Effect of Head Up Tilt on Tumor Perfusion in a Pre-Clinical Model of Prostate Cancer

by

Taylor Anne Rand

B.S., Kansas State University, 2018

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Kinesiology
College of Human Ecology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2018

Approved by:

Major Professor
Bradley J. Behnke

Copyright

Abstract

Introduction: Prostate tumor arterioles lack functional smooth muscle and have a diminished myogenic response. Previous research has demonstrated an enhanced prostate tumor blood flow and oxygenation associated with the augmented mean arterial pressure during exercise. Thus, we tested the hypothesis that elevations in the heart-to-prostate tumor hydrostatic gradient via adoption of the 70˚ head-up tilt (HUT) body position would enhance perfusion of the prostate tumor, which may improve tumor oxygenation and radiation therapy outcomes (Study I). Based upon those findings, we performed a secondary analysis (Study II) on previously published prostate hemodynamic responses to an identical tilt-test between young and aged animals. **Methods:** Study I: Dunning Cell AT-1 tumor cells (100,000) were injected into the ventral lobe of the prostate in male Copenhagen rats (4 mo.; n = 7). Four to six weeks after injection blood flow to the prostate tumor, kidneys, and soleus muscle was measured via the fluorescent microsphere technique in the supine and HUT position. Study II: A secondary analysis was performed on blood flow to the prostate (host tissue of the tumor) in young (6 mo.; n =9) and aged (24 mo.; n=7) male Fisher 344 rats from Ramsey et al., 2007 (39) to determine potential age-associated differences in conductance to this tissue. **Results:** Study I: No significant difference was observed in blood pressure between the two body positions. Compared to the supine posture, there was a significant reduction in blood flow to the soleus muscle. There was no difference in prostate tumor blood flow or vascular conductance between the supine and HUT position. Study II: In response to tilt, there was a significant reduction in prostate vascular conductance in young rats versus that in the supine posture ($P<0.05$). In the aged animals, there was no difference in prostate vascular conductance with tilt. **Discussion:** Contrary to our

hypothesis, we did not see any significant differences in either blood flow or vascular conductance to the prostate tumor with manipulations in body position. Importantly, we believe this may be an age-associated effect. Given tumors both co-opt existing arterioles from the host tissue that retain vasomotor control and develop new vessels that lack functional smooth muscle, the enhanced vascular resistance in the prostate with young animals during tilt likely contributed to the lack of change in tumor perfusion with body position given the rats from study I were also young. Given the lack of change in vascular conductance in the prostate with tilt in aged animals, future studies should be performed in aged models of prostate cancer, of which currently there are no immunocompetent aged rodent models of prostate cancer.

Table of Contents

List of Figures

Acknowledgements

I would first like to acknowledge my mentor, Dr. Bradley Behnke, for allowing me this opportunity and providing me with a prolific laboratory experience. I would also like to thank Drs. Tim Musch and Carl Ade for their contribution to my committee; special thanks to Tim Musch and Sue Hageman for their essential assistance in the experimentation of this project. Many thanks to my laboratory mates Dryden Baumfalk, Alex Opoku-Acheampong, and Joseph Pyle for seeing me through my stressful moments and their quickness to assist wherever needed.

Dedication

I would like to dedicate this thesis to my encouraging and supportive family, especially to those who were unable to see me finish. For your faithful support throughout your lifetime, special thanks to my Great Grandma Evelyn, Grandma Lynna, and Aunt Barbara.

Chapter 1 - Historical Review of Circulatory Changes with Orthostasis

In antiquity, blood was presumed to be produced continuously by the breakdown of food stuffs and the lungs pumped the blood to necessary tissues. It was not until the early 17th century that William Harvey postulated a different circulatory system, one that included the heart as a pump rather than simply a producer of heat and a cardiovascular system that demonstrated unidirectional flow (40)(21). While Harvey also identified the active phase of the heart (systole), blood pressure was not fully understood or quantified until over a century later by the Reverend Stephen Hales. Hales was seemingly a scientific jack-of-all trades but by no means a dilettante; he published in Natural History (e.g., postulated plants use sunlight for energy), Astronomy, Statistic, and Physiology (developed ventilators with large bellows to improve air quality and combat 'bad air' postulated to cause illness). His vast array of intellect raised an interest from the Royal Society to which he was elected to serve in 1718. In 1733 Hales published Volume II of the Statical Essays where he detailed briefly the first experiment that led to the concept of blood pressure. Specifically, Hales restrained a mare on her back and inserted an elongated brass pipe into the crural artery, from which he observed the force of the blood through the pipe. Hales continued his study of blood pressure as well as the anatomy and physiology of the heart (19)(8). Although Hales was the first to describe systolic and diastolic blood pressures, there was not an accurate measurement for these pressures until over a century later when scientists such as Poiseuille, Ludwig, and Vierordt developed direct and indirect measurements of blood pressure (8).

While the knowledge of blood pressure existed, the regulation thereof affording the upright posture was undiscovered until scientists such as John Hunter (1794) and Francois Magendie (1830) demonstrated that muscle movement alleviated swelling in the feet. Further, the studies of French Physician, Pierre Adolphe Piorry, on mechanisms of syncope caused a ripple effect of pressure analysis by means of postural adjustments (44)(22). Perhaps some of the most renowned insights to vasomotor adjustments in upright posture came from the studies of Leonard Hill and Harold Barnard. The two understood that in the upright posture pressure falls in the carotid artery but is compensated by a vasoconstriction of the splanchnic region; however, unlike other scientists of that period, they believed this vasoconstriction did not affect vascular capacity, rather solely vascular resistance (44). We now understand this relationship and the preservation of pressure from the hydraulic resistance equation: MAP = CO x SVR, where MAP is mean arterial pressure, CO is cardiac output, and SVR is systemic vascular resistance. Hence a drop in MAP will result in a compensatory rise in CO and/or SVR and vice versa to regulate pressure.

At the beginning of the 20th century, the English physiologist William Bayliss was studying vasodilator reflexes when he noticed a strange response to pressure changes in an animal after select nerves had been severed. This response led him to believe there was not only neural control of the vasculature, but also local control. Bayliss proceeded to conduct multiple experiments in animals on a variety of tissue where he would denervate the region of interest and examine the local effect to changes in blood pressure. Through these experiments Bayliss discovered a change in vascular tone in response to pressure; he noted “The reaction is, therefore, of peripheral origin, and as I believe, myogenic in nature.” (4). Bayliss is credited with

discovering myogenic autoregulation, which is a change in vascular tone in response to the mechanical stress of pressure, independent of humoral or neural stimuli (32). We now know this relationship to be Q = ΔP/R, where Q is blood flow, ΔP is the change in pressure (also referred to as 'perfusion pressure"), and R is resistance. The importance of myogenic autoregulation is to help maintain blood flow to a tissue through alterations in resistance. For example, an increase in wall tension from greater intraluminal pressures is thought to depolarize smooth muscle cells through stretch-activated cation channels and induce a local vasoconstriction to maintain flow (for review see; (45)). An increase in perfusion pressure, which we see in peripheral tissues below the level of the heart during upright posture, causes an increase in resistance (vasoconstriction) mediated not only through augmented sympathetic stimulation, but also local myogenic autoregulation.

With the expansion of knowledge pertaining to orthostasis, pressure, and blood flow, studies have sought to determine how old age, which is associated with decreased tolerance to orthostasis, impacts many of the aforementioned mechanisms. One such study examined the effect of head up tilt (HUT) on vascular conductance and blood flow in multiple regions and tissues in young and aged rats. The authors found that aged rats were incapable of decreasing vascular conductance to several tissues in HUT, further, mean arterial pressure was compromised speculated to be due to the inability to augment systemic vascular resistance (39). Additionally, there was a reduced myogenic vasoconstrictor response in resistance arterioles from multiple tissues from aged animals versus a robust response in arterioles from young animals. In the same tissues of young animals with tilt there were decrease in blood flow, due to a greater relative vasoconstriction in these tissues versus the level position (39). Importantly, that study

demonstrated that, with age, there is diminished ability to regulate vascular conductance through myogenic autoregulation in several tissues in response to an orthostatic stress ultimately contributing to the increased prevalence of orthostatic intolerance in the aged population.

Considering disease states such as cancer introduce a new component to the vascular system, specifically solid tumors, it is essential to understand how these systems operate comparatively. Tumor vasculature is divergent compared to healthy tissue in that it is unorganized, convoluted, and aberrant (26)(60)(for a review;(25)). Furthermore, solid tumor blood vessels typically lack functional smooth muscle and basement membranes (26)(3)(9), such that the altered morphology impairs function. The tumor microenvironment poses several perturbations in oxygen delivery and tumor perfusion which can result in tumor hypoxia. Importantly, tumor hypoxia is the greatest predictor of anti-cancer treatment failure and when tumor hypoxia is present it requires substantially higher doses of radiation to kill a given population of cells. The overall goal of this project was to acutely augment prostate tumor perfusion with body position and hydrostatic column manipulation. If so, such findings have high clinical value and could rapidly be adopted into practice.

Chapter 2 - Introduction

Significance of prostate cancer and relevance

In men, prostate cancer is the second leading cause of cancer deaths and roughly 1 in 9 men will be diagnosed with the disease in their lifetime (ACS fact sheet; web). There have been significant advances in early detection and treatment of the disease, contributing, in part, to the increased survival rates over the past few decades. Currently, the 5-year survival rate from all stages of prostate cancer patients in the United States is approximately 99% (48). However, if the disease is metastatic this survival rate drops to 30%. Further, there is considerable global variability in the 5-year survival rates from this disease, e.g., rates drop to ~50% in England, and less than 25% in Algeria (10) .

Despite the relatively high survival rates with prostate cancer overall, when patients are stratified based upon specific characteristics of the tumor microenvironment, survival and disease-free rates vary considerably. For example, in patients with hypoxic prostate tumors, within 8 years less than half of them will remain free from biochemical failure, whereas those with less-hypoxic tumors (see below for definitions), ~80% of patients will remain disease free (54). Indeed, tumor hypoxia is one of the strongest prognostic indicators of treatment failure and survival from solid tumor cancers, including prostate cancer (58) .

Hypoxia and Angiogenesis: Effect on Tumor Vasculature

A tumor becomes hypoxic when oxygen supply is not meeting oxygen demand, or vice versa, causing intracellular PO_2 to decrease. Dewhirst and Moeller identified eight primary features of a tumor that result in hypoxia, among those are a drop in intravascular perfusion pressure, lack of oxygen to diffuse into the tumor, inadequate oxygenation of the nutrient bed

due to shunt, low vascular density, random orientation of tumor vessels resulting in inefficient regional oxygenation, high oxygen consumption rate, viscous blood causing reduced flow rate, and finally cyclical hypoxia (13). From cell culture models, when PO_2 decreases below ~ 10 mmHg, there are significant deleterious effects that greatly enhance growth, survivability, and metastatic potential of the tumor cells (see review (49)). Specifically, when tumor cells are exposed to a PO_2 of < 10 mmHg, cell migration is enhanced as well as an increased production of cancer associated fibroblasts (49). Clinically, when patients are stratified based upon having a median tumor PO_2 of > or < 10 mmHg, patients in the latter group have significantly lower 5-year survival rates (23). It should be noted that there are considerable variations in the mechanisms resulting in spatial differences in tumor PO_2; however, there is clear evidence that tumor hypoxia results in an aggressive phenotype and also increases the expression of HIF-1α (55). When HIF-1α is upregulated, it increases the expression of various genes contributing to tumor growth and survival including angiogenesis (46). Although angiogenesis contributes heavily to tumor growth (27), the vessels formed have significant differences when compared to their healthy counterparts. It is well established that the tumor vasculature is aberrant, unorganized, immature, and tortuous (2)(38)(26) typically lacking functional smooth muscle, innervation, basement membranes, and numerous other properties essential for regulating blood flow (9)(26)(3).

Attempts to Mitigate Hypoxia

To target the chaotic tumor microenvironment, many studies have sought to combat hypoxia by augmenting tumor blood flow and oxygenation; through hyperoxic breathing, vasodilatory therapy, and radiosensitizers. The effect of hyperoxic breathing initially failed due

to the vasoconstrictive effect of pure O_2. To offset this, investigators have used carbogen (i.e., any mixture of two gases, initially developed by Ladislas Meduna), with 5% CO_2; 95% O_2 commonly being used to elevate arterial PO_2 and potentially drive more O_2 into the tumor. The effects of carbogen on tumor oxygenation is equivocal with studies showing heterogeneous results, potentially depending upon the tumors adaptation to altered perfusion environments (43)(53)(1). Vasodilators and vasopressors have also been utilized in attempt to attenuate hypoxia (42)(61)(47). However, manipulating arterial PO_2 and regional vascular control mechanisms can have adverse effects on whole body hemodynamics, particularly via alterations in mean arterial pressure (MAP). Cardiac output (Q) and systemic vascular resistance (SVR) are the primary regulators of MAP; a change made to one of these variables will have an inverse and immediate effect upon the other. For example, with the acute use of vasodilators, SVR will drop and the body will respond via an increase in HR in an attempt to elevate Q. If an inadequate venous return is present, MAP may fall, potentially resulting in a life-threatening condition for the patient. Precise regulation of MAP creates a small range of pressures in which therapeutic attempts using pharmacological dilators or constrictors can be utilized, leading to their failure in many instances.

Exercise and Tumor Blood Flow

The effect of exercise on the tumor microenvironment remained widely controversial over the late 20th century. However, recent data demonstrates an augmentation of tumor blood flow to mitigate hypoxia during exercise and after chronic aerobic exercise training. During exercise the body must redistribute cardiac output from compliant tissues to the recruited skeletal muscle to support the metabolic needs of the active muscle and enhance central venous

pressure (CVP)(44). This redistribution is graded dependent upon exercise intensity and approaches directing ~90% of cardiac output to the working muscle during maximal intensity exercise (37),(44).In order to achieve this redistribution of cardiac output there a contraction of the vascular smooth muscle, primarily in arterioles from compliant organs (e.g. skin, splanchnic and renal), to prevent precipitous falls in CVP and re-direct cardiac output to the working skeletal muscle. Given the abnormal microvasculature of a solid tumor (e.g. lack of functional smooth muscle (9), vasoconstriction in resistance vessels of solid tumors is predicted to be attenuated. Indeed, in tumor arterioles from a preclinical prostate cancer model, McCullough and colleagues demonstrated a lack of constriction to increases in intraluminal pressure in the range observed with exercise and to the sympathetic neurotransmitter, norepinephrine (29). Subsequently, during exercise with an augmented MAP, tumor blood flow increased by ~200%. It was hypothesized that with the elevated MAP during exercise, the inability of the tumor vasculature to vasoconstrict resulted in a greater tumor blood flow versus that observed at rest.

While this data is encouraging in altering the tumor microvasculature, it is important to note the increase in blood flow occurred during exercise. Limitations in current technology prevent patients from moving while receiving radiotherapy due to the risk movement poses for irradiating surrounding healthy tissues.

Hydrostatic Pressure Gradients

Radiotherapy for prostate cancer is administered while the patient is in the supine position to reduce motion artifact and simplify positioning the equipment to deliver a given dose. In the supine posture pressure gradients throughout the body are relatively homogenous. However, in the upright posture, hydrostatic gradients result in heterogeneous pressure

gradients depending upon the position of an organ relative to the level of the heart. For example, versus the supine posture there would be reduced vascular pressures in the brain and increased pressures in any organ below the level of the heart. This would result in an enhanced perfusion pressure to organs below the level of the heart. As the body rises from supine to standing, a multitude of changes occur to maintain CVP and prevent syncope. To prevent an over-perfusion of organs located below the heart level, regional increases in vascular resistance through enhanced myogenic and sympathetically-mediated vasoconstriction must occur. However, a lack of functional smooth muscle in the prostate tumor may impair local increases in vascular resistance which may result in an enhanced tumor perfusion in the upright versus supine posture. In animal models, the 70˚ head-up tilt position has been used to test orthostatically induced alterations in systemic and regional hemodynamics (39). Despite an absolute lower heart-to-prostate distance in the rat versus human, clear differences in below heart level tissue to tilt in the animal model can be observed (39), making it an appropriate model to test differences in prostate tumor blood flow with the upright posture.

Purpose

As stated above, the vasculature of solid tumors lacks functional smooth muscle and innervation requisite to vasoconstrict. Therefore, based on the location of the prostate and the diminished myogenic response in tumor arterioles, we hypothesize that an increase in pressure of the hydrostatic column caused by an orthostatic stress would result in enhanced blood flow to a prostate tumor in a rat preclinical model of prostate cancer. Given prostate tumor preclinical models in immunocompetent rodents are limited to younger animals, we also sought to compare potential age-associated differences in prostate vascular conductance with tilt. When solid

tumors develop they not only develop arterioles that lack function smooth muscle, but also co-opt existing organ mature blood vessels that maintain vasomotor control. Hence, age-related changes in host-tissue (i.e. prostate) may impact blood flow responses within the tumor. Therefore, we performed a secondary analysis to determine prostate vascular conductance with old age and tilt on previously published prostate blood flow responses (39).

Chapter 3 - Methods

Study I: Orthostatic Challenge and Prostate Tumor Perfusion

Animals

Young adult (n = 7) male Copenhagen rats were obtained from Charles River, Inc. Rats were housed individually at 23º C and were maintained on a 12:12-h light-dark cycle and fed rat chow and water *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committees at Kansas State University and the University of Florida.

Tumor Model

Orthotopic Model of Cancer

The cell line utilized in this study was the Dunning R-3327 (AT-1) strain of rat prostate adenocarcinoma cells characterized by high growth rate, low metastatic potential, with similar growth characteristics as human prostate cancer (24). A T-1 cells were cultured in RPMI-1640 media (GE Healthcare Life Sciences, Marlborough, MA) containing 10% fetal bovine serum (FBS) RMBIO, Missoula, MT), 2 mM L-glutamine (Fisher Scientific), 100 mM sodium pyruvate (Thermo Fisher Scientific), 1% penicillin/streptomycin (Thermo Fisher Scientific), and 0.025 mM dexamethasone (Cayman Chemical) and incubated at 37˚C with 5% CO_2. Once cells reached ~80-90% confluence, a sample of the cells were counted via hemocytometer to calculate proper dilution (100,000 cells/ml) of the viable cells for a tumor cell stock solution in physiological salt solution (PSS). This solution was aliquoted into 0.1 mL increments containing ~10^4 AT-1 cells**.** These methods have been used previously to induce the development of prostate tumors (16)(30).

In tumor-bearing rats, animals were anesthetized (2-5% isoflurane, oxygen balance) and a small incision of ~ 1cm or less was made in the abdomen, lateral of the midline. The bladder/prostate complex was exposed and, through this incision, the ventral lobe of the prostate was isolated and 10^4 AT-1 cells were injected using a sterile 26G insulin syringe. To prevent leakage of cells to the tissue surrounding the prostate, a sterile cotton tipped applicator was placed alongside the needle during removal. Immediately following injection, the abdominal wall was closed with sterile 3–0 polyglycolic acid coated suture (DemeTECH, Miami Lakes, FL) and the overlying skin/fascia was closed with sterile 3–0 nylon monofilament; (DemeTECH) and sealed with skin adhesive (3M, Vet-Bond). Rats were then injected with 0.05 ml/kg buprenorphine (S.C.) to control any post-operative pain and 0.5 mg/kg acepromazine (S.C.) as a sedative. This combination of analgesic and sedatives abolished any signs of pain or discomfort and prevented dehiscence by the rats. All procedures were performed under aseptic conditions with daily postoperative monitoring performed until animals were placed into sedentary or exercise-trained groups ~7 days post injection.

Surgical Procedures

Prior to the surgical procedure, animals were habituated to the tilt apparatus at 0˚tilt for 20 min per day for at least 3 days. At the conclusion of the habituation regimen, the rats were anesthetized with isoflurane (2%/O_2 balance) and a catheter (Dow Corning, Silastic; ID 0.6 mm, OD 1.0 mm) filled with heparinized saline solution (Elkins-Sinn Inc., 100 U/mL) was advanced into the ascending aorta via the right carotid artery as previously described (39). This catheter was used for infusion of fluorescent microspheres for tissue blood flow measurement and for monitoring MAP. The carotid catheter was externalized at the base of the tail and secured on the

underside of the tail. A second polyurethane catheter (Braintree Scientific; ID 0.36 mm; OD 0.84 mm) was implanted in the caudal tail artery as described previously (11) and externalized at the tail. This catheter was used to obtain a reference blood sample which serves as an artificial organ for calculating tissue flows. At the end of the surgical procedure the animals were injected with a low-dose of acepromazine (0.5 mg/kc, s.c.) to minimize stress and rolling within the tilt apparatus. The animals were then given 4 hours to recover as this time frame allows for re-establishment of basal systemic hemodynamics (14).

Experimental Protocol

After a 4 hr recovery period the animals were gently restrained in a Plexiglas canopy (Rodent ECU, Braintree Scientific) hinged to a tilting Plexiglas support base in a horizontal-standing position (0˚ tilt) and allowed 20 min of quiet standing before the first microsphere infusion. The head end of the canopy had a tapered opaque plastic hood to protect the rodents from visual disturbances. This served to minimize the ocular postural input, which may alter cardiovascular reflex responses. The animals were placed so that the thorax is at the same level as the tilting axis. Tilt time was ~ 1-2 s from the level (0˚) baseline condition to 70˚ head-up tilt. Arterial pressure and heart rate were measured in each rat at 0˚ tilt for baseline data and every minute up to 10 min immediately after the onset of tilt at 70˚. Tissue blood flows were measured during 0˚ tilt and after 10 min of 70˚ tilt. During the experiments animals remained in the tilted position for 10 min prior to the infusion of the microspheres as previous research indicates this time is representative of a relative steady state measure of the cardiovascular response to head-up tilt. (39). At the end of the experiment, the animals were anesthetized with isoflurane (5%/O_2 balance) and euthanized via removal of the heart. Thereafter the anatomical distance between

the carotid artery cannula and the prostate tumor was measured. The kidneys, soleus muscles, prostate tumor and viable prostate were then excised, weighed, and placed into 50 mL conicals for determination of blood flow as described below.

Blood Flow and Vascular Conductance Measures

Fluorescent microspheres (ThermoFisher Scientific) with a 15.0 ± 0.5 µm diameter were used for blood flow measurements using similar methods to Ramsey et al. 2007. Specifically, microspheres were suspended in physiological saline with <0.5% Tween 80 and mixed prior to infusion by 10 min sonication (FS20 Sonicator, Fisher Scientific). Red (at 0˚) and blue-green (at 70˚) microspheres were used as the excitation and emission wavelengths did not overlap. A reference blood sample was taken from the caudal artery at a rate of 0.618 ml/min with a Harvard withdrawal pump (model 907, Cambridge, MA) while simultaneously ~2.5 X 10^5 microspheres suspended in 0.2 ml saline were infused into the carotid catheter over a 10- to 15-s period. Warm (37°C) saline (0.5 ml) was infused over a 30-s period immediately after microsphere infusion to clear the catheter of residual microspheres; withdrawal of the reference blood sample continued for 20 sec after the saline flush. After euthanasia and tissue dissection, tissue microsphere extraction was performed based upon the manufacturer guidelines. Thereafter, sample fluorescent was measured in a spectrometer and flows were calculated from fluorescence and tissue wet weights and reported in ml/min/100 g. Adequate mixing of the microspheres was verified by demonstrating a <15% difference in blood flows to the right and left kidneys or the left and right soleus muscles. Mean arterial pressure was electronically averaged from pulsatile pressure measurements via a pressure transducer (BP100, ADInstruments). Tissue vascular

conductance (ml/min/100 g/mmHg) was calculated by dividing tissue flows (ml/min/ 100 g) by the MAP (mmHg).

Study II: Effects of age on prostate vascular conductance with an orthostatic challenge

In the absence of pre-clinical models of prostate cancer in aged animals we sought to determine postural changes with old-age in healthy host tissue of prostate cancer, i.e., the prostate. This was accomplished by analysis of previously published blood flow data from Ramsey et al. 2007 to calculate prostate vascular conductance and resistance to 70˚ HUT in young and aged animals, which has never been reported. To determine this, prostate blood flow reported by Ramsey et al. 2007 was divided by the MAP from each animal of that study in the level and tilt positions. The general methods (i.e., tilt, instrumentation, post-analysis, etc.) were identical between study II and study I above, with the exception of the following:

Animals:

Young (6 mo; n=9) and aged (24 mo; n=7) male Fisher 344 rats were used to determine blood flow responses to supine and 70˚ HUT as previously reported from Ramsey et al., 2007.

Blood flow: Instrumentation for the animals was identical to study I.

Radiolabeled (^{46}Sc and ^{85}Sr) microspheres (Perkin Elmer NEN) with a 15.5 ± 0.2 µm diameter were used for blood flow measurements, as described in Ramsey et al., 2007, at level and 70˚ HUT positions. Microspheres were suspended in physiological saline with 0.5% Tween 80 and mixed prior to infusion by 10 min sonication (FS20 Sonicator, Fisher Scientific). A reference blood sample was taken from the caudal artery at a rate of 0.618 ml/min with a Harvard withdrawal pump (model 907, Cambridge, MA) while simultaneously ~2.5 X 10^5 microspheres suspended in 0.2 ml saline were infused into the carotid catheter over a 10- to 15-s period. Warm

(37˚C) saline (~0.5 ml) was infused over a 30-s period immediately after microsphere infusion to clear the catheter of residual microspheres; withdrawal of the reference blood sample continued for 20 sec after the saline flush. After euthanasia and tissue dissection, tissue samples were counted in a gamma counter (Packard Auto Gamma Spectrometer, model 5780), and prostate flow was computed from counts per min and tissue wet weights (blood flow reported in ml/min/100 g). Mean arterial pressure was electronically averaged from pulsatile pressure measurements via a pressure transducer (BP200, ADInstruments). To determine age-related effects on prostate vascular conductance we took the individual prostate blood flows from Ramsey et al., 2007 and divided those by the individual MAP responses for each animal immediately preceding microsphere infusion and is reported as ml/min/100 g/mmHg. To calculate prostate vascular resistance, MAP was divided by blood flow and is reported as mmHg/ml/min/100 g. Importantly, whereas the average prostate blood flow values with age have been published previously (39), prostate vascular conductance and resistance with age and tilt have not been and were determined for the first time in the present study.

Statistical analysis:

Study I: A two-tailed paired T-test was used to determine differences in blood flow and vascular conductance with tilt to the kidney, soleus, prostate tumor. All data are presented as a mean ± SEM. Significance was set at $P \leq 0.05$.

Study II: A two-way, repeated measures ANOVA was used to compare prostate vascular conductance between groups (young vs. aged) and within groups across conditions (rest vs. 70˚ tilt). Duncan's multiple range test was used to determine the significance of difference among treatment means. All data are presented as mean ± SEM. Significance was set at $P \leq 0.05$.

Chapter 4 - Results

Study I:

Animals

Successful blood flow experiments were completed in six rats (final age ~6 mo.) with an average body mass of 272 ± 22 g.

Heart Rate and MAP

Values for heart rate were not different between level and tilt (426 ± 24 beats/min vs. 416 ± 26 beats/min, $P > 0.05$, respectively). There was no change in MAP between level and tilt (80 ± 15 mmHg vs. 78 ± 13 mmHg, respectively) (Figure 1).

Tissue Blood Flow

Average kidney blood flow was not different between body positions (Figure 2). Blood flow to the soleus muscle decreased significantly between the level and tilt position ($P \leq 0.018$; Figure 2).

Prostate Tumor Arterial Pressure, Blood Flow, Vascular Conductance, Vascular Resistance

The average anatomical distance between the prostate tumor and the carotid cannula (where pressure was determined) was 9.1 ± 0.7 cm. In the supine posture the average prostate tumor arterial pressure would be similar to mean arterial pressure. However, in the upright posture, a hydrostatic gradient would be present which would elevate the prostate tumor average arterial pressure above mean arterial pressure measured near the heart. We chose to calculate prostate tumor vascular resistance and conductance on the estimated arterial pressure at the tumor. Assuming an increase of 2 mmHg per 2.54 cm of vertical distance between the cannula tip and prostate tumor (see discussion), at 90° this would increase pressure by an

average of 7.1 mmHg. At the 70° angle this cannula tip-to prostate tumor vertical distance would be reduced to 8.6 cm resulting in an increased pressure of 6.7 mmHg. Thus, despite no change in MAP between body positions (Figure 1), there would be an increased average arterial pressure at the prostate tumor in the tilt versus level position (Figure 3A). Average prostate tumor blood flow did not did not differ between body positions (Figure 3B). Further, despite an increase in average pressure at the level of the prostate tumor with tilt vs. level body position, there were no differences in prostate tumor vascular conductance (Figure 4A) or vascular resistance (Figure 4B) with body position.

Study II:

Data were collected from 9 young animals and 7 aged animals. Young animals demonstrated a higher MAP versus aged animals in the level and tilt positions (Figure 5A). In response to tilt, arterial pressure increased in the young animals but did not change in the aged group versus the level position. When calculating prostate vascular resistance to tilt between groups, there was a significant reduction in conductance with tilt in the prostate of young animals whereas prostate conductance did not change in the aged group (Figure 5B). Thus, in young animals there is a significant increase in healthy prostate vascular resistance to tilt (level; 7.4 ± 1.5 vs. tilt, 10.1 ± 2.2 mmHg/ml/min/100 g; $P<0.05$) not present with old age (level, 11.1 ± 2.1 vs. tilt, 13.5 ± 3.5 mmHg/ml/min/100 g; $P>0.05$).

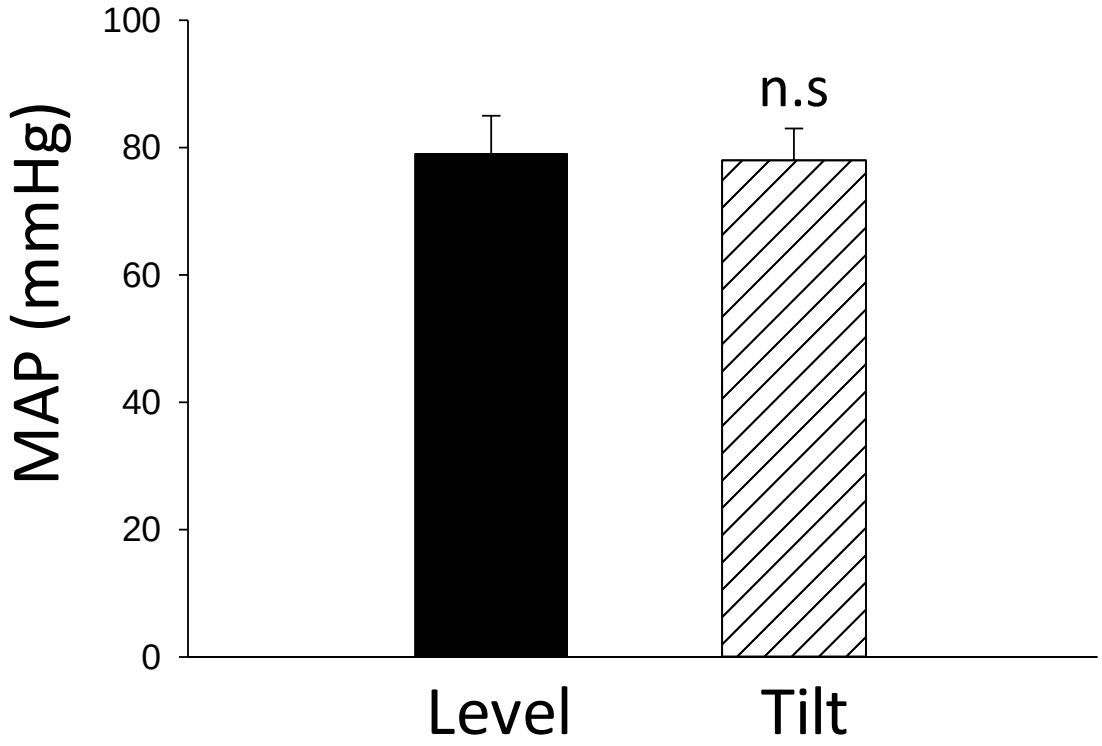

Figure 1 MAP during level and tilt

Average MAP did not change between level and tilt positions. Data are mean ± SE.

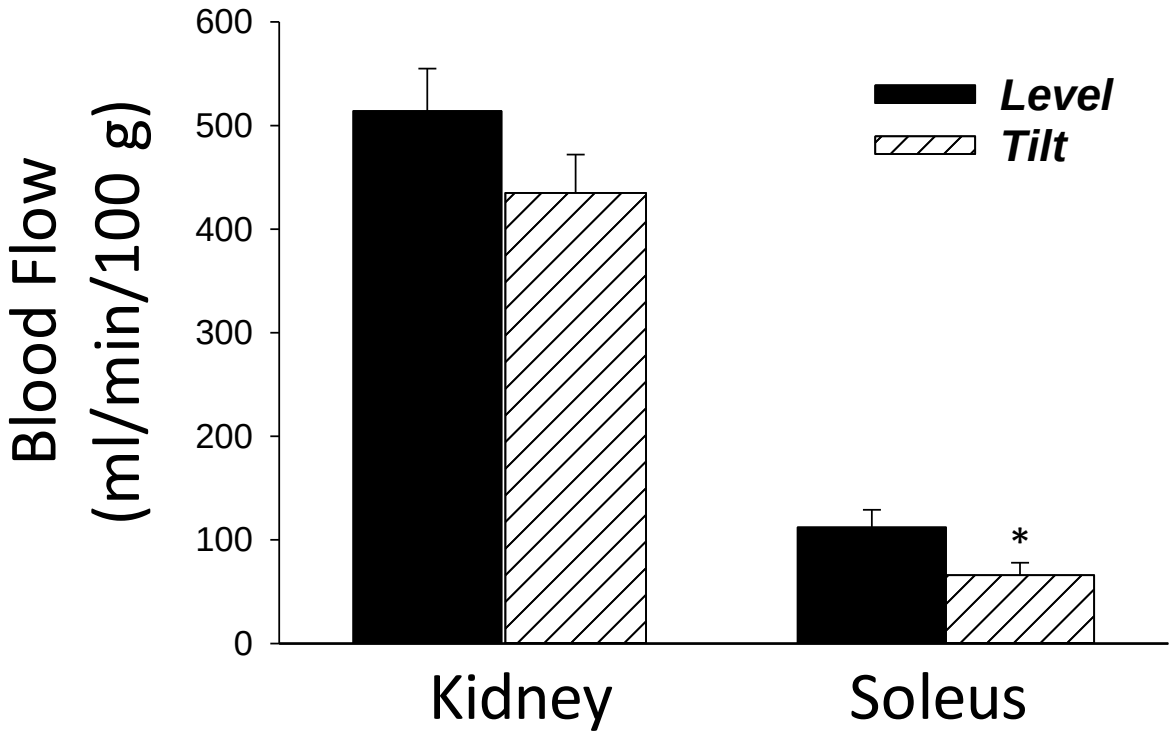

Figure 2 Kidney and soleus blood flows during level and tilt

Data are mean ± SE. * P < 0.05 vs. level in same tissue.

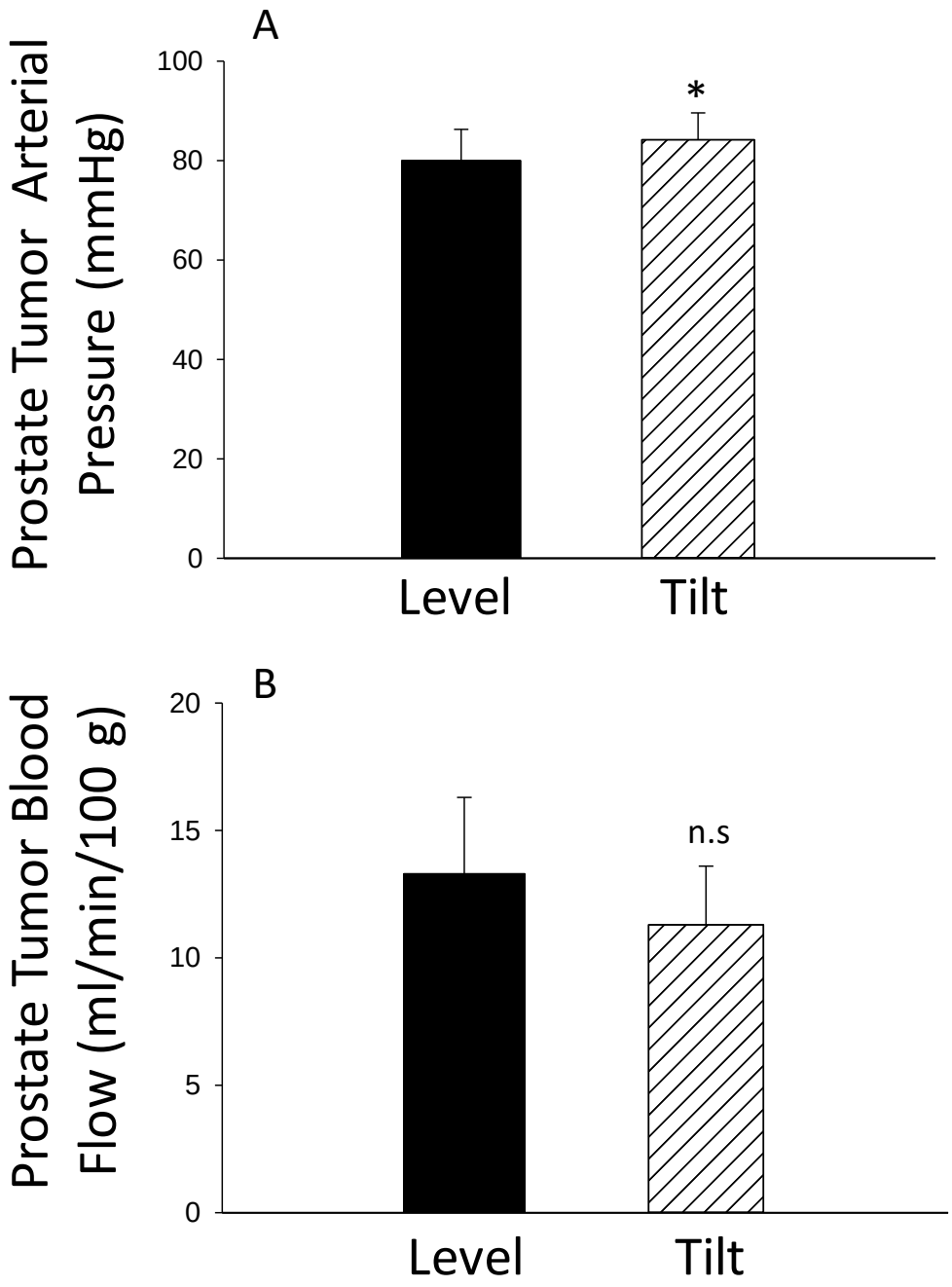

Figure 3 Prostate tumor MAP and blood flow during level and tilt

A. Average prostate perfusion pressure in the level and tilt body position. B. Prostate tumor blood flow did not increase with tilt. *P < 0.05 versus level. Data are mean ± SE.

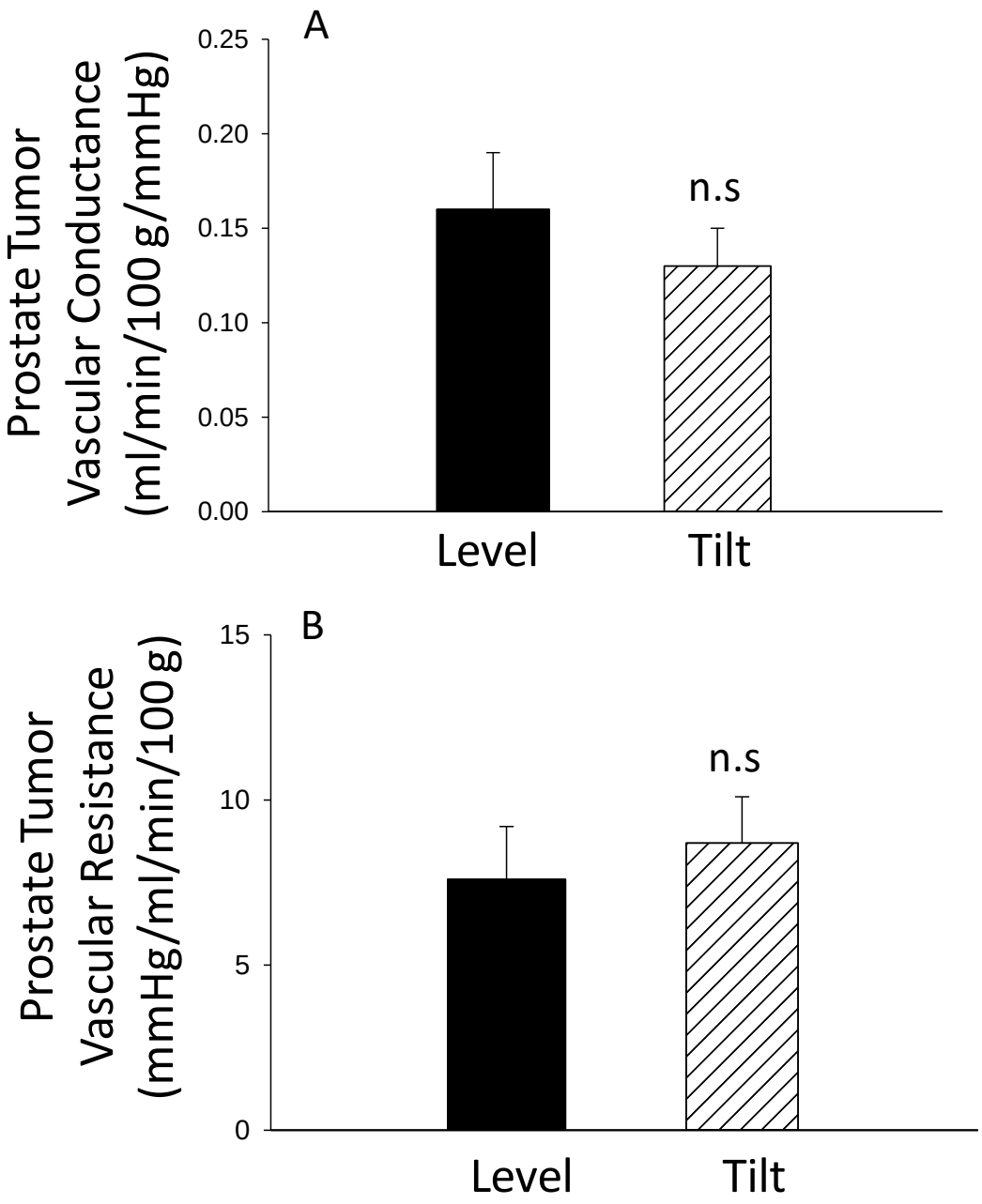

Figure 4 Prostate tumor vascular resistance during level and tilt

A. Average prostate tumor vascular conductance did not change with tilt. B. Average prostate vascular resistance did not change with tilt. Data are mean ± SE.

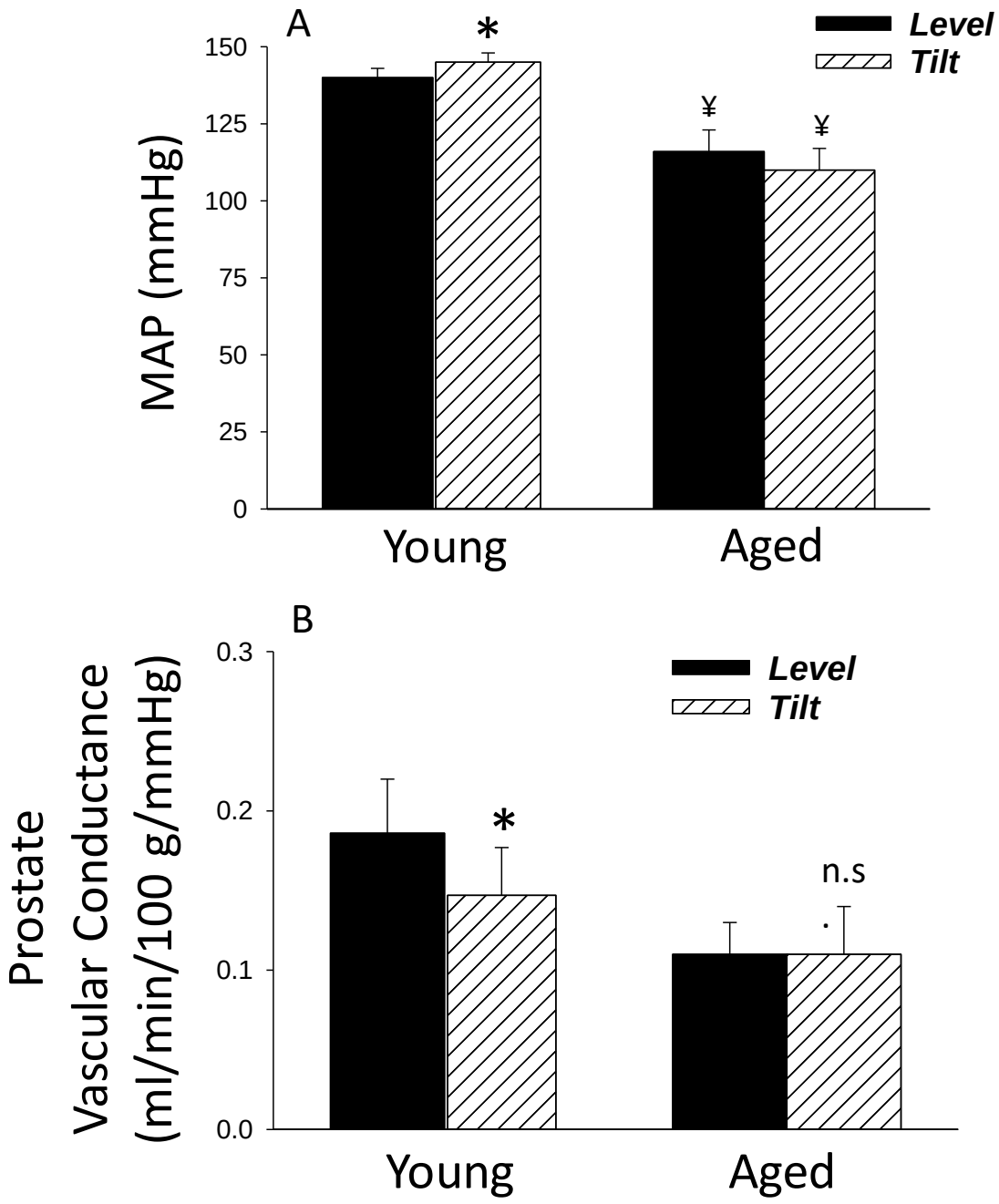

Figure 5 MAP and prostate vascular conductance for young and aged animals

A. Young animals demonstrated a significant increase in MAP during tilt while aged animals demonstrated a significantly lower MAP than young animals in both level and tilt, but no change in MAP between level and tilt positions. B. Young animals demonstrated a significantly diminished vascular conductance to the prostate with tilt whereas aged animals showed no change in vascular conductance with tilt. * P < 0.05 vs. level with age groups. ¥ P < 0.05 vs. young for same body position.

Chapter 5 - Discussion

In this study we sought to determine whether alterations in body position may acutely increase prostate tumor blood flow as a means to increase the efficacy of radiation therapy. Contrary to our hypothesis, we did not observe any change in tumor blood flow between the supine and upright (via 70˚ head-up tilt; HUT) body position in a preclinical model of prostate cancer. Relative to the level (supine) body position there were no differences in calculated prostate tumor vascular resistance or conductance versus the HUT position. Importantly, when interpreting these results it is necessary to understand the immunocompetent model of prostate cancer used herein consisted of male Copenhagen rats of a relatively young age (6 mo). Based upon the longevity curve for this strain of rat, this age would be similar to a young-to-middle aged human, considerably younger than the average age of humans at diagnosis of prostate cancer (i.e., 66 yrs; ACS). Given clear age-associated effects on cardiovascular hemodynamics (36), resistance vascular architecture (5), material properties (20), and function (34), we performed another study to determine whether old age may compromise the ability of the host-tissue (prostate) to change vascular conductance with postural changes. To accomplish this we used published prostate blood flow values in young and aged animals in response to 70˚ HUT (39) to calculate prostate vascular conductance between age groups. Importantly, with tilt there was a significant reduction in vascular conductance to the prostate in young animals, whereas no change was observed in the aged animals. As tumors grow the arterial input comprises co-opted mature, functional vessels from the host tissue as well as immature, dysfunctional vessels. With the current orthotopic cancer model, the co-opted vessels would originate from the young, healthy prostate and would likely maintain adequate myogenic vasoconstriction to offset

pressure changes in the animals used in our initial study, contributing to the lack of change in prostate tumor blood flow with tilt (Figure 2). In aged animals, which demonstrate an inability to diminish vascular conductance or augment vascular resistance in the prostate with tilt (Figure 5B), the co-opted vessels in a prostate tumor would expectedly also demonstrate such age-associated contractile dysfunction. Unfortunately, there are no preclinical models of prostate cancer in aged immunocompetent rats currently available. Therefore, future studies need to be designed to determine the effect of age on prostate tumor vasculature function as well as implications of transmural pressure changes with orthostasis.

Tumor hemodynamics

Compared to healthy tissue, tumor arterioles are typically unorganized, lack innervation, and acquire a poorly developed medial layer lacking functional smooth muscle (26). Hostile tumor microenvironments are largely a result of this abnormal tumor vasculature (56). Without adequate blood flow and vascularization, hypoxia often occurs in solid tumors and is associated with negative clinical outcomes. Tumor hypoxia is established as a key indicator of poor patient prognosis, increased risk of metastasis, and desensitization to radiotherapy (56). One of the earliest non-pharmacological approaches to mitigating hypoxia was by means of increasing tumor blood flow. Specifically, in a preclinical model of cancer, the prostate tumor vasculature lacks the ability to adjust to increases in cardiac output and arterial pressure occurring with aerobic exercise (30). In that study there was a significant exercise pressor reflex that, coupled with the inability of the tumor resistance vessels to constrict at such pressures, resulted in a significant increase in tumor perfusion during exercise versus rest. Therefore, we sought to capitalize on this tumor vascular dysfunction by increasing perfusion pressure at the tumor with

altered body position as many patients with cancer will not be able to exercise. While our study did not demonstrate an augmented tumor blood flow during an orthostatic stress (HUT), it is important to acknowledge several differences between the McCullough et al., 2014 (30) and the current study. Whereas tumor resistance vessels demonstrate a diminished myogenic tone, those differences (versus healthy prostate) manifest at pressures greater than that observed at rest (30). At pressures consistent with level or tilt in the current study, based upon previous data from this strain of rats and this tumor type (30), there would not be any difference in myogenic autoregulation within the tumor resistance vasculature. Given the high interstitial pressure (i.e., reduced transmural pressure) within the tumor, it is likely that the small, but significant, increase in arterial pressure at the tumor with tilt was not enough to overcome such vascular resistance within the tumor. It is unknown, however, whether the greater heart-to-tumor distance and hydrostatic column with the assumption of the upright posture in humans can provide enough of an elevation in perfusion pressure to augment tumor blood flow and is discussed below.

Postural position

The effect of orthostasis on the hydrostatic pressure gradient within the vasculature must be taken into account. For example, in the supine posture intravascular pressure is relatively homogenous in the large arteries throughout the body. With a change in posture to the upright position there will be an increase in intravascular pressure below the level of the heart that is dependent upon the weight of the column of blood proportional to its vertical distance from the heart, assuming the heart as the 'zero' reference point. It could be argued that the relatively small heart-to-prostate tumor distance in the rat versus human may not be enough to induce a hydrostatic difference with orthostatic challenges. However, for several decades rats have been

the model of choice to allow invasive studies of cardiovascular response and regional hemodynamics with postural changes; in particular head-down tilt (HDT) and HUT (59)(39)(35)(57)(18). With the introduction of space flight in the 1960's, the impact of microgravity on the cardiovascular system became a topic of intense study. HDT simulates cephalic fluid shift experienced during microgravity allowing observers to study possible adverse effects. For example, Wilkerson and colleagues used HDT to study vascular changes within the cephalic region (cerebral circulation) in response to increased transmural pressure (59), demonstrating that prolonged HDT (14 days) induces remodeling (hypertrophy) of cerebral arteries. Further, the importance of cerebral vascular myogenic autoregulation has been demonstrated in HDT rat models (17). Whereas HDT has been used to simulate microgravity and/or prolonged bed rest, HUT in rats has been used to simulate the upright posture in humans. For example, studies using HUT have been conducted to examine the effect of age on regional hemodynamics (39).

Within large arteries, for every 2.54 cm (1 inch) between the tissues, there is a 2 mmHg increase in pressure in the distal tissue (41) in the upright posture. The average distance between the carotid catheter (where MAP was measured) and the center of the prostate tumor was 9.1 cm. This would produce an approximate pressure increase of 7 mmHg within the tumor *if* the rat was truly vertical; however, the degree of HUT was 70˚, therefore, the true distance from the carotid catheter and the center of the prostate tumor is 8.6 cm. This distance would produce an approximate pressure increase of 6.7 mmHg. Although this was a relatively small distance it did increase the estimated perfusion pressure at the level of the prostate in the tilt vs. level position (Figure 3A). Based upon the varying heights of humans, it is difficult to find an average heart-to-

prostate distance. However, anatomical measures indicate a range of 12-18 inches, corresponding to an increased hydrostatic pressure to the prostate in the upright versus supine posture of 24-36 mmHg. Importantly, this pressure is independent of dynamic pressure changes with posture. It is currently unknown whether the greater hydrostatic gradient in humans (versus rats) is enough to offset the high interstitial pressure within the prostate tumor to acutely augment flow, and its potential effects on radiation therapy outcomes as discussed below.

Radiation outcomes

According to the American Cancer Society there are a variety of conventional treatment options for prostate cancer patients including, but not limited to, radical prostatectomy, hormone therapy, and radiation. Treatment suggestions differ based on the stage of prostate cancer, however, radiation is included as an option at every stage. External Beam Radiation Therapy (EBRT), and Brachytherapy (internal radiation therapy) are the two primary form of radiation (ACS). Radiation therapy lasts a few seconds to minutes and is commonly administered 5 days a week for several weeks. Importantly, from the classic work of Gray and colleagues it has been known for > 100 years that the presence of oxygen drastically lowers the concentration of radiation needed to kill a given population of tumorous cells (7), which is the main reason why hypoxic tumor are radio-resistant. For this reason we were particularly interested in transiently increasing tumor blood flow with postural changes to maximize tumor cell death to a given concentration of radiation. Potential contributions of HUT combined with radiation therapy are, therefore, dependent upon an increase in vascular conductance within the prostate tumor. As described previously, the tumor vasculature and microcirculation contribute substantially to the aggressive microenvironment of solid tumors (55), therefore, increased tumor blood flow is

essential in diminishing the adverse effects of hypoxia and promoting radiotherapy responsiveness. Importantly, an increase in vascular conductance may augment tumor PO_2 through exercise or body position, hence improving the fixation of free radicals formed during ionizing radiation.

Age-associated changes in hemodynamics

Knowledge of age - associated changes in hemodynamics is important when considering the effect of stressors such as exercise or HUT. Folkow and Svanborg (15) identified four key indicators of aging that negatively impact cardiovascular function. Foremost, a gradual decline in neuronal networks with age effects the central integration of neural and humoral networks in regulating cardiovascular performance. Second, cardiac and vascular muscle cells demonstrate a slow decline in number, strength and speed of contraction with age. Third, a steady decline in tissue and vasculature compliance (31) impairs cardiovascular function. Finally, the increase in adipose tissue and loss of skeletal muscle associated with age decreases total oxygen consumption, not to be mistaken as a change in cellular metabolic rate (15). Importantly, simply measuring tissue hemodynamics in the absence of a stress may lead to erroneous conclusions regarding vascular responsiveness of tissues. This is epitomized in a study by Musch and colleagues where they did not observe gross differences in whole hindlimb or individual skeletal muscle blood flow at rest between young and aged subjects (36). However, during aerobic exercise, despite no difference in bulk hindlimb blood flow, there were significant differences both between and within muscle. This resulted in a relative under and overperfusion of many muscles comprised of primarily oxidative and glycolytic fiber types, respectively. This highlights two important factors; 1) that vascular structure and function largely adapt to the resting

condition and, 2) stresses to the cardiovascular system are required to determine how age and or pathological conditions may impair local vascular function and hemodynamics.

As stated above, in rats of a similar age and identical cancer model, McCullough et al. 2014 demonstrated a diminished myogenic response of tumor arterioles, albeit at higher intraluminal pressures than that which would have occurred with tilt from the current study. It is important to note the tumor microenvironment is contingent upon that of the host tissue (28), and the tumor resistance vasculature is a combination of co-opted mature and neo-vessels, with the former retain vascular responsiveness (50) and the latter demonstrating severely perturbed morphology (33). Therefore, it is necessary to assess the vasculature of the prostate itself, especially in an aged model (avg. age of prostate cancer ~66 yrs). With age, prostate hemodynamics are altered; for example, in animal models there is a significant decrease in prostate blood flow at rest (12). Delp and colleagues used radioactive microspheres to examine the effects of aging on central and regional hemodynamics. Importantly, they found no change in prostate tissue mass between juvenile, adult, and aged rats, however, they did observe a diminished prostate tissue blood flow between groups (24, 22, and 14 ml/min/100 g, respectively). Further, the percent of cardiac output to the prostate decreased with age (12). Importantly, these studies in animals preceded similar findings in humans (6), indicating the utility of preclinical animal models to assess age-associated changes in tissue perfusion in the human patient.

Ramsey and colleagues compared tissue blood flow in young and aged rats at level and HUT, and found that with old age there is an unchanged vascular conductance to several tissues with HUT, conjectured to be a result of diminished myogenic autoregulation and vasoconstrictive

responsiveness (39). Our second study divided prostate blood flow with old age and HUT from Ramsey et al. (2007) by MAP to determine old-age associated changes in vascular conductance within the prostate. Interestingly, with old age there was no change in prostate vascular conductance from level to HUT, whereas young animals demonstrated a significant decrease in conductance (Figure 5B). As stated previously, co-opted vessels from host tissue play a critical role in tumor blood flow. Considering the aged rat model demonstrated an inability to regulate vascular conductance in the HUT position, it can be postulated that vascular structure and function of the prostate is altered with age. Specifically, in young animals, with tilt the significant decrease in prostate vascular conductance (Figure 5B) and increase in vascular resistance indicates a strong local vasoconstriction resulting in a diminished perfusion. However, with old age the unchanged vascular conductance or resistance in the prostate with tilt indicates an impaired ability to augment vascular resistance to increased pressure observed with tilt (Figure 3A). Co-opted vessels from the host tissue are vital in supporting tumor growth (for review see (28)), therefore, it is reasonable to assume that an aged model of pre-clinical prostate cancer may demonstrate an augmented tumor blood flow during orthostatic stress. Unfortunately, there are no old-age models of prostate cancer in immunocompetent rat models currently available.

Limitations

There are several limitations that need to be addressed in the current study. The relative age of the animals and lack of old-age associated cardiovascular dysfunction limits the translation of these studies to the human patient. The sample size used was relatively small (n = 6). However, a paired analysis was used to increase the statistical strength of the study. It is

possible that, since blood flow was always measured first in the level position followed by the tilt position, that the some of the capillaries in the tumor would have been blocked by the first microsphere label and may not accurately reflect tissue blood flow during tilt. However, we injected a total of 500,000 microspheres (sum of both labels) into each animal, well below the threshold (>1 million) where hemodynamics are altered (51). Finally, we used a mild sedative (acepromazine) to 'calm' the animal while in the tilt apparatus. This was based upon previous experience with animals potentially spinning and occluding the indwelling catheters needed for injection of microspheres and reference sample collection. Although the dose of acepromazine used was at the lowest end of recommended dose, MAP was considerably lower in the sedated (Study I) versus non-sedated (Study II) animals. Given myogenic contractile dysfunction in tumor arterioles manifests at higher intraluminal pressures (30), the lower MAP in study I likely contributed to the lack of change with posture in tumor blood flow(52).

Conclusions

To conclude, study I demonstrated a lack of augmented tumor blood flow in response to orthostatic stress in a relatively young model of pre-clinical prostate cancer. Importantly, tumor vasculature adopts host arterioles while also developing its own immature, dysfunctional vessels via angiogenesis. Therefore, study II holds promising implications given the impact of age on host vasculature (prostate) which demonstrated an inability to reduce vascular conductance with HUT. Considering the average age of prostate cancer diagnoses (66 yrs), it can be postulated that with the advanced age of prostate cancer diagnoses, prostate vasculature would demonstrate similar dysfunction. Further, given the hydrostatic column within the rat is smaller than that of a human, it raises the question as to whether the increased distance from the heart to tumor in

humans would elicit a sufficient increase in tumor perfusion pressure and in turn increase blood flow. Finally, considering the multiple benefits of augmented blood flow for radiosensitivity stated previously, future studies should focus on developing an immunocompetent model of prostate cancer to further investigate the effect of age on prostate tumor vasculature and response of such vasculature to orthostatic stress.

Chapter 6 - References

1. **Alonzi R, Padhani AR, Maxwell RJ, Taylor NJ, Stirling JJ, Wilson JI, Collins DJ, Saunders MI and Hoskin PJ.** Carbogen breathing increases prostate cancer oxygenation: a translational MRI study in murine xenografts and humans. *Br.J.Cancer* 100: 4: 644, 2009.

2. **Baish JW, Gazit Y, Berk DA, Nozue M, Baxter LT and Jain RK.** Role of tumor vascular architecture in nutrient and drug delivery: an invasion percolation-based network model. *Microvasc.Res.* 51: 3: 327-346, 1996.

3. **Baluk P, Morikawa S, Haskell A, Mancuso M and McDonald DM.** Abnormalities of basement membrane on blood vessels and endothelial sprouts in tumors. 163: 5: 1801-1815, 2003.

4. **Bayliss WM.** On the local reactions of the arterial wall to changes of internal pressure. *J.Physiol.(Lond.)* 28: 3: 220-231, 1902.

5. **Behnke BJ, Prisby RD, Lesniewski LA, Donato AJ, Olin HM and Delp MD.** Influence of ageing and physical activity on vascular morphology in rat skeletal muscle. *J.Physiol.(Lond.)* 575: 2: 617-626, 2006.

6. **Berger AP, Horninger W, Bektic J, Pelzer A, Spranger R, Bartsch G and Frauscher F.** Vascular resistance in the prostate evaluated by colour Doppler ultrasonography: is benign prostatic hyperplasia a vascular disease? *BJU Int.* 98: 3: 587-590, 2006.

7. **Bertout JA, Patel SA and Simon MC.** The impact of O 2 availability on human cancer. 8: 12: 967, 2008.

8. **Booth J.** No title. 1977.

9. **Carmeliet P and Jain RK.** Angiogenesis in cancer and other diseases. 407: 6801: 249, 2000.

10. **Coleman MP, Quaresma M, Berrino F, Lutz J, De Angelis R, Capocaccia R, Baili P, Rachet B, Gatta G and Hakulinen T.** Cancer survival in five continents: a worldwide population-based study (CONCORD). 9: 8: 730-756, 2008.

11. **Copp SW, Hirai DM, Hageman KS, Poole DC and Musch TI.** Nitric oxide synthase inhibition during treadmill exercise reveals fiber-type specific vascular control in the rat hindlimb. 298: 2: R485, 2009.

12. **Delp MD, Evans MV and Duan C.** Effects of aging on cardiac output, regional blood flow, and body composition in Fischer-344 rats. *J.Appl.Physiol.* 85: 5: 1813-1822, 1998.

13. **Dewhirst MW, Cao Y and Moeller B.** Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. 8: 6: 425, 2008.

14. **Flaim SF, Nellis SH, Toggart EJ, Drexler H, Kanda K and Newman ED.** Multiple simultaneous determinations of hemodynamics and flow distribution in conscious rat. *J.Pharmacol.Methods* 11: 1: 1-39, 1984.

15. **Folkow B and Svanborg A.** Physiology of cardiovascular aging. *Physiol.Rev.* 73: 4: 725-764, 1993.

16. **Garcia E, Becker VG, McCullough DJ, Stabley JN, Gittemeier EM, Opoku-Acheampong AB, Sieman DW and Behnke BJ.** Blood flow responses to mild-intensity exercise in ectopic vs. orthotopic prostate tumors; dependence upon host tissue hemodynamics and vascular reactivity. *J.Appl.Physiol.* 121: 1: 15-24, 2016.

17. **Geary GG, Krause DN, Purdy RE and Duckles SP.** Simulated microgravity increases myogenic tone in rat cerebral arteries. *J.Appl.Physiol.* 85: 5: 1615-1621, 1998.

18. **Golin RM, Keil LC and Ganong WF.** Effect of head-up tilt on vasopressin secretion and arterial pressure in anesthetized rats. 54: 1: 42-48, 1991.

19. **Hales S.** *Statical essays.* 1733.

20. **Hanna M, Taylor C, Chen B, La H, Maraj J, Kilar C, Behnke B, Delp M and Muller-Delp J.** Structural remodeling of coronary resistance arteries: effects of age and exercise training (1079.24). 28: 1_supplement: 1079.24, 2014.

21. **Harvey W.** 1628. 2006.

22. **Hill L.** The influence of the force of gravity on the circulation of the blood. *J.Physiol.(Lond.)* 18: 1-2: 15-53, 1895.

23. **Hockel M and Vaupel P.** Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J.Natl.Cancer Inst.* 93: 4: 266-276, 2001.

24. **Isaacs JT, Heston WD, Weissman RM and Coffey DS.** Animal models of the hormone-sensitive and-insensitive prostatic adenocarcinomas, Dunning R-3327-H, R-3327-HI, and R-3327-AT. *Cancer Res.* 38: 11 Part 2: 4353-4359, 1978.

25. **Jain RK.** Determinants of tumor blood flow: a review. *Cancer Res.* 48: 10: 2641-2658, 1988.

26. **Konerding MA, Malkusch W, Klapthor B, Van Ackern C, Fait E, Hill SA, Parkins C, Chaplin DJ, Presta M and Denekamp J.** Evidence for characteristic vascular patterns in solid tumours: quantitative studies using corrosion casts. *Br.J.Cancer* 80: 5-6: 724, 1999.

27. **Li C, Shan S, Huang Q, Braun RD, Lanzen J, Hu K, Lin P and Dewhirst MW.** Initial stages of tumor cell-induced angiogenesis: evaluation via skin window chambers in rodent models. *J.Natl.Cancer Inst.* 92: 2: 143-147, 2000.

28. **Liotta LA and Kohn EC.** The microenvironment of the tumour–host interface. 411: 6835: 375, 2001.

29. **McCullough DJ, Nguyen LM, Siemann DW and Behnke BJ.** Effects of exercise training on tumor hypoxia and vascular function in the rodent preclinical orthotopic prostate cancer model. *J.Appl.Physiol.* 115: 12: 1846-1854, 2013.

30. **McCullough DJ, Stabley JN, Siemann DW and Behnke BJ.** Modulation of blood flow, hypoxia, and vascular function in orthotopic prostate tumors during exercise. 106: 4: 2014.

31. **McVeigh GE, Bratteli CW, Morgan DJ, Alinder CM, Glasser SP, Finkelstein SM and Cohn JN.** Age-related abnormalities in arterial compliance identified by pressure pulse contour analysis: aging and arterial compliance. 33: 6: 1392-1398, 1999.

32. **Meininger GA and Davis MJ.** Cellular mechanisms involved in the vascular myogenic response. 263: 3: H659, 1992.

33. **Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK and McDonald DM.** Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. 160: 3: 985-1000, 2002.

34. **Muller-Delp JM, Spier SA, Ramsey MW and Delp MD.** Aging impairs endothelium-dependent vasodilation in rat skeletal muscle arterioles. 283: 4: H1672, 2002.

35. **Musacchia XJ, Steffen JM and Dombrowski J.** Rat cardiovascular responses to whole body suspension: head-down and non-head-down tilt. *J.Appl.Physiol.* 73: 4: 1504-1509, 1992.

36. **Musch TI, Eklund KE, Hageman KS and Poole DC.** Altered regional blood flow responses to submaximal exercise in older rats. *J.Appl.Physiol.* 96: 1: 81-88, 2004.

37. **Musch TI, Friedman DB, Pitetti KH, Haidet GC, Stray-Gundersen J, Mitchell JH and Ordway GA.** Regional distribution of blood flow of dogs during graded dynamic exercise. *J.Appl.Physiol.* 63: 6: 2269-2277, 1987.

38. **Pries AR, Cornelissen AJ, Sloot AA, Hinkeldey M, Dreher MR, Höpfner M, Dewhirst MW and Secomb TW.** Structural adaptation and heterogeneity of normal and tumor microvascular networks. 5: 5: e1000394, 2009.

39. **Ramsey MW, Behnke BJ, Prisby RD and Delp MD.** Effects of aging on adipose resistance artery vasoconstriction: possible implications for orthostatic blood pressure regulation. *J.Appl.Physiol.* 103: 5: 1636-1643, 2007.

40. **Ribatti D.** William Harvey and the discovery of the circulation of the blood. 1: 1: 3, 2009.

41. **Riess ML, Connolly LA and Woehlck HJ.** Two millimeters per inch. *J.Clin.Anesth.* 21: 1: 72, 2009.

42. **Robinson SP, Howe FA, Stubbs M and Griffiths JR.** Effects of nicotinamide and carbogen on tumour oxygenation, blood flow, energetics and blood glucose levels. *Br.J.Cancer* 82: 12: 2007, 2000.

43. **Robinson SP, Howe FA and Griffiths JR.** Noninvasive monitoring of carbogen-induced changes in tumor blood flow and oxygenation by functional magnetic resonance imaging. 33: 4: 855-859, 1995.

44. **Rowell LB.** *Human cardiovascular control.* Oxford University Press, USA, 1993.

45. **Schubert R, Lidington D and Bolz S.** The emerging role of Ca2 sensitivity regulation in promoting myogenic vasoconstriction. *Cardiovasc.Res.* 77: 1: 8-18, 2008.

46. **Semenza GL.** Targeting HIF-1 for cancer therapy. 3: 10: 721, 2003.

47. **Shankar A, Loizidou M, Burnstock G and Taylor I.** Noradrenaline improves the tumour to normal blood flow ratio and drug delivery in a model of liver metastases. *Br.J.Surg.* 86: 4: 453-457, 1999.

48. **Siegel RL, Miller KD and Jemal A.** Cancer statistics, 2015. 65: 1: 5-29, 2015.

49. **Siemann DW.** *Tumor microenvironment.* John Wiley & Sons, 2011.

50. **Sonveaux P.** Provascular strategy: targeting functional adaptations of mature blood vessels in tumors to selectively influence the tumor vascular reactivity and improve cancer treatment. 86: 3: 300-313, 2008.

51. **Stanek KA, Smith TL, Murphy WR and Coleman TG.** Hemodynamic disturbances in the rat as a function of the number of microspheres injected. 245: 6: H923, 1983.

52. **Stanek KA, Smith TL, Murphy WR and Coleman TG.** Hemodynamic disturbances in the rat as a function of the number of microspheres injected. 245: 6: H923, 1983.

53. **Taylor NJ, Baddeley H, Goodchild KA, Powell ME, Thoumine M, Culver LA, Stirling JJ, Saunders MI, Hoskin PJ and Phillips H.** BOLD MRI of human tumor oxygenation during carbogen breathing. 14: 2: 156-163, 2001.

54. **Turaka A, Buyyounouski MK, Hanlon AL, Horwitz EM, Greenberg RE and Movsas B.** Hypoxic prostate/muscle PO2 ratio predicts for outcome in patients with localized prostate cancer: long-term results. 82: 3: e439, 2012.

55. **Vaupel P.** The role of hypoxia-induced factors in tumor progression. *Oncologist* 9: Supplement 5: 10-17, 2004.

56. **Vaupel P.** Tumor microenvironmental physiology and its implications for radiation oncology. 14: 198-206, 2004.

57. **Vaziri ND, Ding Y, Sangha DS and Purdy RE.** Upregulation of NOS by simulated microgravity, potential cause of orthostatic intolerance. *J.Appl.Physiol.* 89: 1: 338-344, 2000.

58. **Walsh JC, Lebedev A, Aten E, Madsen K, Marciano L and Kolb HC.** The clinical importance of assessing tumor hypoxia: relationship of tumor hypoxia to prognosis and therapeutic opportunities. 21: 10: 1516-1554, 2014.

59. **Wilkerson MK, Muller-Delp J, Colleran PN and Delp MD.** Effects of hindlimb unloading on rat cerebral, splenic, and mesenteric resistance artery morphology. *J.Appl.Physiol.* 87: 6: 2115-2121, 1999.

60. **Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ and Holash J.** Vascular-specific growth factors and blood vessel formation. 407: 6801: 242, 2000.

61. **Zlotecki RA, Baxter LT, Boucher Y and Jain RK.** Pharmacologic modification of tumor blood flow and interstitial fluid pressure in a human tumor xenograft: network analysis and mechanistic interpretation. *Microvasc.Res.* 50: 3: 429-443, 1995.