. We also evaluated aerobic capacity both before tumor cell injections and post exercise training period, using a standardized maximal treadmill running protocol [70], to assess the impact of loss of heart mass on aerobic capacity. Specifically, the smaller heart mass would likely result in a reduced stroke volume, that could potentially reduce cardiac output during exercise, which would decrease $\dot{V}_{o2peak}$ (seen in this study), ($\dot{V}_{O2}$= HR*SV* (A_{O2}-V_{O2}) if there was no compensatory increase in HR. The difference between groups in cardiac mass was expected, and had a negative correlation with tumor mass, and a positive correlation with $\dot{V}_{o2peak}$, which support previous findings [23,67,] that prostate cancer, independent of therapy induces cardiac atrophy that could be playing a role in fatigue associated with prostate cancer and its treatments [21,22]. There are several potential mechanisms for this cardiac atrophy, through necrotic and/or apoptotic pathways, as discussed in

recent reviews [33,43,61]. There is considerable evidence that both chronic exercise training and acute exercise affect circulating cytokine levels potentially providing a mitigating effect on multiple pathways of muscle wasting [61,68,69]. However, the single bout of exercise used herein would not be enough to alter chronic tumor growth or potential malignant systemic effects. Although no mechanisms of atrophy were measured in this series of studies, 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 [66]. 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, that could potentially be altered via exercise [61,66,69]. Exercise training is known to be efficacious in increasing LV mass in health as well as multiple disease states and additional studies are needed to investigate mechanism of cardiac atrophy associated with prostate cancer, and exercises sparring effect on cardiac tissue.

Limitations

Although this study provides many novel findings, several limitations need addressed. Food consumption and cage activity was not measured and could lead to decreased levels of protein synthesis, and ultimately contribute to the cardiac atrophy/loss of aerobic capacity in the tumor-bearing groups if caloric demands are not met. However, the continued increase in mass (data not shown) and that femur length was not different between groups (data not shown) suggests growth rates of the cancer group were of similar proportion. From animal studies, the ideal length of training is typically 6-8 weeks of constant load moderate intensity exercise to induce an exercise phenotype [70]. 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. The lack of mechanisms for some of these findings is evident, however, this study sought to first establish impact exercise on prostate cancer treatment and provides a direction for future studies. Lastly, the lack of a sham or sedentary control group is noted and could provide further insights into the atrophy observed herein, and the impact on aerobic capacity, as the loss of aerobic capacity could potentially be impacted by the length of study.

Conclusions

Overall, this investigation gives insights into the impact of aerobic exercise use as a therapeutic modulator of radiosensitization in solid prostate tumors in rats. Specifically, both a single acute bout, and chronic exercise training enhanced radiosensitivity of prostate tumors to a single 2Gy dose or radiation, that resulted in reduced survival fractions and viability of prostate cancer cells from both *in vivo* and *in vitro* experiments. Further, that chronic aerobic exercise can mitigate cardiac atrophy that is associated with a reduced aerobic capacity, providing further evidence of its utility to prevent fatigue occurring in prostate cancer patients. However, these findings are lacking mechanistic underpinnings, and additional studies investigating the potential of exercise for improving prostate cancer therapeutic outcomes and cardiac atrophy mitigation in both pre-clinical and clinical models is warranted.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA: A Cancer Journal for Clinicians 2019; 69:7–34.

2. Miller KD, Nogueira L, Mariotto AB, Rowland JH, Yabroff KR, Alfano CM, Jemal A, Kramer JL, Siegel RL. Cancer treatment and survivorship statistics, 2019. CA: A Cancer Journal for Clinicians 2019; 69:363–385.

3. Stephen W. Leslie, Taylor L. Soon-Sutton;, Hussain Sajjad;, Larry E Siref. Prostate cancer. Elsevier; 2020.

4. Feng LR, Wolff BS, Liwang J, Regan JM, Alshawi S, Raheem S, Saligan LN. Cancer related fatigue during combined treatment of androgen deprivation therapy and radiotherapy is associated with mitochondrial dysfunction. International Journal of Molecular Medicine 2020; 45:485–496.

5. Eisemann N, Nolte S, Schnoor M, Katalinic A, Rohde V, Waldmann A. The ProCaSP study: quality of life outcomes of prostate cancer patients after radiotherapy or radical prostatectomy in a cohort study. BMC Urology 2015; 15.

6. Smith DP, King MT, Egger S, Berry MP, Stricker PD, Cozzi P, Ward J, O'Connell DL, Armstrong BK. Quality of life three years after diagnosis of localised prostate cancer: population based cohort study. The BMJ 2009; 339:195.

7. Vaupel P. Tumor microenvironmental physiology and its implications for radiation oncology. Seminars in Radiation Oncology; Tumor Physiology 2004; 14:198–206.

8. Vaupel P. Hypoxia and Aggressive Tumor Phenotype: Implications for Therapy and Prognosis. The Oncologist 2008; 13:21–26.

9. Milosevic M, Warde P, Meńard C, Chung P, Toi A, Ishkanian A, McLean M, Pintilie M, Sykes J, Gospodarowicz M, Catton C, Hill RP, Bristow R. Tumor Hypoxia Predicts Biochemical Failure following Radiotherapy for Clinically Localized Prostate Cancer. Clinical Cancer Research 2012; 18:2108–2114.

10. Turaka A, Buyyounouski MK, Hanlon AL, Horwitz EM, Greenberg RE, Movsas B. Hypoxic Prostate/Muscle Po2 Ratio Predicts for Outcome in Patients With Localized Prostate Cancer: Long-Term Results. International Journal of Radiation Oncology*Biology*Physics 2012; 82:e433–e439.

11. Rockwell S, Dobrucki IT, Kim EY, Marrison ST, Vu VT. Hypoxia and radiation therapy: Past history, ongoing research, and future promise. Current Molecular Medicine 2009; 9:442.
12. Winkler F, Kozin S v., Tong RT, Chae SS, Booth MF, Garkavtsev I, Xu L, Hicklin DJ, Fukumura D, di Tomaso E, Munn LL, Jain RK. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: Role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell 2004; 6:553–563.
13. Henke M, Laszig R, Rübe C, Schäfer U, Haase KD, Schilcher B, Mose S, Beer KT, Burger U, Dougherty C, Frommhold H. Erythropoietin to treat head and neck cancer patients with anaemia undergoing radiotherapy: Randomised, double-blind, placebo-controlled trial. Lancet 2003; 362:1255–1260.
14. Dewhirst MW, Vujaskovic Z, Jones E, Thrall D. Re-setting the biologic rationale for thermal therapy. International Journal of Hyperthermia 2005; 21:779–790.
15. Cairns R, Papandreou I, Denko N. Overcoming Physiologic Barriers to Cancer Treatment by Molecularly Targeting the Tumor Microenvironment Unique Microenvironment of Solid Tumors. Mol Cancer Res 2006; 4:61–70.
16. Vaupel P, Kallinowski F, Okunieff P. Blood Flow, Oxygen and Nutrient Supply, and Metabolic Microenvironment of Human Tumors: A Review. Cancer Res 1989; 49:6449.
17. Wang Y, Jacobs EJ, Gapstur SM, Maliniak ML, Gansler T, McCullough ML, Stevens VL, Patel A v. Recreational Physical Activity in Relation to Prostate Cancer-specific Mortality Among Men with Nonmetastatic Prostate Cancer. European Urology 2017; 72:931–939.
18. Harber MP, Kaminsky LA, Arena R, Blair SN, Franklin BA, Myers J, Ross R. Impact of Cardiorespiratory Fitness on All-Cause and Disease-Specific Mortality: Advances Since 2009. Progress in Cardiovascular Diseases 2017; 60:11–20.
19. Kenfield SA, Stampfer MJ, Giovannucci E, Chan JM. Physical activity and survival after prostate cancer diagnosis in the health professionals follow-up study. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology 2011; 29:726–732.
20. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee I-M, Nieman DC, Swain DP. 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. Medicine and Science in Sports and Exercise 2011; 43:1334–1359.

21. Galvao DA, Taaffe DR, Spry N, Joseph D, 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. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology 2010; 28:340–347.
22. Wall BA, Galvão DA, Fatehee N, Taaffe DR, Spry N, Joseph D, Newton RU. Reduced Cardiovascular Capacity and Resting Metabolic Rate in Men with Prostate Cancer Undergoing Androgen Deprivation: A Comprehensive Cross-Sectional Investigation. Advances in Urology 2015
23. Segal RJ, Reid RD, Courneya KS, Sigal RJ, Kenny GP, Prud'Homme DG, Malone SC, Wells GA, Scott CG, Slovinec D'Angelo ME. Randomized controlled trial of resistance or aerobic exercise in men receiving radiation therapy for prostate cancer. Journal of Clinical Oncology 2009; 27:344–351.
24. Opoku-Acheampong AB, Baumfalk DR, Horn AG, Kunkel ON, Ganta CK, McCullough DJ, Siemann DW, Muller-Delp J, Behnke BJ. Prostate cancer cell growth characteristics in serum and prostate-conditioned media from moderate-intensity exercise-trained healthy and tumor-bearing rats. American Journal of Cancer Research 2019; 9:650.
25. Scott JM, Nilsen TS, Gupta D, Jones LW. Exercise therapy and cardiovascular toxicity in cancer. Circulation 2018; 137:1176–1191.
26. Parsons JK. Prostate cancer and the therapeutic benefits of structured exercise. Journal of Clinical Oncology 2014; 32:271–2.
27. McCullough DJ, Stabley JN, Siemann DW, Behnke BJ. Modulation of blood flow, hypoxia, and vascular function in orthotopic prostate tumors during exercise. Journal of the National Cancer Institute 2014.
28. Wiggins JM, Opoku-Acheampong AB, Baumfalk DR, Siemann DW, Behnke BJ. Exercise and the Tumor Microenvironment: Potential Therapeutic Implications. Exercise and Sport Sciences Reviews 2018; 46:56–64.
29. Schadler KL, Thomas NJ, Galie PA, Bhang DH, Roby KC, Addai P, Till JE, Sturgeon K, Zaslavsky A, Chen CS, Ryeom S. Tumor vessel normalization after aerobic exercise enhances chemotherapeutic efficacy. Oncotarget 2016; 7:65429.

30. Morrell MBG, Alvarez-Florez C, Zhang A, Kleinerman ES, Savage H, Marmonti E, Park M, Shaw A, Schadler KL. Vascular Modulation Through Exercise Improves Chemotherapy Efficacy in Ewing Sarcoma. Pediatric Blood & Cancer 2019; 66:e27835.
31. Antunes JMM, Ferreira RMP, Moreira-Gonçalves D. Exercise Training as Therapy for Cancer-Induced Cardiac Cachexia. Trends in Molecular Medicine 2018; 24:709–727.
32. Baumfalk DR, Opoku-Acheampong AB, Caldwell JT, Ade CJ, Copp SW, Musch TI, Behnke BJ. Effects of prostate cancer and exercise training on left ventricular function and cardiac and skeletal muscle mass. Journal of Applied Physiology 2018; 126:668–680.
33. Esau PJ, Gittemeier EM, Opoku-Acheampong AB, Rollins KS, Baumfalk DR, Poole DC, Musch TI, Behnke BJ, Copp SW. Prostate cancer reduces endurance exercise capacity in association with reductions in cardiac and skeletal muscle mass in the rat. American Journal of Cancer Research 2017; 7:2566.
34. Baumfalk DR, Opoku-Acheampong AB, Caldwell JT, Butenas ALE, Horn AG, Kunkel ON, Copp SW, Ade CJ, Musch TI, Behnke BJ. Effects of high-intensity training on prostate cancer-induced cardiac atrophy. American Journal of Translational Research 2021; 13:197–209.
35. McCullough DJ, Nguyen LM-D, Siemann DW, Behnke BJ. Effects of exercise training on tumor hypoxia and vascular function in the rodent preclinical orthotopic prostate cancer model. Journal of Applied Physiology 2013; 115:1846–1854.
36. Copp SW, Davis RT, Poole DC, Musch TI. Reproducibility of endurance capacity and V̇O2peak in male Sprague-Dawley rats. Journal of Applied Physiology 2009; 106:1072–1078.
37. Musch TI, Bruno A, Bradford GE, Vayonis A, Moore RL. Measurements of metabolic rate in rats: a comparison of techniques. Journal of Applied Physiology (Bethesda, Md: 1985) 1988; 65:964–970.
38. Franken NAP, Rodermond HM, Stap J, Haveman J, van Bree C. Clonogenic assay of cells in vitro. Nature Protocols 2006 1:5 2006; 1:2315–2319.
39. Brix N, Samaga D, Belka C, Zitzelsberger H, Lauber K. Analysis of clonogenic growth in vitro. Nature Protocols 2021 16:11 2021; 16:4963–4991.
40. PA., Srere. Citrate synthase: [EC 4.1. 3.7. Citrate oxaloacetate-lyase (CoA-acetylating)]. Methods Enzymol 1969; 3–11.

41. Elming PB, Sørensen BS, Oei AL, Franken NAP, Crezee J, Overgaard J, Horsman MR. Hyperthermia: The Optimal Treatment to Overcome Radiation Resistant Hypoxia. Cancers 2019, Vol 11, Page 60 2019; 11:60.
42. Siemann DW, Rojiani AM. Enhancement of radiation therapy by the novel vascular targeting agent ZD6126. International Journal of Radiation Oncology Biology Physics 2002; 53:164–171.
43. Ashcraft KA, Peace RM, Betof AS, Dewhirst MW, Jones LW. Efficacy and Mechanisms of Aerobic Exercise on Cancer Initiation, Progression, and Metastasis: A Critical Systematic Review of In Vivo Preclinical Data. Cancer Research 2016; 76:4032–4050.
44. Jones LW, Viglianti BL, Tashjian JA, Kothadia SM, Keir ST, Freedland SJ, Potter MQ, Moon EJ, Schroeder T, Herndon JE, Dewhirst MW. Effect of aerobic exercise on tumor physiology in an animal model of human breast cancer. Journal of Applied Physiology 2010; 108:343–348.
45. Dufresne S, Richard C, Dieumegard A, Orfila L, Delpon G, Chiavassa S, Martin B, Rouvière L, Escoffre J-M, Oujagir E, Denis de Senneville B, Bouakaz A, Rioux-Leclercq N, Potiron V, Rébillard A. Voluntary Wheel Running Does Not Enhance Radiotherapy Efficiency in a Preclinical Model of Prostate Cancer: The Importance of Physical Activity Modalities? Cancers 2021; 13:5402.
46. Morrell MBG, Alvarez-Florez C, Zhang A, Kleinerman ES, Savage H, Marmonti E, Park M, Shaw A, Schadler KL. Vascular modulation through exercise improves chemotherapy efficacy in Ewing sarcoma. Pediatric Blood & Cancer 2019; e27835.
47. Dufresne S, Guéritat J, Chiavassa S, Noblet C, Assi M, Rioux-Leclercq N, Rannou-Bekono F, Lefeuvre-Orfila L, Paris F, Rébillard A. Exercise training improves radiotherapy efficiency in a murine model of prostate cancer. FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology 2020; 34:4984–4996.
48. Moulder JE, Rockwell S. Hypoxic fractions of solid tumors: Experimental techniques, methods of analysis, and a survey of existing data. International Journal of Radiation Oncology*Biology*Physics 1984; 10:695–712.
49. Vaupel P, Kelleher DK. Blood Flow and Oxygenation Status of Prostate Cancers. Oxygen Transport to Tissue XXXIV 2013; 299–305.

50. Schumacher O, Galvão DA, Taaffe DR, Chee R, Spry N, Newton RU. Exercise modulation of tumour perfusion and hypoxia to improve radiotherapy response in prostate cancer. Prostate Cancer and Prostatic Diseases 2021; 24.
51. Garcia E, Becker VGC, McCullough DJ, Stabley JN, Gittemeier EM, Opoku-Acheampong AB, Sieman DW, Behnke BJ. Blood flow responses to mild-intensity exercise in ectopic vs. orthotopic prostate tumors; dependence upon host tissue hemodynamics and vascular reactivity. Journal of Applied Physiology 2016; 121:15-24.
52. Petersen AMW, Pedersen BK. The role of IL-6 in mediating the anti-inflammatory effects of exercise. Journal of Physiology and Pharmacology 2006.
53. Pedersen BK, Saltin B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. Scandinavian Journal of Medicine & Science in Sports 2015; 25:1–72.
54. Rundqvist H, Augsten M, Strömberg A, Rullman E, Mijwel S, Kharaziha P, Panaretakis T, Gustafsson T, Östman A. Effect of Acute Exercise on Prostate Cancer Cell Growth. PLoS ONE 2013; 8: e67579.
55. Ngo TH, Barnard RJ, Leung PS, Cohen P, Aronson WJ. Insulin-Like Growth Factor I (IGF-I) and IGF Binding Protein-1 Modulate Prostate Cancer Cell Growth and Apoptosis: Possible Mediators for the Effects of Diet and Exercise on Cancer Cell Survival. Endocrinology 2003; 144:2319–2324.
56. Orange ST, Jordan AR, Saxton JM. The serological responses to acute exercise in humans reduce cancer cell growth in vitro: A systematic review and meta-analysis. Physiological Reports 2020; 8(22): e14635.
57. Hackney A. Endurance Exercise Training and Reproductive Endocrine Dysfunction in Men Alterations in the Hypothalamic-Pituitary-Testicular Axis. Current Pharmaceutical Design 2005; 7:261–273.
58. Isaacs JT, Heston WDW, Weissman RM, Coffey DS. Animal Models of the Hormone-sensitive and-insensitive Prostatic Adenocarcinomas, Dunning R-332T-H, R-3327-HI, and R-3327-AT. Cancer Research 1978; 38:4353–4359.
59. Cumming DC, Brunsting LA, Strich G, Ries AL, Rebar RW. Reproductive hormone increases in response to acute exercise in men. Medicine and Science in Sports and Exercise 1986; 18:369–373.

60. Hart NH, Galvão DA, Newton RU. Exercise medicine for advanced prostate cancer. Current Opinion in Supportive and Palliative Care 2017; 11:247–257.
61. Powers SK, Lynch GS, Murphy KT, Reid MB, Zijdewind I. Disease-induced skeletal muscle atrophy and fatigue. Medicine and Science in Sports and Exercise 2016; 48:2307.
62. Murphy KT. The pathogenesis and treatment of cardiac atrophy in cancer cachexia. American Journal of PhysiologyHeart and Circulatory Physiology 2016; 310:466.
63. Yuan L, Han J, Meng Q, Xi Q, Zhuang Q, Jiang Y, Han Y, Zhang B, Fang J, Wu G. Muscle-specific E3 ubiquitin ligases are involved in muscle atrophy of cancer cachexia: An in vitro and in vivo study. Oncology Reports 2015; 2261-2268.
64. Barkhudaryan A, Scherbakov N, Springer J, Doehner W. Cardiac muscle wasting in individuals with cancer cachexia. ESC Heart Failure 2017; 458-467.
65. 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, Anker SD. Prevention of liver cancer cachexia-induced cardiac wasting and heart failure. European Heart Journal 2014; 35:932–941.
66. Bodine SC, Baehr LM. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. American Journal of Physiology Endocrinology and Metabolism 2014; 307:E469-84.
67. 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, Ferreira R. Exercise training protects against cancer-induced cardiac remodeling in an animal model of urothelial carcinoma. Archives of Biochemistry and Biophysics 2018; 645:12–18.
68. Zhou J, Liu B, Liang C, Li Y, Song YH. Cytokine Signaling in Skeletal Muscle Wasting. Trends in Endocrinology and Metabolism 2016; 27:335-347.
69. Souza RWA, Piedade WP, Soares LC, Souza PAT, Aguiar AF, Vechetti-Júnior IJ, Campos DHS, Fernandes AAH, Okoshi K, Carvalho RF, Cicogna AC, Dal-Pai-Silva M. Aerobic exercise training prevents heart failure-induced skeletal muscle atrophy by anti-catabolic, but not anabolic actions. PloS One 2014; 9:e110020.

70. Kregel KC, Allen DL, Booth FW, Fleshner MR, Henriksen EJ, Musch TI, O'Leary DS, Parks CM, Poole DC, Ra'anan AW. Resource book for the design of animal exercise protocols. American Physiological Society 2006; 152.

Table 3.1 Animal Characteristics

Animal Characteristics	Sedentary Tumor- Bearing (n=9)	Acute Tumor-Bearing (n=9)	Exercise Tumor-Bearing (n=7)
Absolute Tissue Mass (g)			
Heart	0.94±0.03	0.96±0.04	1.05±0.02
Left Ventricle	0.61±0.01	0.60±0.03	0.71±0.05
Gastrocnemius	1.85±0.10	1.86±0.05	1.90±0.09
Soleus	0.16±0.01	0.15±0.01	0.18±0.01
Plantaris	0.21±0.01	0.22±0.01	0.26±0.01
Tumor Mass	-	-	-
Tissue Mass Normalized to Body Mass (mg/g)			
Heart/Body mass	2.78±0.12	2.85±0.09	3.20±0.07* **
Left Ventricle/Body mass	1.98±0.05	2.01±0.08	2.26±0.08* **
Gastrocnemius/Body mass	5.72±0.24	5.67±0.32	5.89±0.22
Soleus/Body mass	0.43±0.02	0.45±0.04	0.48±0.03
Plantaris/Body mass	0.69±0.03	0.72±0.06	0.79±0.04
Tumor/Body mass (%)	2.01±0.21	1.94±0.23	1.73±0.14
Skeletal Muscle Citrate Synthase Activity (μmol/min/g)	n=8	n=8	n=7
Soleus	14.8±1.1	15.2±1.0	19.8±1.0* **
Red gastrocnemius	19.7±1.9	18.9±1.5	24.8±1.8* **

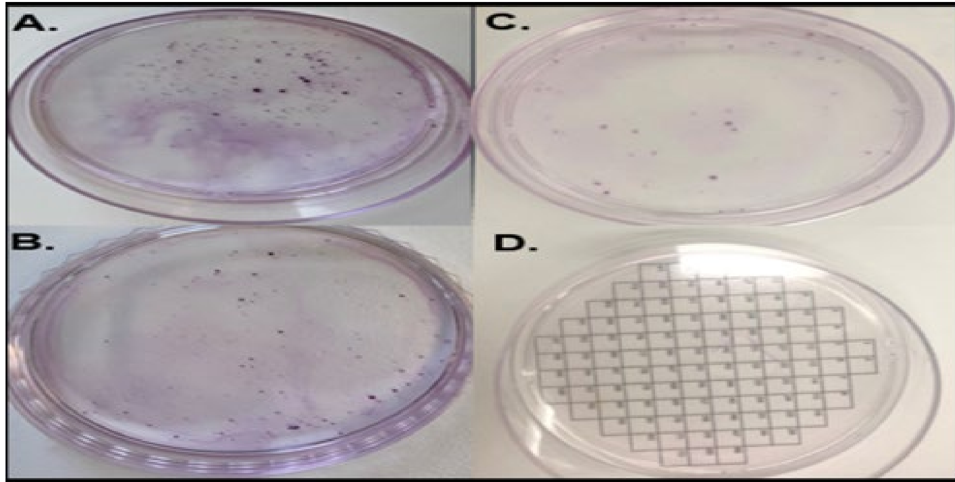
Data are mean±SEM *= $p<0.05$ vs. Sedentary Tumor-Bearing **= $p<0.05$ vs. Acute Tumor-Bearing

Table 3.2 Survival Curve Parameters

Survival Curve Parameters	TBS	TBAEX	TBEX
MATLyLu Cells			
Alpha	0.286±0.07	0.322±0.06	0.361±0.06
Beta	0.0434±0.02	0.0791±0.02	0.0715±0.01
Alpha/Beta	6.634	4.051	5.082
PC-3 Cells			
Alpha	0.151±0.06	0.270±0.09	0.307±0.11
Beta	0.036±0.02	0.015±0.01	0.016±0.02
Alpha/Beta	4.210	18.25	19.07

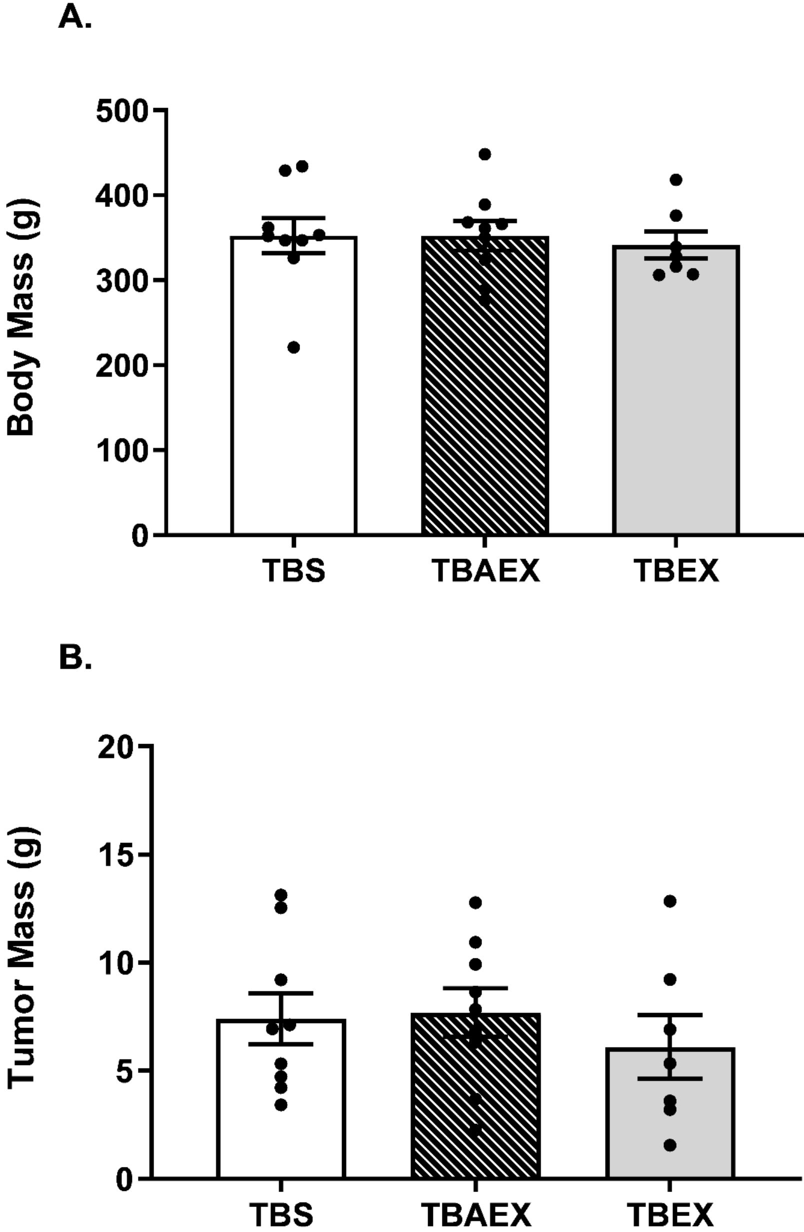
***** Values are means ± 95% confidence intervals

Figure 3.1 Representative Plates



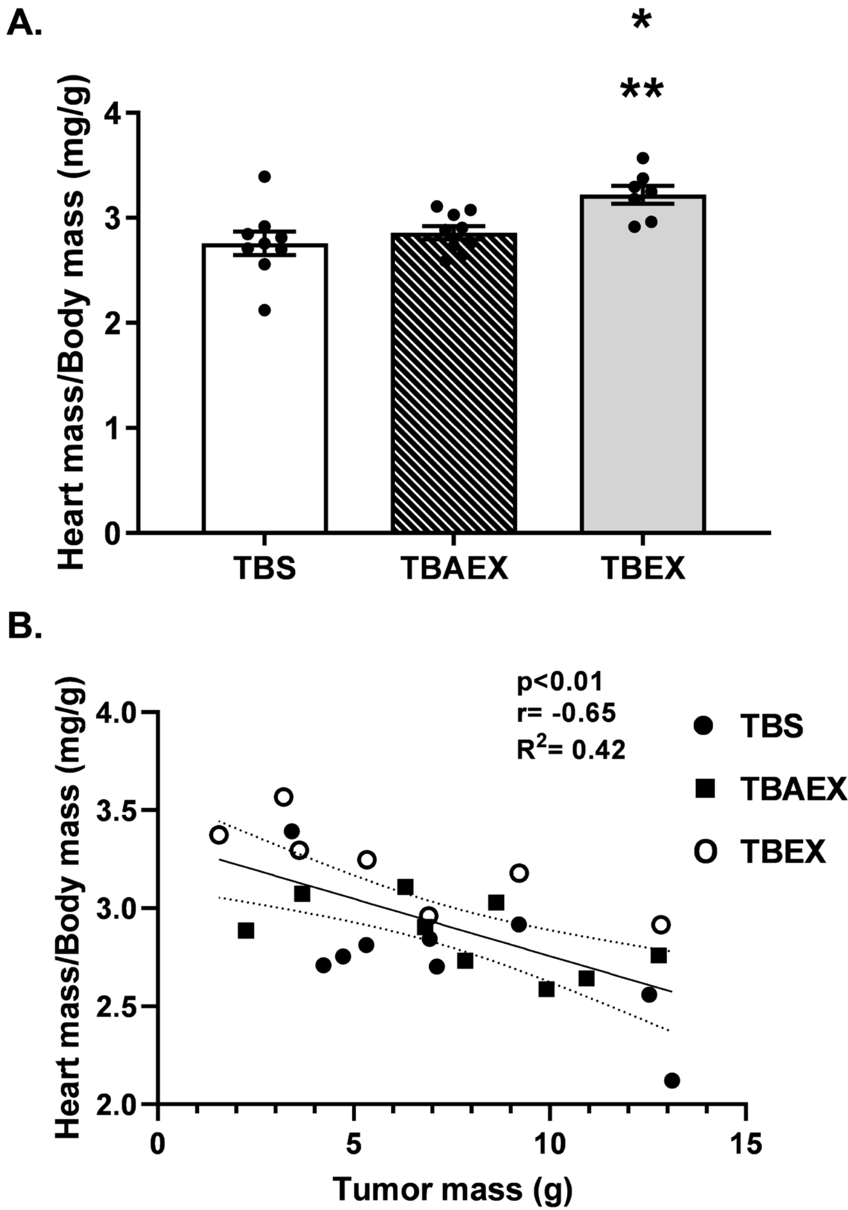
Representative plates for clonogenic cell growth. Panel A. represents TBS, B. is TBAEX, and panel C. is an example of plating from a TBEX animal. The plate in panel D. is an example of the grid pattern we used to assist in counting colonies.

Figure 3.2 Body Mass and Tumor Mass



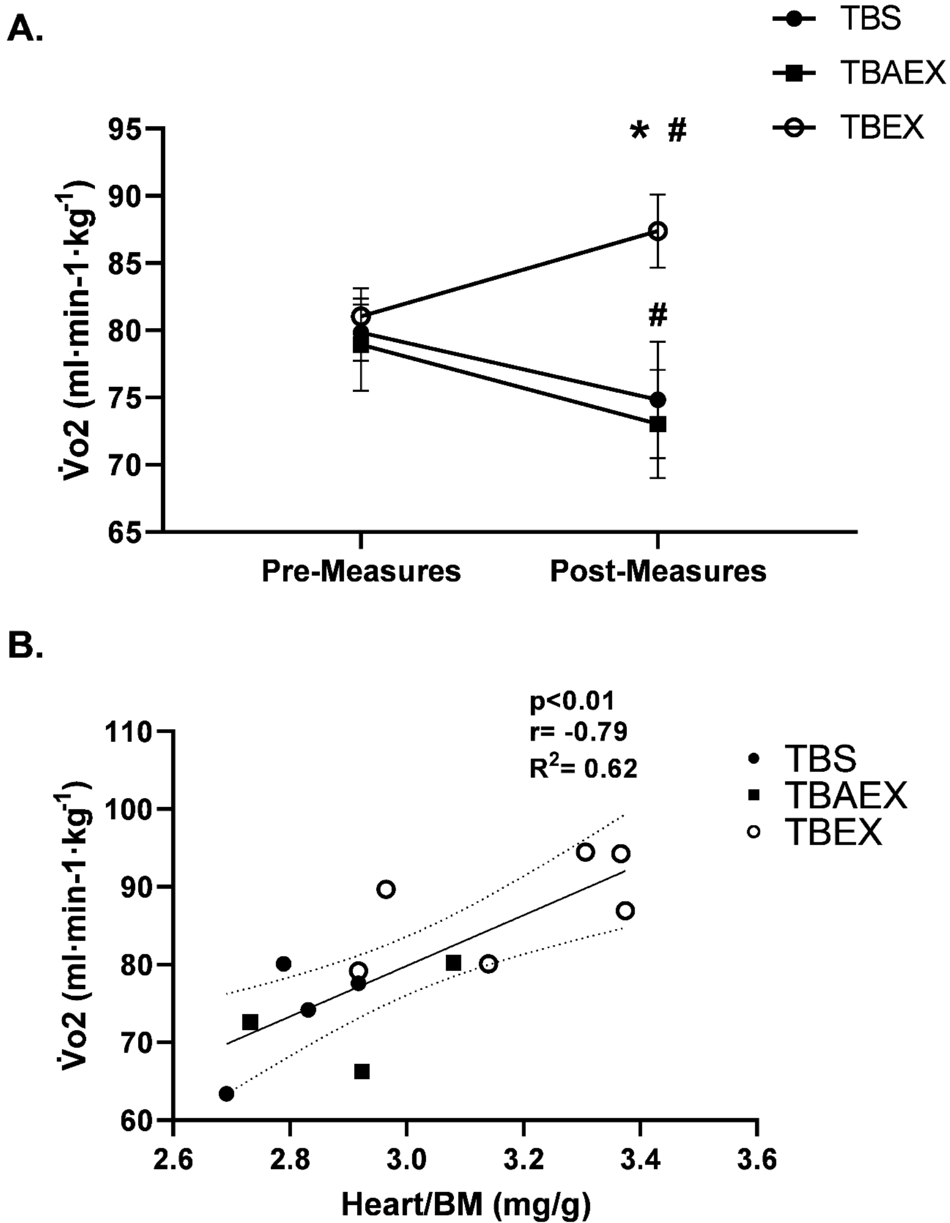
There were no significant differences in (A.) body mass, or tumor mass (B.) between groups, $p > 0.05$.

Figure 3.3 Cardiac Mass and Correlation



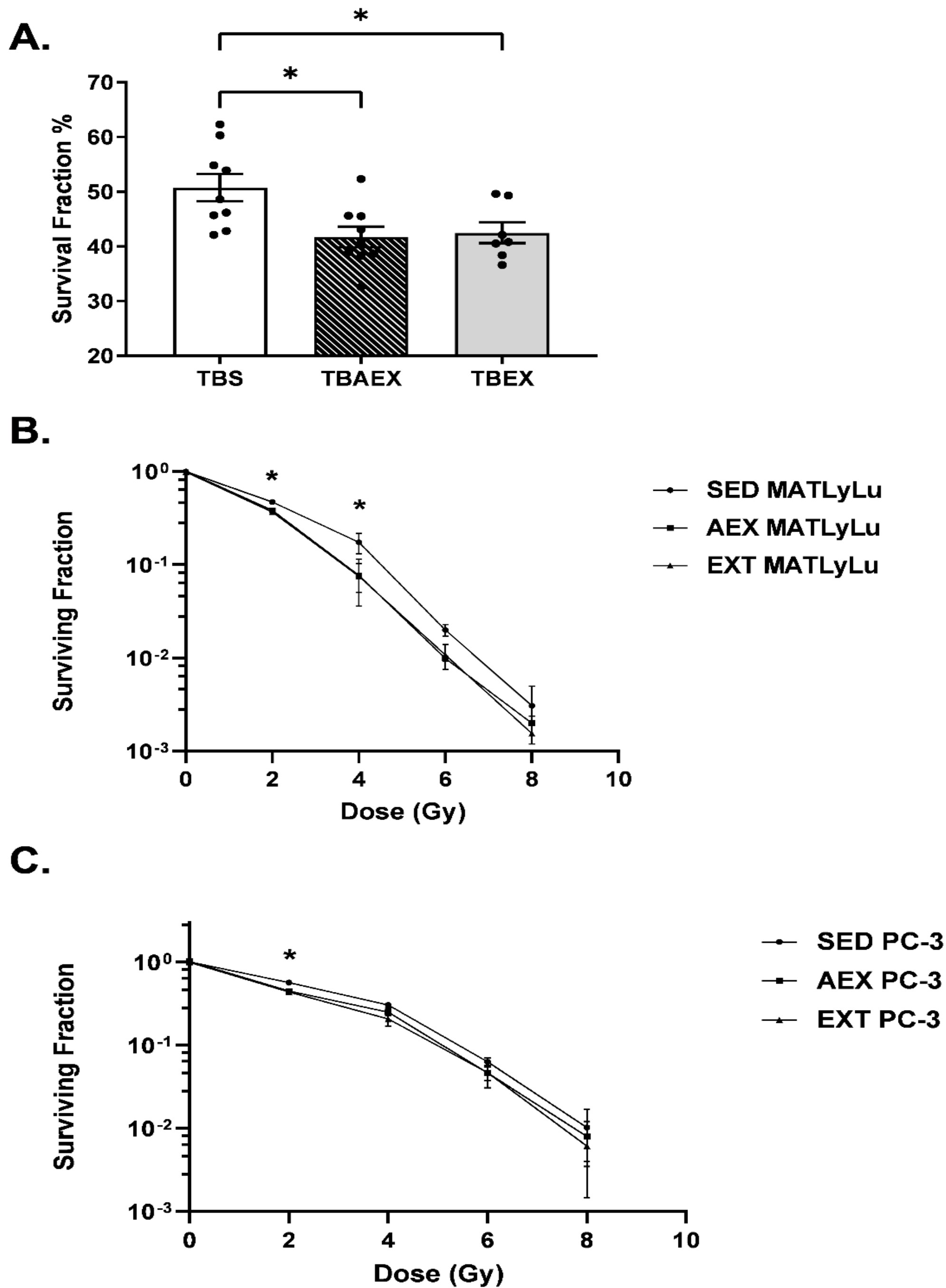
The heart to body mass ratio (A.) was assessed with a One-way ANOVA and Holm-Sidak post hoc tests) between groups. *, $p<0.05$ vs. TBS; ** $p<0.05$ vs TBAEX. There was a negative correlation (B.) between heart to body mass ratio and tumor mass.

Figure 3.4. Aerobic Capacity and Correlations



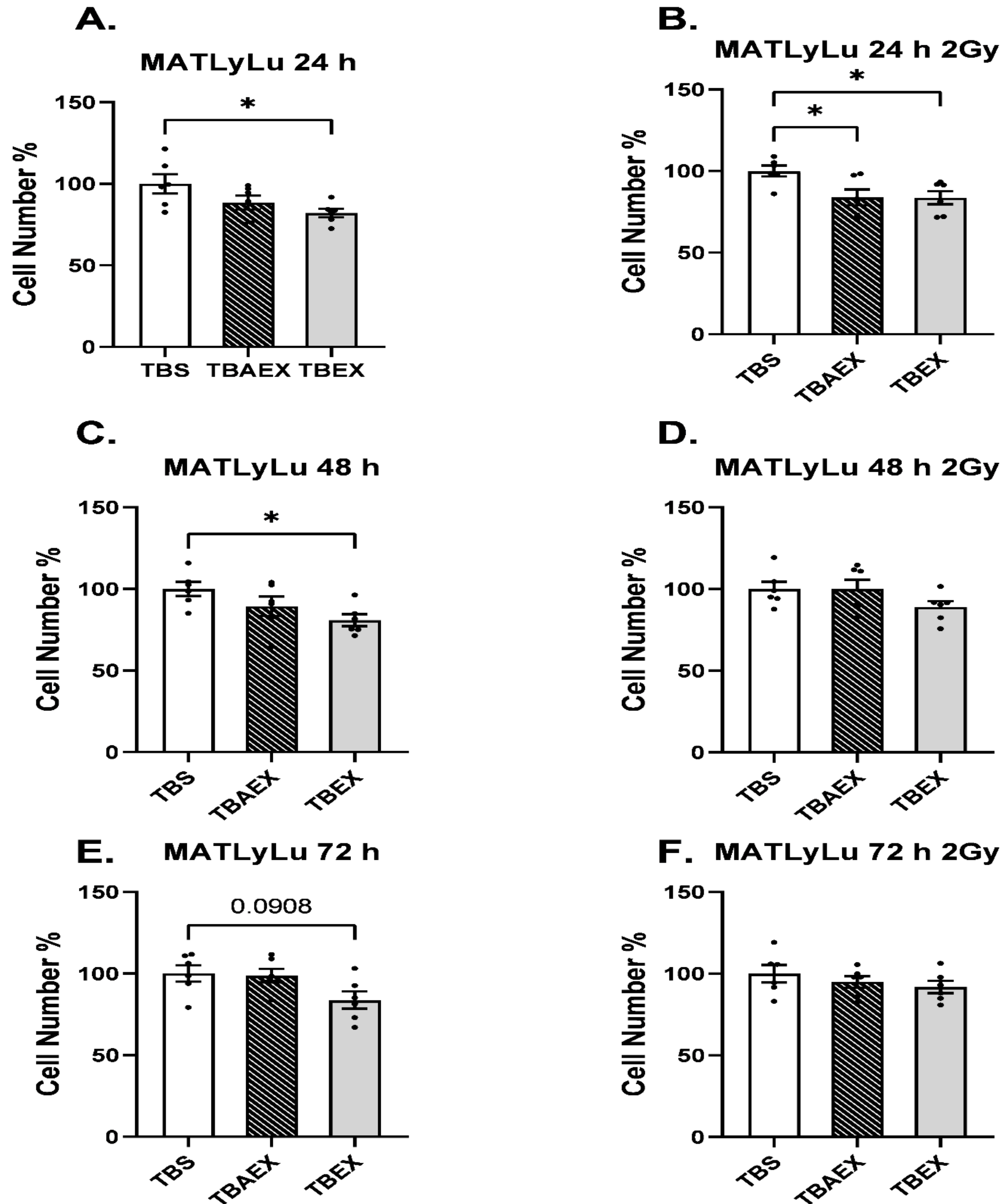
The Pre- and Post-measures of aerobic capacity (A.) were assessed with a One-way ANOVA repeated measures and Holm-Sidak post hoc tests) between groups. *, p<0.05 vs. TBS&TBAEX; # p<0.05 Pre-measure vs Post-measures. There was a positive correlation (B.) between heart to body mass ratio and $\dot{V}_{O2peak}$.

Figure 3.5 Clonogenic Survival Results



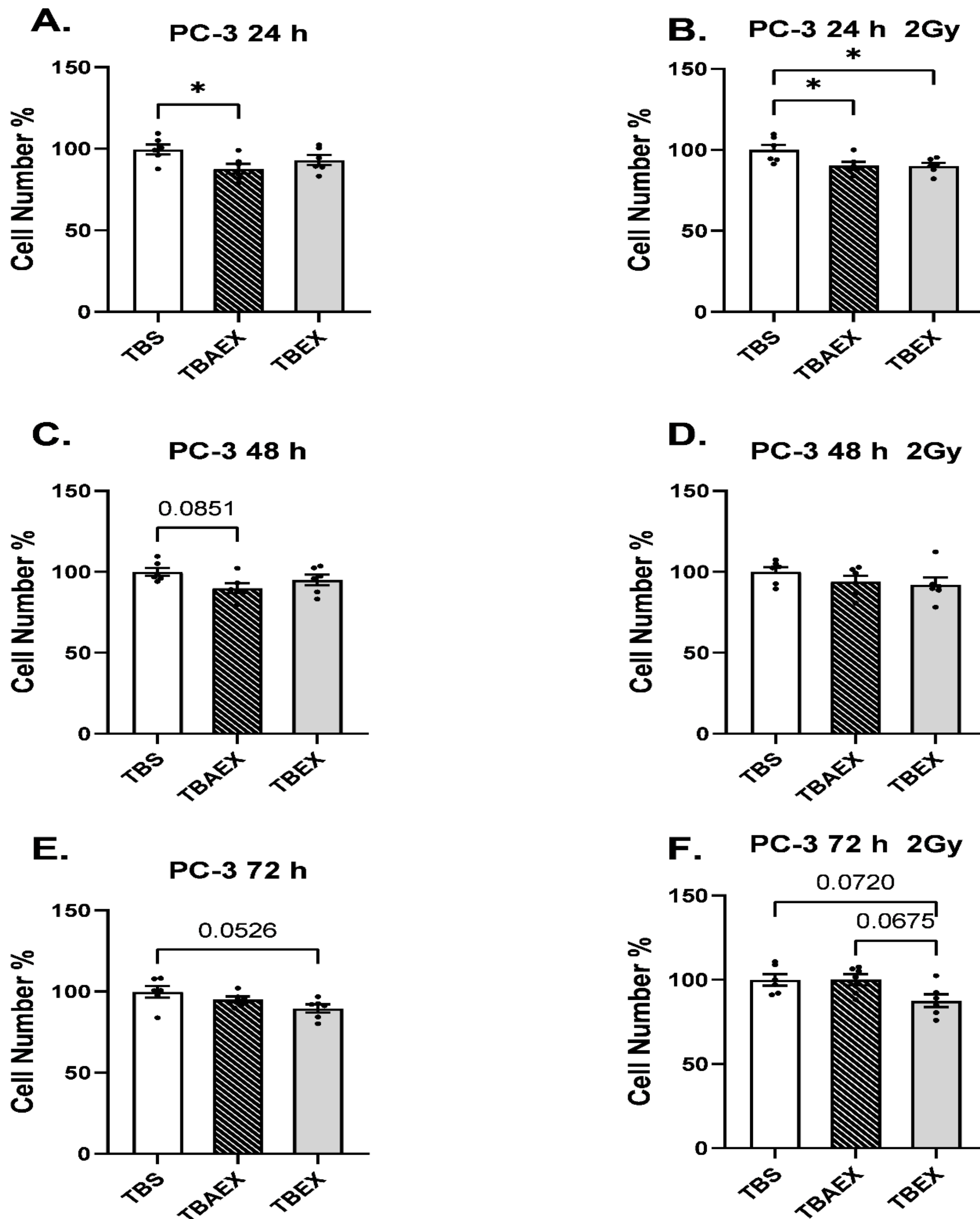
Clonogenic survival *in vivo* to a single 2Gy dose of radiation (A.) was assessed with a One-way ANOVA and Holm-Sidak post hoc tests) between groups. *In vitro* surviving fractions of MATLyLu and PC-3 prostate cancer cells cultured in serums supplemented media from each tumor-bearing group (TBS, TBAEX, TBEX). *, $p<0.05$ vs. TBS

Figure 3.6 Viable Cell Number MATLyLu



Effect of 24, 48, and 72 h incubation with pooled serum supplemented media from each group of tumor-bearing animals (TBS, TBAEX, TBEX) using MATLyLu cancer cells. Results are from groups of normal (A, C, E) and 2Gy irradiated (B, D, F) cell studies. Values are expressed as mean cell number (%) normalized to TBS. *, $p<0.05$ vs. TBS.

Figure 3.7. Viable Cell Number PC-3



Effect of 24, 48, and 72 h incubation with pooled serum supplemented media from each group of tumor-bearing animals (TBS, TBAEX, TBEX) using PC-3 cancer cells. Results are from groups of normal (A, C, E) and 2Gy irradiated (B, D, F) cell studies. Values are expressed as mean cell number (%) normalized to TBS. *, $p<0.05$ vs. TBS.

Chapter 4 - Effects of high-intensity training on prostate cancer-induced cardiac atrophy

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 ($1x10^5$) 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.

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 regimes, 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) atrophy [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 anti-cancer 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, Waltham, MA), 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 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 ~$1x10^5$ 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 26G 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 are demonstrated in Figure 4.1. 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.

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, Waltham, MA) 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 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, Ann Arbor, MI), and 30 µl of DTNB (Thermo Fisher Scientific, Waltham, MA) were added to each sample in duplicate in a 96-well plate. Samples were analyzed in a spectrophotometer (accuSkan GO; Thermo Fisher Scientific, Waltham, MA) 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 4.2**). Tumor mass and tumor burden (tumor mass/body mass) were not different between TBEX and TBS groups (**Table 4.1**). 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 4.2**). Longitudinal increases Pre-Post in volume (LVEDV, SV) for the TBEX group were significant ($p < 0.05$, **Figure 4.3**), 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 4.1**) and relative (normalized to body mass) heart and LV masses (**Figure 4.4**). There was no significant difference in RV mass normalized to body mass (**Figure 4.4**). 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 4.1**).

Left Ventricle Protein Expression

There was no difference in CTSB or CTSL protein expression of the left ventricle from the TBEX versus TBS (**Figure 4.5; A and B**, $p = 0.71$ and $p = 0.62$ respectively). Further, there were no differences in MAFbx, or Nrf-2 protein expression between groups (**Figure 4.5; C and D**, $p = 0.93$ 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 4.6**), 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 4.6**); 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 form 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 induction 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 Murph-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 difference between groups for MAFbx was unexpected however, there are other UPS targets, such as 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-$_k$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-$_k$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-κB 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 4.3**), which was likely a function of the larger LV mass (**Figure 4.4**). Subsequently, the measures of LV function herein reflect a non-stressed condition, from which subclinical alterations in function 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 4.1**). The lack of

differences in markers of atrophy between groups may be due to the low tumor burden in these animals (**Table 4.1**). 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 4.6**) 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.

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References

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

2. Morrow GR, Andrews PLR, Hickok JT, Roscoe JA, Matteson S. Fatigue associated with cancer and its treatment. Support Care Cancer 2002; 389–398.

3. Galvao DA, Taaffe DR, Spry N, Joseph D, 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, 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:

5. Ewer MS, Ewer SM. Cardiotoxicity of anticancer treatments. Nat Rev Cardiol 2015; 7:564.

6. O'Farrell S, Garmo H, Holmberg L, Adolfsson J, Stattin P, 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, 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, Morrow GR. Cancer-Related Fatigue: The Scale of the Problem. Oncologist 2007; 12:4.

9. McCullough DJ, Nguyen LM-D, Siemann DW, 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, 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, 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 CRR, Da Cunha TF, Da Paixão NA, 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, 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, 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; 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, 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, 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, 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, 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, Schadler KL. Vascular modulation through exercise improves chemotherapy efficacy in Ewing sarcoma. Pediatr Blood Cancer 2019; e27835.
21. Antunes JMM, Ferreira RMP, 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 I-M, Nieman DC, Swain DP. 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, 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, 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, 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 WDW, Weissman RM, Coffey DS. Animal Models of the Hormone-sensitive 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, 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, 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 X-L, Bolli R, 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, Davidson WR. Echocardiographic evaluation of size, function, and mass of normal and hypertrophied rat ventricles. J Appl Physiol (Bethesda, Md 1985) 1993; 74:2598–2605.

32 Craig JC, Colburn TD, Caldwell JT, Hirai DM, Tabuchi A, Baumfalk DR, Behnke BJ, Ade CJ, Musch TI, 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 M V., 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, 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, Moore RL. Measurements of metabolic rate in rats: a comparison of techniques. J Appl Physiol (Bethesda, Md 1985) 1988; 65:964–970.
36. PA., Srere. Citrate synthase: [EC 4.1. 3.7. Citrate oxaloacetate-lyase (CoA-acetylating)]. Methods Enzymol 1969; 3–11.
37. Moreira JBN, Bechara LRG, Bozi LHM, Jannig PR, Monteiro AWA, Dourado PM, Wisløff U, 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, 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, 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, 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, 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, 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, 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, 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, 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, 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, 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, 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, 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, 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, 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 - Gen Subj 2013; 1830:4137–4146.
52. Smuder AJ, Hudson MB, Nelson WB, Kavazis AN, 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, 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; 2:6.
55. Fonović M, Turk B. Cysteine cathepsins and their potential in clinical therapy and biomarker discovery. Proteomics - Clin Appl 2014; 8:416–426.
56. Löser R, 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, 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, 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, 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, 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, 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, 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, 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, Anker SD. Prevention

of liver cancer cachexia-induced cardiac wasting and heart failure. Eur Heart J 2014; 35:932–941.

Table 4.1. Cardiac and 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*

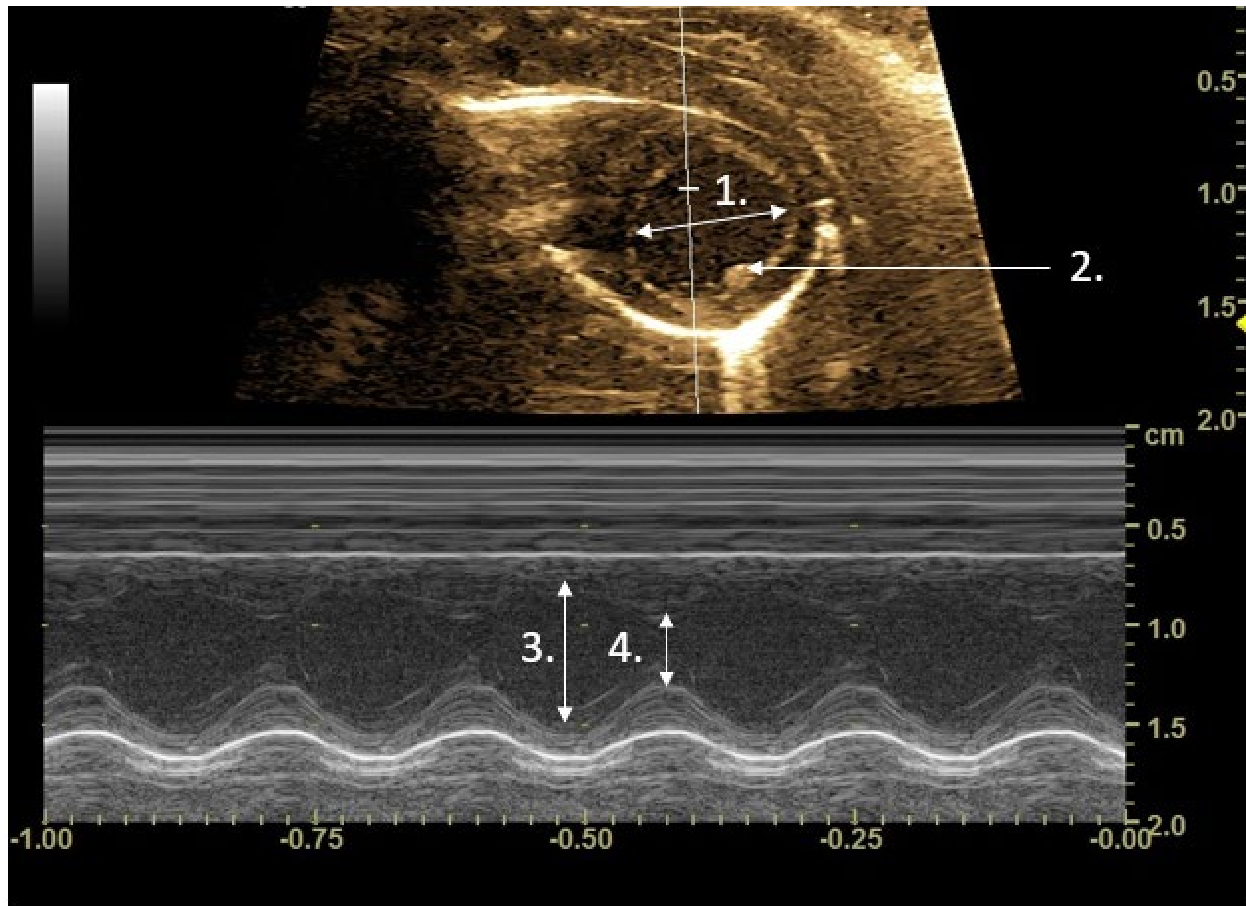
Table 4.2. Echocardiographic measures

Left Ventricle Measures	Sedentary Tumor-Bearing (n=9)	Exercise Tumor-Bearing (n=15)
<u>Pre-Measures</u>		
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
<u>Post-Measures</u>		
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; PWTD, posterior wall thickness end-diastole; PWTS, 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.

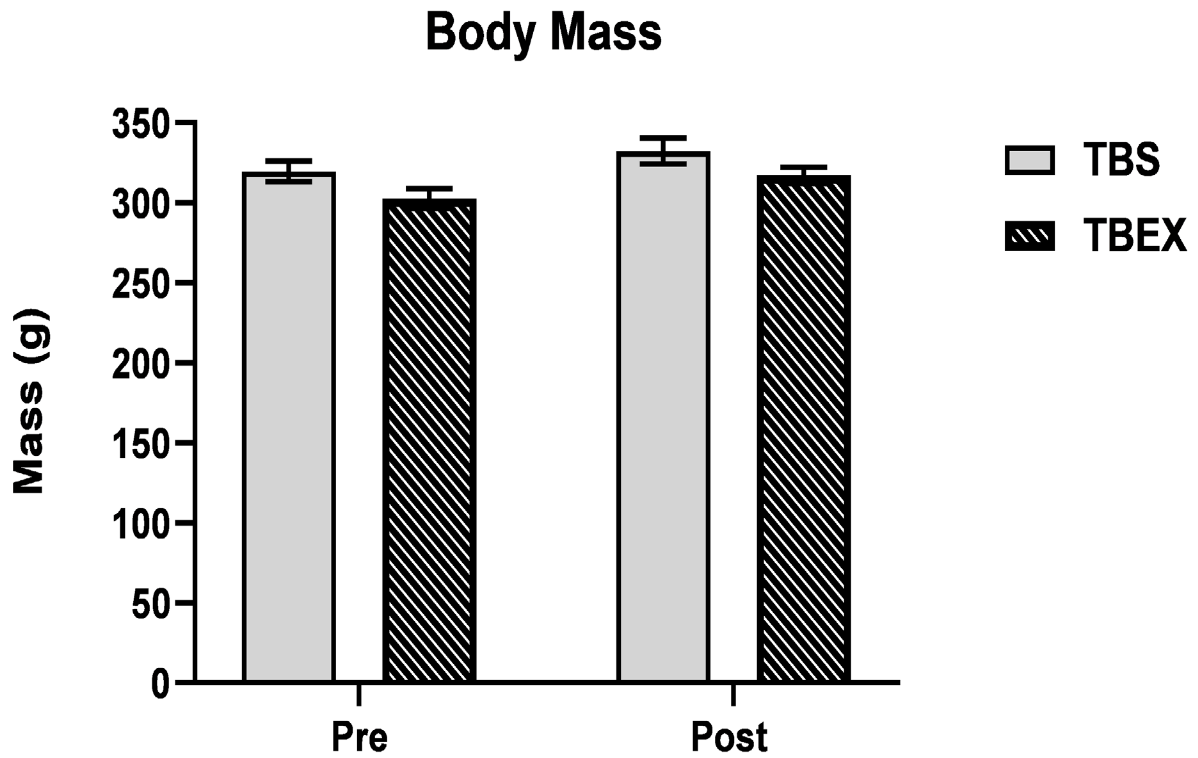
# $p< 0.05$ Pre vs. Post

Figure 4.1. Representative Echocardiography Image



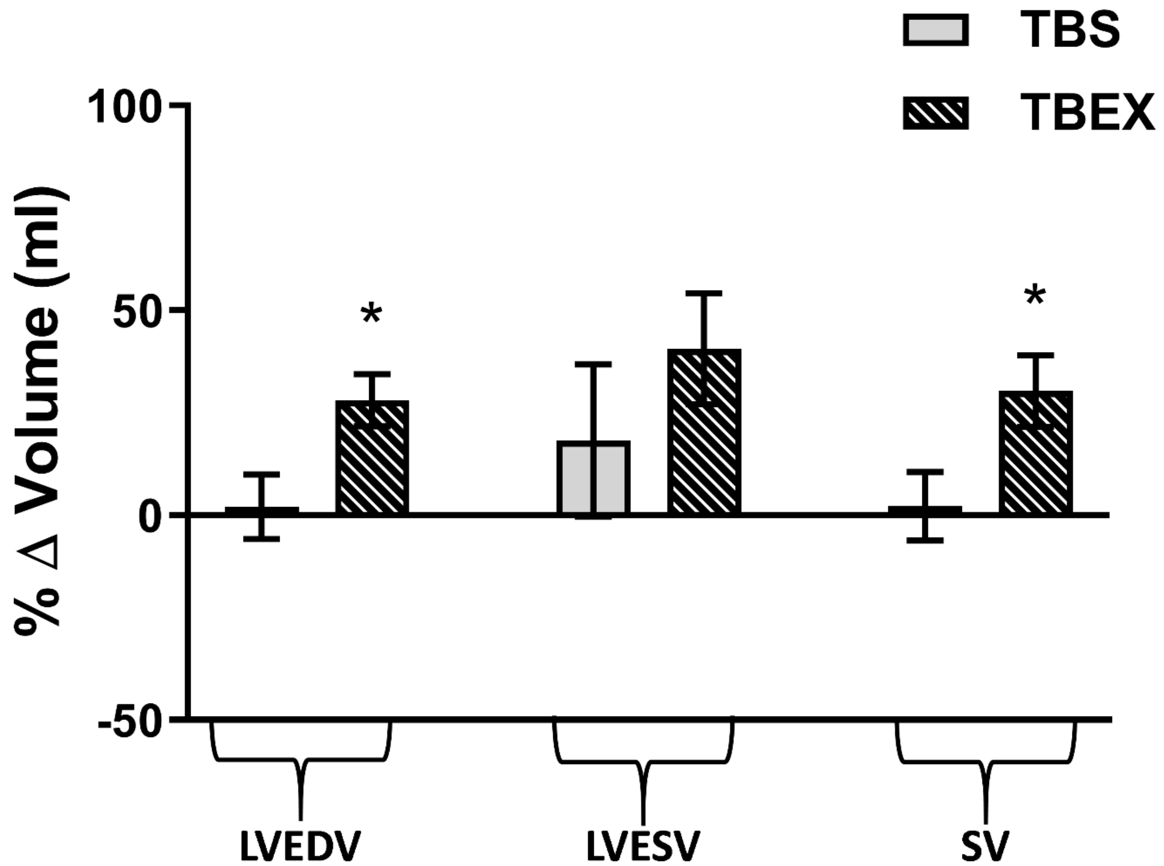
A representative 2D image (Top) and M-mode tracing (bottom) of the rat left ventricle at the level of the papillary muscle in diastole. Arrows represent: 1. the left ventricle chamber, 2. papillary muscle, 3. left ventricular diastolic dimension, and 4. left ventricular systolic dimension.

Figure 4.2. Change in Body Mass



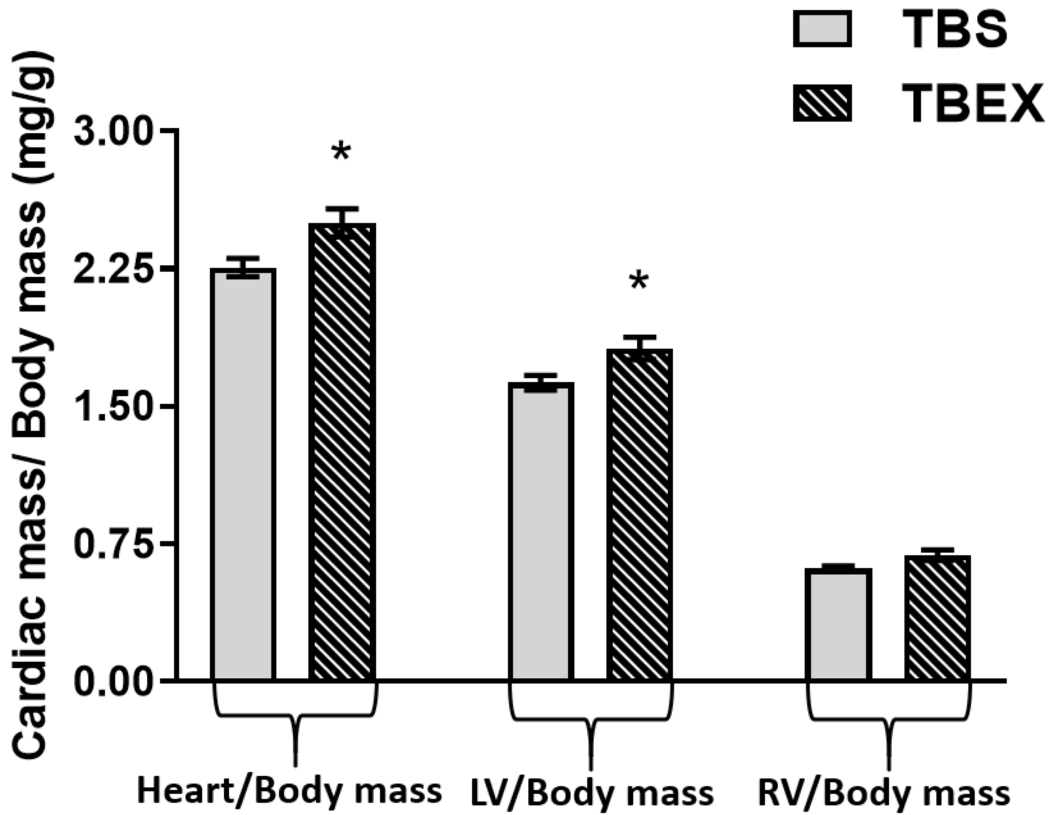
Body mass for both sedentary (n=9) and exercise (n=15) groups were not significantly different at Pre or Post measures of echocardiography.

Figure 4.3. Percent Change in Volume



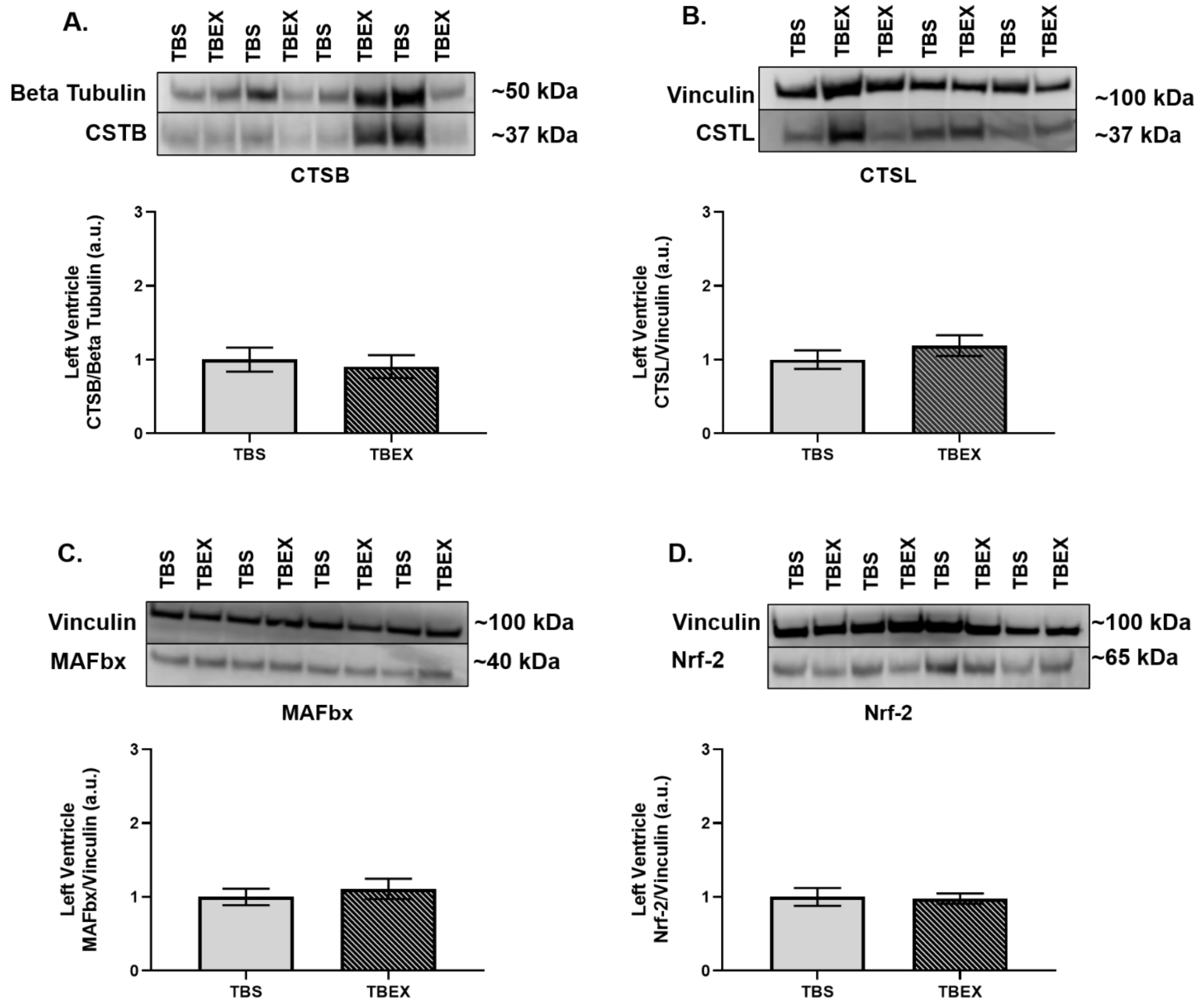
Pre-Post % change of cardiac measures of left ventricle diastolic volume (LVEDV), left ventricle systolic volume (LVESV), and stroke volume (SV) were compared between tumor groups (TBS; n=9 vs. TBEX; n=15). *$p < 0.05$ vs. TBS.

Figure 4.4. 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.

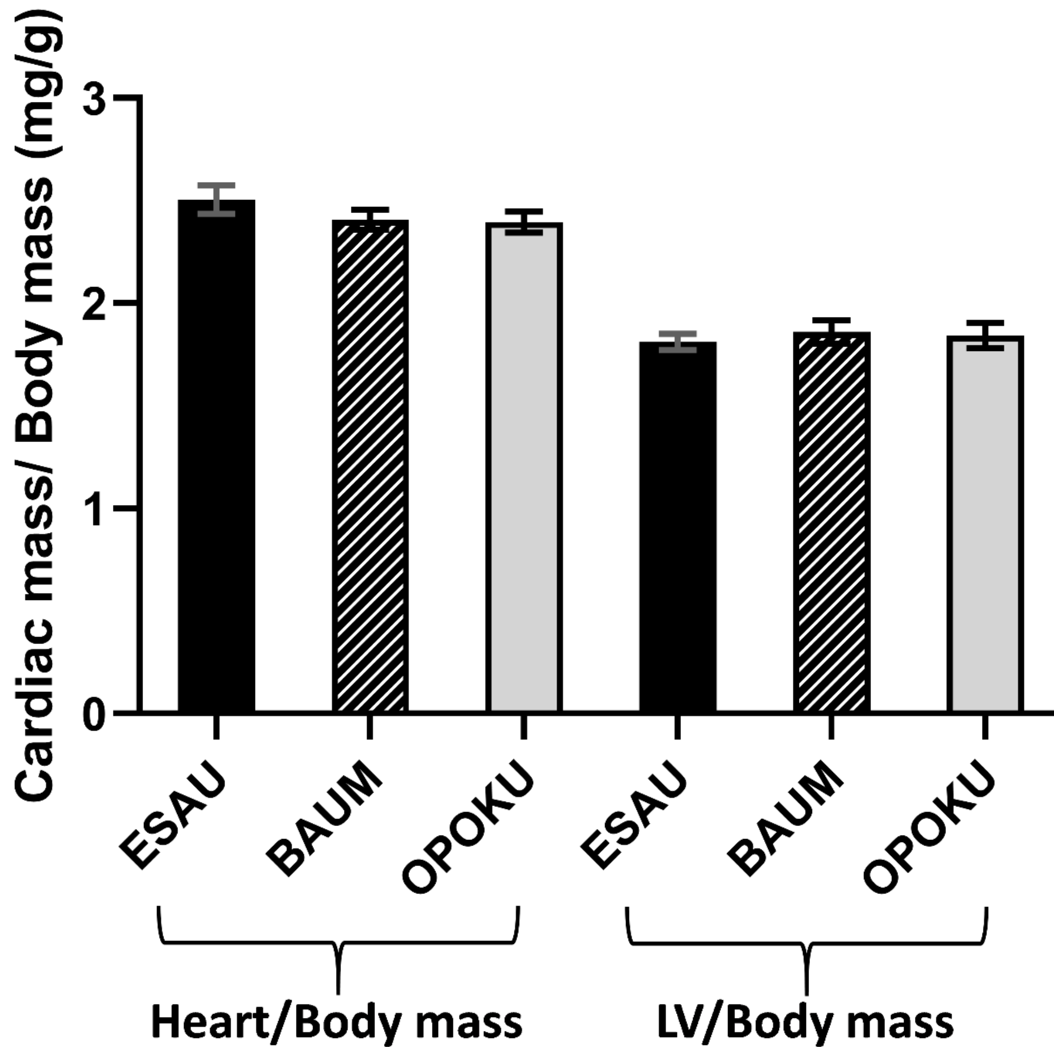
Figure 4.5. Left Ventricle Protein Expression



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 MAFbx and Nrf-2 protein expression were similar between groups as well (TBS; n=9 vs. TBEX; n=15).

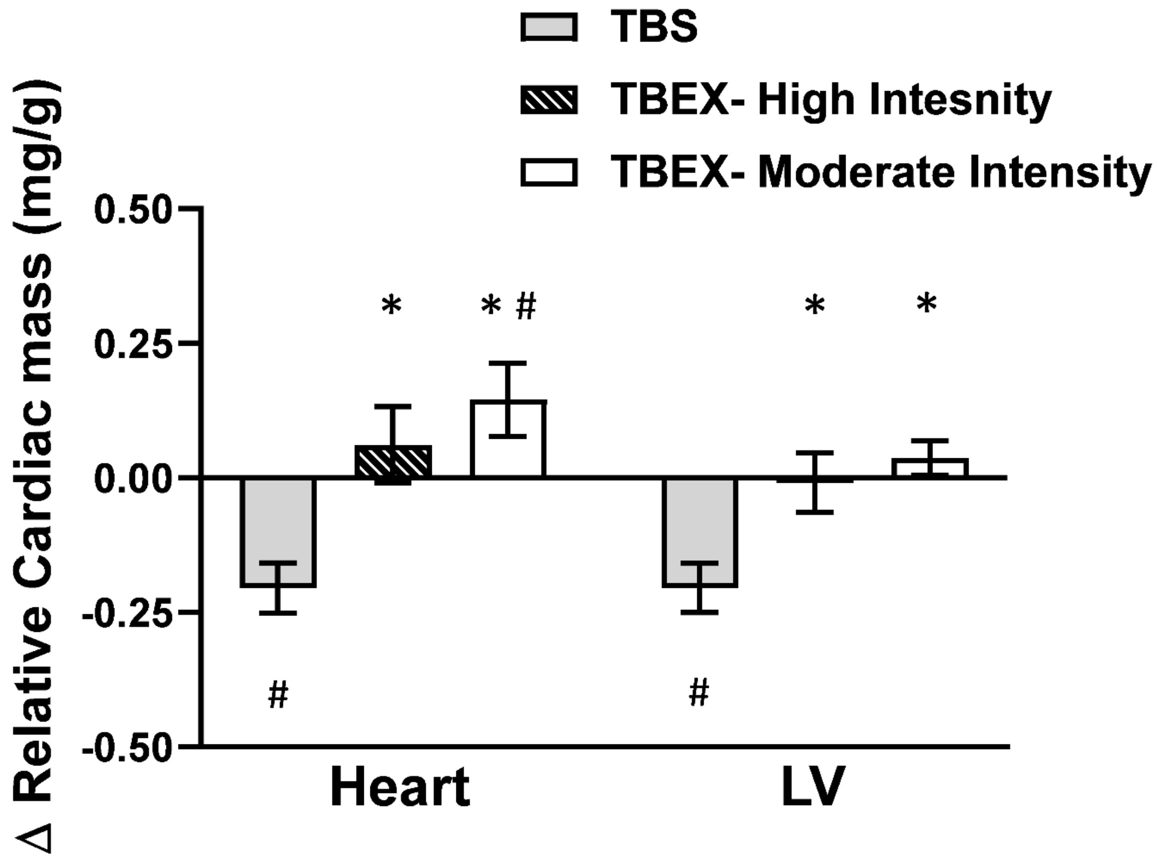
Figure 4.6. Comparison to Non-Tumor Bearing Groups

A.



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

B.



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

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Chapter 5 - Conclusions

This collection of studies presented here as part of my doctoral work was to determine the efficacy of aerobic exercise (with specifics in terms of intensity, and acute vs chronic duration) as an adjuvant therapy in a pre-clinical model of prostate cancer. Specifically, to understand its value in terms of improving radiotherapy outcomes, and investigate its use for prostate induced cardiac atrophy mitigation. In Chapter 2, we found that exercise was able to mitigate levels of hypoxia assessed via IHC in solid tumors at both a high, and moderate intensity of exercise of similar duration, that was corroborated by a decrease in the cell survival fractions of both exercise treatment groups to a similar degree after a single 2Gy dose of radiation. In chapter 3 we expanded upon the findings in chapter two and evaluated if a single moderate-intensity acute bout of exercise would have a similar effect as chronic moderate-intensity exercise training on radio-sensitizing prostate tumor cells. With reductions in cell survival in both acute and chronic exercise groups, these data suggest exercise can potentially be used to radiosensitize solid prostate tumors and improve the efficacy of cancer therapy. Further, prostate cancer induced cardiac alterations can be mitigated with moderate aerobic exercise, confirming findings of our prior work in a different strain of animal with a similar cell line. Chapter 4 we focused primarily on the ability of high-intensity exercise to induce a cardiac atrophy sparing phenotype to possibly preserve alterations in left ventricle function in an expedited manner to moderate-intensity exercise. Although limited functional alterations were seen in these animals, high-intensity exercise did spare cardiac atrophy seen in sedentary tumor bearing animals in a shorter period of time than moderate-intensity

exercise. Together, the pre-clinical evidence presented herein introduce novel findings of exercises ability to improve prostate cancer associated maladies that prostate cancer patients present clinically, while also improving anti-tumor therapeutic outcomes in this model. These findings could potentially lead to future clinical studies to evaluate efficacy of utilizing aerobic exercise in prostate cancer patients to improve cancer patients' cardiovascular health, quality of life, and possibly improvement in the treatment of solid prostate tumors requiring radiation therapy.

Appendix A - Curriculum Vitae

Dryden Baumfalk
Kansas State University

Department of Kinesiology

Email: dryden32@ksu.edu

Education

PhD- Kansas State University 2018-2021

Major: Physiology

Dissertation Title: Prostate Cancer, The Heart, and Exercise: Cardiovascular Complications and Therapeutic Implications

MS- Kansas State University, 2017-2018
Major: Physiology
Thesis Title: Effects of a Pre-Clinical Model of Prostate Cancer on Left Ventricular Function in Sedentary and Exercise Trained Rats

BS- Kansas State University, 2013-2016
Major: Kinesiology
Supporting Areas of Emphasis: Exercise Physiology

BS- Kansas State University, 2013-2016
Major: Human Nutrition

AS- Colby Community College 2011-2013
Major: Business

Professional Experience

Kansas State University:

Exercise Physiology Lab Coordinator, Dept. Kinesiology 2020-2021

Graduate Teaching/Research Assistant, Dept. Kinesiology 2017-Present

Basketball Instructor, Dept. Kinesiology 2016-2017

Undergraduate Teaching Assistant, Dept. Human Nutrition 2015-2016

Undergraduate Research Assistant, Dept. Human Nutrition 2014-2017

Courses Taught:

- Kinesiology 609- Environmental Physiology 2021-Present
- Kinesiology 220- Bio-Behavioral Basis of Exercise Laboratory 2017-2021
- Kinesiology 336- Exercise Physiology Laboratory 2017-2021
- Kinesiology 360- Anatomy Physiology Laboratory 2018-2020
- Kinesiology 625- Exercise Testing and Prescription Laboratory 2018-Present
- Kinesiology 101- Intro to Basketball 2016-2017
- Human Nutrition 510- Life Span Nutrition 2015-2016
    - Teaching Aid
- Human Nutrition 110- Introduction to Public Health 2015-2016
    - Teaching Aid

Invited Lectures:

- Kinesiology 603- Cardiovascular Physiology: Peripheral Vasculature 2019
- Kinesiology 360- Anatomy & Physiology: Reproductive System 2019

Mentorship and Training

Undergraduate Research Assistants

Torian Simpson (Summer 2021-December 2021)

Taylor Rand (Fall 2017-Spring 2019)- M.S. KSU, University of Kansas Medical School

Joseph Pyle (Fall 2017-Spring 2019)- M.S., KSU.

Charles Schneider (Fall 2018-Summer 2019)

Savannah Ardery (Summer 2017-Spring 2018)- Wichita State Physician's Assistant Program

Jennifer Thompson (Spring 2017-Fall 2017)- Dietitian at HCA Mid-West Health

Eric Heffern (Spring 2017-Spring 2018)- University of Kansas Medical School

University, College, Departmental Service

Kansas State University:

University Town Hall Panelist: Academic Integrity and Covid-19 2020

Graduate Student Council Honor Council Chair 2017-2021

College of Human Ecology Graduate Student Advisory Council 2017-2020

College of Human Ecology Ambassador Selections Chair	2014-2016
Kappa Omicron Nu Selections Induction Chair	2014-2015

Awards and Honors

Kansas State Distinguished Professor Graduate Student Award	2021
College of Human Ecology Dissertation Award	2021
APS Translation Physiology Showcase Best Abstract Award	2021
College of Health and Human Sciences Outstanding Graduate Student	2021
Graduate Teaching Assistant of the Year Award Kinesiology	2021
Outstanding Doctoral Student Award Kinesiology	2021
APS Cardiovascular Research Recognition Award	2021
Kansas State Publication Reimbursement Award	2020
College of Health and Human Sciences Outstanding Research Award	2020
Environmental and Exercise Physiology Gatorade Pre-doctoral award	2020
17th Capitol Graduate Research Summit Presentation Winner	2020
Selected as a Kansas State University's Capital Research Representative	2020
Johnson Cancer Research Center Heart Grant Award	2019
Journal of Applied Physiology APS Select Article	2019
Outstanding Master Student Kinesiology	2018
Johnson Cancer Research Center Travel Award (2019, 2020)	2018
CHE Travel Grant (2019, 2020)	2018
Marth L. Dunlap Memorial Scholarship	2017
Outstanding Senior award for Leadership, Kansas State University	2016
Inaugural Bill Snyder Leadership Legacy Fellow	2015

Funded Grant Work

1. Johnson Cancer Research Center Heart Grant Award ($5,000) 2019
"Prevention of 5-flurouracil Cardiotoxicity with Exercise Therapy"

The major goal of this project is to prevent cardiotoxic effects of 5-flurouracil cardiotoxicity with acute bouts of exercise that are translatable to cancer patient care programs.

2. Johnson Cancer Research Center Summer Research Stipend ($12,000) 2018-2021
"Prostate cancer, the heart, and exercise: Cardiovascular dysfunction and therapeutic outcomes" ($5,000, 2021)

"Prostate Cancer and Exercise: Therapeutic Implications" ($5,000, 2020)

"The effect of moderate intensity exercise in prostate tumor-bearing rats" ($2,000, 2018)

Professional Memberships

American College of Sports Medicine (ACSM) 2019-Present

American Physiological Society (APS) 2017-Present

Ad-hoc Journal Reviewer

Frontiers in Physiology 2021-Present

Medicine and Science in Sports and Exercise 2019-Present

Journal of Applied Physiology 2018-Present

Invited Oral Presentations

1. Kansas State College of Health and Human Sciences, Department of Kinesiology seminar series. "Effect of High Intensity Exercise on Prostate Cancer induced Cardiac Atrophy.", 2020.

2. NIH NIEHS, Department of Pathology, "Moderate- and High-intensity exercises impact on the tumor microenvironment: Molecular evaluations." January 2021.

3. APS Translation Physiology Interest Group Symposium at Experimental Biology: "Effect of Moderate and High Intensity Exercise on Hypoxia and Radiosensitivity in Tumor--Bearing Rats." April 2021.

4. University Distinguished Professor showcase, Kansas State University. "Prostate Cancer, Exercise and the Hear: Therapeutic Implications, and Cardiovascular Complications." September 2021

Published Intellectual Contributions

Refereed Journal Articles:

* *Manuscripts in preparation or review*

1. ***Baumfalk, D. R.,** Opoku-Acheampong, A. B., Horn, A. G., Kunkel, O. N., Siemann, D. W ., & Behnke, B. J., (2021). "Acute and Chronic effects of Exercise on Prostate Tumor Radiosensitivity." In-Preparation 2021
2. ***Baumfalk, D. R.,** Opoku-Acheampong, A. B., Ganta, C. K., Siemann, D. W ., & Behnke, B. J., (2021). "Effects of Moderate- and High-Intensity Training on Prostate Tumor Microenvironment and Radiosensitivity." In-Preparation 2021
3. *Kunkel, O. N., Rand, T. A., Pyle, J. G., **Baumfalk D. R.,** Horn, A. G., Opoku-Acheampong, A. B., Ade, A. J., Musch, T. I,, Ramsey, M. W., Delp, M, D., Behnke, B. J., " Increased vascular hydrostatic pressure gradient with head-up tilt does not enhance prostate tumor perfusion or oxygenation in young rats" In Review, PlosOne, 2021
4. *Hammond, S.S., **Baumfalk, D. R.**, Parr, S. K., Behnke B. J., Ade, C. J.,"Impaired Endothelium-Dependent Microvascular Reactivity in Cancer Patients Treated with 5-Fluorouracil Chemotherapy: The Case for eNOS Dysfunction" In review, *American Journal of Physiology-Heart and Circulatory Physiology*, 2021
5. Horn, A. G., Kunkel, O. N., **Baumfalk, D. R.,** Simon, M. E., Schulze, K. M., Muller-Delp, J., Poole, D. C., Behnke, B. J., "The effects of prolonged mechanical ventilation on the material and structural properties of diaphragm arterioles." (2021) Microcirculation, e12727
6. **Baumfalk, D. R.,** Opoku-Acheampong, A. B., Caldwell, J, T., Butenas, A. L. E., Ade, C. J., Copp S. W., Musch, T. I., & Behnke, B. J., (2020). "High-Intensity Training and Prostate Cancer-Induced Cardiac Atrophy in a Pre-Clinical Model." (2020) American Journal of Translational Research, 13(1) 197.
7. Horn, A. G., **Baumfalk, D. R.,** Kunkel, Olivia N., Bruells, Christian S., Musch, T. I., Poole, David C., Behnke, Bradley J., (2020) Effects of Low-PEEP and High-PEEP on Diaphragm blood flow during Mechanical Ventilation. Journal of Applied Physiology, 129(3), 626-635.
8. Butenas, A. L., Colburn, T. D., **Baumfalk, D. R**., Ade, C. J., Hageman, K. S., Copp, S. W., ... & Musch, T. I. (2021). Angiotensin converting enzyme inhibition improves cerebrovascular control during exercise in male rats with heart failure. Respiratory Physiology & Neurobiology, 286, 103613.
9. Horn, Andrew G., Davis III, Robert T., **Baumfalk, Dryden R**., Kunkel, Olivia N., Bruells, Christian S., McCullough, Danielle J., Opoku-Acheampong, Alex B., Poole, David C., Behnke, Bradley J. (2019). Impaired diaphragm resistance vessel vasodilation with prolonged mechanical ventilation. *Journal of Applied Physiology*, 127(2), 423-431.
10. Craig, J. C., Colburn, T. D., Caldwell, J. T., Hirai, D. M., Tabuchi, A., **Baumfalk, D. R**., ... & Poole, D. C. (2019). Central and peripheral factors mechanistically linked to exercise intolerance in heart failure with reduced ejection fraction. *American Journal of Physiology-Heart and Circulatory Physiology*, *317*(2), H434-H444.
11. Caldwell, J. T., Sutterfield, S. L., Post, H. K., Craig, J. C., **Baumfalk, D. R**., Copp, S. W., & Ade, C. J. (2019). Impact of Acute Dietary Nitrate Supplementation during

Exercise in Hypertensive Women. *Medicine and science in sports and exercise*, *51*(5), 1014-1021.

12. Opoku-Acheampong, A. B., **Baumfalk, D. R**., Horn, A. G., Kunkel, O. N., Ganta C. K., McCullough, D. J., Siemann, D. W., Muller-Delp, J., & Behnke, B. J (2019) "Prostate cancer cell growth characteristics in serum and prostate-conditioned media from moderate-intensity exercise-trained healthy and tumor-bearing rats" American Journal of Cancer Research.
13. **Baumfalk, D.R**., Opoku-Acheampong, A. B., Caldwell, J, T., Ade, C. J., Copp S. W., Musch, T. I., & Behnke, B. J., (2019). "Effects of prostate cancer and exercise training on left ventricular function and cardiac and skeletal muscle mass." Journal of Applied Physiology. 126 (3), 668-680 **APS Select**
14. Wiggins, J. M., Opoku-Acheampong, A. B., **Baumfalk, D. R**., Siemann, D. W., & Behnke, B. J. (2018). Exercise and the Tumor Microenvironment: Potential Therapeutic Implications. Exercise and sport sciences reviews, 46(1), 56-64.
15. Smith, J. R., Sutterfield, S. L., **Baumfalk, D. R**., Didier, K. D., Hammer, S. M., Caldwell, J. T., & Ade, C. J. (2017). Left ventricular strain rate is reduced during voluntary apnea in healthy humans. Journal of Applied Physiology, 123(6), 1730-1737.
16. Esau, P. J., Gittemeier, E. M., Opoku-Acheampong, A. B., Rollins, K. S**., Baumfalk, D. R**., Poole, D. C., ... & Copp, S. W. (2017). Prostate cancer reduces endurance exercise capacity in association with reductions in cardiac and skeletal muscle mass in the rat. American journal of cancer research, 7(12), 2566.

Scientific Abstracts

** Indicates Poster Presentation*

1. ***Baumfalk, D. R**., Opoku-Acheampong, A., Horn, A. G., Kunkel, O. N., Musch, T., Siemann, D. W., & Behnke, B. J. (2021) Effect of Moderate and High Intensity Exercise on Hypoxia and Radiosensitivity in Tumor-Bearing Rats. *The FASEB Journal.* Accepted.
2. Horn, A. G., **Baumfalk, D. R**., Schulze, K. M., Colburn, T. D., Weber, R. E., Kunkel, O. N., ... & Behnke, B. J. (2020). Effects of Supplemental Oxygen During Mechanical Ventilation on Diaphragmatic Blood Flow. *The FASEB Journal.* Accepted.
3. Kunkel, O. N., **Baumfalk, D. R**., Horn, A. G., Siemann, D. W., & Behnke, B. J. (2021) Effects of Heat Therapy on Prostate Cancer Cells Subjected to 4Gy Radiation. *The FASEB Journal.* Accepted.
4. Parr, S. K., Hammond, S. T., **Baumfalk D. R**., Kunkel, O. N., Behnke B. J., & Ade, C. J.(2021) 5-Fluorouracil Induces Vascular Calcification in Rat Aortic Smooth Muscle Cells. *The FASEB Journal.* Accepted.
5. ***Baumfalk, D. R.**, Opoku-Acheampong, A., Colburn, T. D., Horn, A. G., Kunkel O. N., Musch, T. I., Siemann, D. W., Behnke, B. J. (2020) Effect of Acute and chronic Exercise on Radiosensitivity in Tumor-Bearing Rats. Integrative Physiology of Exercise Conference.
6. Parr, S. K., Hammond, S. T., **Baumfalk D. R**., Kunkel, O. N., Steele, C. C., Behnke B. J., & Ade, C. J. (2020) Protein Kinase C Activity is Increased in Human Coronary Smooth Muscle Cells following Exposure to 5-FU Chemotherapy. The FASEB Journal

7. ***Baumfalk, D. R.,** Colburn, T. D., Horn, A. G., Kunkel, O. N., Weber, R. E., Musch, T. I., & Behnke, B. J. (2020). Effect of Prostate Cancer and Endurance Exercise Training on Aerobic Capacity. *The FASEB Journal.* 34(S1), 1-1.
8. Kunkel, O. N., **Baumfalk, D. R**., Horn, A. G., & Behnke, B. J. (2020). Effects of Acute and Repeated Heat Therapy on Prostate Cancer Cell Characteristics. *The FASEB Journal*, *34*(S1), 1-1.
9. ***Baumfalk, D. R.,** Colburn, T. D., Horn, A. G., Kunkel, O. N., Musch, T. I., & Behnke, B. J. (2020). Immunocompromised Rats Have a Decreased Aerobic Capacity. *The FASEB Journal.* 34(S1), 1-1.
10. Horn, A. G., **Baumfalk, D. R**., Schulze, K. M., Colburn, T. D., Weber, R. E., Kunkel, O. N., ... & Behnke, B. J. (2020). Effects of Intrathoracic Pressure changes on Diaphragmatic Blood Flow during Mechanical Ventilation. The FASEB Journal, 34(S1), 1-1.
11. Horn, A. G**., Baumfalk, D. R.,** Kunkel, O. N., Poole, D. C., & Behnke, B. J. (2020) The Effects of Prolonged Mechanical Ventilation on Structural and Material Properties of Diaphragm Arterioles. *The FASEB Journal.* 34(S1), 1-1.
12. Hirai, D. M., Tabuchi, A., Craig, J. C., Colburn, T. D., Caldwell, J. T., Ade, C. A., **Baumfalk, D. R.**, Opoku-Acheampong, A. B., Behnke, B. J., Musch, T. I., & Poole, D. C. (2020). Skeletal muscle capillary hemodynamics in rats with heart failure with preserved ejection fraction. *The FASEB Journal.* 34(S1), 1-1.
13. Kunkel, O. N., **Baumfalk, D. R.**, Horn, A. G., & Behnke, B. J. (2020). Effects of Acute and Repeated Heat Therapy on Prostate Cancer Cell Characteristics. *The FASEB Journal. Accepted*
14. ***Baumfalk, D. R**., Opoku-Acheampong, A., Caldwell, J., Ade, C., Musch, T., & Behnke, B. (2019). High Intensity Training Impacts on Prostate Cancer Induced Cardiac Atrophy in a Pre-Clinical Model. *The FASEB Journal*, *33*(1_supplement), 535-6.
15. Kunkel, O., Opoku-Acheampong, A., **Baumfalk, D. R.**, Horn, A., Muller-Delp, J., & Behnke, B. (2019). Effect of Hypoxia on Prostate Cancer Cell Viability in Serum and Prostate Conditioned Media from Mixed-Intensity Exercise Trained Rats. *The FASEB Journal*, *33*(1_supplement), 536-13.
16. Horn, A. G., Opoku-Acheampong, A. B., **Baumfalk, D. R**., Kunkel, O. N., Caldwell, J. T., Poole, D. C., & Behnke, B. J. (2019). Effects of Prolonged Mechanical Ventilation on Diaphragm Arteriolar Response to Alpha-Adrenergic Agonists. *The FASEB Journal*, *33*(1_supplement), 541-11.
17. ***Baumfalk, D.R**., Opoku-Acheampong, A. B., Caldwell, J. T., Ade, C. J., Musch, T. J., Behnke, B. J. (2018). *Effects of a Pre-Clinical Model of Prostate Cancer on Left Ventricular Function in Sedentary and Exercise Trained Rats. The FASEB Journal, 32(1 Supplement), 854-5*
18. Opoku-Acheampong, A., **Baumfalk, D. R**., Ganta, C. K., & Behnke, B. J. (2018). *Prostate cancer cell viability and migration in serum and prostate conditioned media from moderate-intensity exercise trained rats. The FASEB Journal, 32(1), 588.32.*
19. Ardery, S., Horn, A., Opoku-Acheampong, A., **Baumfalk, D, R.,** & Behnke, B. (2018). *Exercise training does not alter prostate tumor cell growth in rat serum or prostate conditioned media. The FASEB Journal, 32(1), 855.19.*

20. Rand, T., Pyle, J., **Baumfalk, D, R**., Opoku-Acheampong, A., Musch, T., & Behnke, B. (2018). *No effect of head up tilt on tumor perfusion in a rat pre-clinical model of prostate cancer. The FASEB Journal, 32(1), 722.9.*
21. Caldwell, J. T., Craig, J. C., Post, H. K., Lovoy, G. M., Sutterfield, S. L., Banister, H. R. **Baumfalk, D, R**., . . . Ade, C. J. (2018). *Effect of dietary nitrate on blood pressure and vascular control in post-menopausal hypertensive women. The FASEB Journal, 32(1), 847.15.*
22. Alexander, A. M., Hammer, S. M., Didier, K. D., **Baumfalk, D. R**., & Barstow, T. J. (2017). *Muscle Recruitment Patterns Above Critical Power. The FASEB Journal, 31(1 Supplement), 710-2.*

23. Hammer, S. M., Alexander, A. M., Didier, K. D., **Baumfalk, D. R**., & Barstow, T. J. (2017). *Oxygenation Characteristics of the Vastus Lateralis During Cycling Exercise Performed Above Critical Power. The FASEB Journal, 31(1 Supplement), 831-14.*
24. Emmanuel G., Becker, V.G.C., McCullough, D., Stabely, J, N., Gittemeier, E, M., **Baumfalk, D. R**., Opoku-Acheampong, A. B., Dietmar. S, W., Behnke, B. J. (2017) *Blood flow responses to mild-intensity exercise in ectopic versus orthotopic prostate tumors dependence upon host-tissue hemodynamics and vascular reactivity.*
25. Sutterfield, S. L., Smith, J. R., **Baumfalk, D. R.,** Didier, K. D., & Ade, C. J. (2017). *Acute Changes in Left Ventricular Mechanics During Voluntary Apnea. The FASEB Journal, 31(1 Supplement), 843-16.*