BLOOD FLOW RESPONSES TO MILD-INTENSITY EXERCISE IN ECTOPIC VERSUS
ORTHOTOPIC PROSTATE TUMORS; DEPENDENCE UPON HOST-TISSUE
HEMODYNAMICS AND VASCULAR REACTIVITY

by

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Abstract

Given the critical role of tumor O_2 delivery on patient prognosis and the rise in preclinical exercise-oncology studies, we investigated tumor and host-tissue blood flow at rest and during exercise as well as vascular reactivity using a rat prostate cancer model grown in two transplantation sites. **Methods**. In male COP/CrCrl rats, blood flow (via radiolabeled microspheres) to prostate tumors (R3327-MatLyLu cells injected in the left flank (ectopic) or ventral prostate (orthotopic)) and host-tissue was measured at rest and during a bout of mild-intensity exercise. Alpha-adrenergic vasoconstriction to norepinephrine (NE: 10^{-9} to 10^{-4} M) was determined in arterioles perforating the tumors and host-tissue. To determine host-tissue exercise hyperemia in healthy tissue, a sham-operated group was included. **Results**. Blood flow was lower at rest and during exercise in ectopic tumors and host-tissue (subcutaneous adipose) versus the orthotopic tumor and host-tissue (prostate). During exercise, blood flow to the ectopic tumor significantly decreased by $25 \pm 5\%$, whereas flow to the orthotopic tumor increased by $181 \pm 30\%$. Maximal vasoconstriction to NE was not different between arterioles from either tumor location. However, there was a significantly higher peak vasoconstriction to NE in subcutaneous adipose arterioles ($92 \pm 7\%$) versus prostate arterioles ($55 \pm 7\%$). Establishment of the tumor did not alter host-tissue blood flow from either location at rest or during exercise. **Conclusion**. These data demonstrate blood flow in tumors is dependent on host-tissue hemodynamics and that the location of the tumor may critically affect how exercise impacts the tumor microenvironment and treatment outcomes.

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Introduction

Ancient Egyptians described what historians and physiologists believe to be cancer as early as ~3,000 BC (13,24). When describing the physiology of cancer, Greek philosopher Hippocrates (460–375 BC) described what we know today as cancerous tumors as having crab-like movements in their spread throughout the body; hence when his works were translated into Latin they called this malady: *cancer,* translating to "crab" (32). Claudius Galen (131–200 AD), another physician who gained acclaim during his time, named what we now know as tumors: *oncos* (Greek for swelling), this term is now given to specialists who study cancer: Oncologist. Even as far back as Hippocrates' time, the "Father of Medicine" noticed that cancers most often happened in adults (32), this observation has been proven to hold true with the risk of developing cancer increasing greatly as people age (1).

All cancers begin when a gene mutation causes a protein that participates in the process of cell reproduction to change the rate of cell division and apoptosis. Cells divide, grow, and die in a process called the cell-cycle, a tightly regulated process with "checkpoints" to ensure normal cell functioning. Proper replication of DNA during the S phase of Interphase is one of the most important jobs of these checkpoints and ensures that daughter cells behave like the previous parent cell. However, even when all of the checkpoints function properly, a small percentage of replication errors (gene mutations) in the form of a DNA nucleotide sequence can still be passed to daughter cells. These mutations can be minor and result in changes in the cell that do no harm; nevertheless, the possible accumulation of these mutations with each subsequent generation of daughter cells can allow for the checkpoints between cell phases to decrease in their effectiveness, and eventually the pace of the cell cycle speeds up with no effective checkpoints attenuating the process.

When cells undergo a cancerous mutation, three main types of genes become affected: DNA repair genes, tumor suppressor genes, and proto-oncogenes. DNA repair genes are responsible for fixing any damaged DNA inside cells and any mutations in these genes usually means additional mutations in other genes will follow. Proto-oncogenes are normal genes that have the potential to become oncogenes and are involved in cell growth and division, mutations to these genes accommodate uncontrollable cell division and longer than normal cell life. Mutations in tumor suppressor genes allow for the uncontrollable cell division.

The uncontrolled division of the mutated cells coupled with the absent or belated apoptosis outpaces the normal cell-cycle of cells in the area resulting in an accumulation of cells called a neoplasm, or more commonly called: tumor. When these tumors grow slowly and do not spread onto adjacent or distant tissues they are said to be benign and non-cancerous thanks to various mechanisms that anchor the uncontrolled growth to the site of origin. Cancerous, malignant tumors are those that grow with the potential to spread to other parts of the body causing harm to the normal functions of various tissues and/or organs. While cancer has been found to start in almost all areas of the body, the cancer is named after the organ or tissues where the cancer first arose from i.e. prostate cancer first starts in cells from the prostate but can spread to other parts of the body causing harm elsewhere. To put it simply: cancer, a term used to describe more than 100 different diseases, is an accumulation of cell mutations that cause uncontrolled cell division and longer than normal cell life with the potential to spread to other parts of the body.

The spread of cancer, metastasis, happens when cancer cells dislodge from the original tumor and go to other parts of the body using the bloodstream and/or lymph system. Many cancers metastasize to the same regions in various cases due to the origin of the primary cancer.

The cancer cells that end up breaking free will get carried in the bloodstream or lymph until they attach somewhere "downstream"; explaining why many cancers frequently spread to the lungs. However, as is the case in advanced prostate cancer, sometimes the metastases of the cancer cannot be explained by the location of the primary tumor, e.g., advanced prostate cancer often spreads to bones before spreading to other organs in the body. This could be due to certain substances on the cancer cells that attach more readily to certain organs. Thus, understanding site-specific tumor-host interactions is critical to understanding the pathogenesis of advanced disease as well as developing useful therapeutic interventions.

Chapter 1 - Literature Review

The chance of an American man being diagnosed with prostate cancer is 1 in 7, with 60% of those diagnoses being in those over 65 years of age (1); and while only 1 in 39 men diagnosed with prostate cancer die from this disease, this disease still holds the second leading mortality rate amongst cancers in men, only behind lung cancer (1). While other cancers seem to have shown decreases in their incidence rates, prostate cancer incidence rates in the United States have remained relatively stable (1).

The prostate, a walnut-shaped gland located inferiorly to the bladder, is part of the male reproductive system, where its main function is to make a fluid that, along with sperm from the testicles and fluid from other glands, makes up semen. The muscles in the prostate are responsible for pushing semen out of the urethra during ejaculation. The prostate gland is made up of three different zones that go around the urethra in layers: the innermost layer, the transition zone surrounds the urethra and makes up ~10% of the total prostate mass; the middle layer, the central zone is where the ejaculatory duct is found and makes up ~25% of the total mass; the outermost layer is called the peripheral zone and accounts for the remaining mass (43). During a male's lifetime, the prostate gland experiences two growth periods. The first growth period happens during the early stages of puberty when the prostate doubles in size. The second growth period happens around the age of 25 and usually continues for most of a man's life. While benign prostatic hyperplasia usually occurs in the transition zone, prostate cancer usually develops in the peripheral zone (43).

The diagnosis for prostate cancer typically begins with a prostate-specific antigen (PSA) test taken from a blood sample and/or a digital rectal exam (DRE) where the doctor puts a gloved lubricated finger into the rectum and feels the prostate gland for any abnormalities, given that

prostate cancer presents itself with solid tumor growth. Both of these tests are not 100%

accurate, with research indicating that PSA levels in men are subject to many variables and often

leading to false-negatives and even false-positives (70). Prostate biopsies and urine tests are

often needed in conjunction for an accurate diagnosis.

Tumor Microenvironment

The blood vessels in the body are made and regulated by a balance between both pro- and

anti-angiogenic factors; therefore, the normal vasculature seen in the body is organized in a

hierarchal way with mature vessels that allow for perfusion of oxygen to all cells. Tumors that

have undergone angiogenesis have been known to contain irregular vascular networks (42,82),

which lead to impaired blood flow and inadequate O_2 delivery.

Tumors continue to grow by feeding off their host tissue until the point where the

division of cells on the outside of the tumor is balanced by the death of cells near the center of

the tumor (50). The typical size of a tumor before beginning angiogenesis has approximately 10^6

cells or a ~2 mm^3 tumor size (60). At this point the tumors do not have an adequate amount of

nutrients being supplied from the host tissue, so continued growth is not possible. These tumors

cause few problems in the body and it is not until they secrete angiogenic hormones that they

continue to spread and increase in size, with blood vessels in close proximity helping facilitate

angiogenesis (60). The steps involved in angiogenesis are complex, but they are initiated by the

breakdown of the basal lamina that surround capillaries. Endothelial cells from the capillaries

migrate and attach to the tumor through cell division followed by the formation of a new

basement membrane (60). After the primary blood supply has been established, new vessels can

be formed from existing ones at the tumor.

There are many tumors that have the potential to secrete growth factors necessary for angiogenesis. Basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), are growth factors that have been shown to have angiogenic properties (14,41). Current research indicates primary tumors secrete substances that actually inhibit angiogenesis around secondary metastases (49). With the possible removal of the primary tumor, stimulation for angiogenesis for the secondary metastases can occur (49).

The great overexpression of angiogenic agents leads to the development of very tortuous, hyper-permeable, and disorganized vascular networks in which the heterogeneity of arteries and venules is not seen. The resulting lymphatic vessels in the tumor are also dilated, permeable and often times discontinuous (73). Given the highly dysfunctional and immature vasculature seen in tumors, the ability to adequately deliver oxygen, nutrients, and remove waste products is severely reduced. The increased permeability of the blood vessels coupled with the irregularity of the new vasculature leads to hypoxia within the tumor (73).

Hypoxia

The development of tumor hypoxia modifies the composition and key signaling components of the tumor microenvironment (73), promotes the adoption of an aggressive tumor phenotype (28,80), affects tumor progression (58), and enhances tumor cell dissemination (22,86), all of which culminate in poor patient prognosis (33,34). Therefore, cancer treatment outcomes in solid tumors are highly dependent upon the tumor microenvironment and associated perfusion and local hemodynamics in and around the tumor (34,74). Given the undesirable consequences of hypoxia, approaches that can mitigate tumor hypoxia are of high clinical significance.

Blood Flow Distribution During Exercise

Dynamic exercise involving large muscle masses causes cardiac output and oxygen consumption to increase significantly (8,68). There is a rapid increase in cardiac output that is sustained by the contracting muscle acting as a peripheral pump to drive venous blood flow back to the right ventricle and maintain central venous pressure (68). In concurrence with the elevated cardiac output, during exercise there is an increase in mean arterial pressure, and a resetting of the baroreflex (67) such that a higher blood pressure is tolerated. Coinciding with the increased blood pressure and cardiac output, there is a redistribution of regional blood flow which is primarily regulated by resistance arterioles due to enhanced sympathetic nerve activity eliciting the release of the neurotransmitter norepinephrine (NE) which can bind to alpha-adrenergic receptors on the vascular smooth muscle, inducing a vasoconstriction in inactive tissues (6,56). This vasoconstriction increases vascular resistance in these tissues, thus 'shunting' blood flow to active tissues (23). The degree of vasoconstriction in these tissues depends largely upon autonomic innervation, alpha-adrenergic receptor density, and the magnitude of sympathetic outflow, which is non-uniform at the onset of exercise (10). Thus, the extent of vasoconstriction is tissue-specific (i.e., different responses in skin, muscle, and viscera (4,10)), and collectively 'shunt' blood flow to metabolically active tissues (e.g., heart, brain, bone, respiratory and skeletal muscles). Therefore, the ability to alter vascular resistance largely resides in the capacity of vascular smooth muscle to constrict (or dilate) when exposed to vasoactive stimuli and neurotransmitters. Given the vasodilation and vasoconstriction properties in both active and inactive tissue, respectively, blood flow levels can experience as much as a 1000% increase in the working skeletal muscle as well as a 90% decrease in inactive tissue.

Tumor Blood Flow During Exercise

The tumor vasculature often possesses a poorly developed medial layer lacking in functional smooth muscle (20) and contractile wall components (42). The pathophysiological consequences of these structural abnormalities include an impaired regulation of blood flow and heterogeneous blood flow patterns, which may result in impaired tissue oxygenation. McCullough et. al., previously demonstrated dysfunctional vasoconstriction within the arteries perforating orthotopic prostate tumors (53) which was associated with an enhanced blood flow during exercise (53). Analogous to Ohms law in response to exercise, an inability to increase tumor vascular resistance (due to dysfunctional arteriolar vasoconstriction) concomitant to the augmented cardiac output and systemic blood pressure (comparable to voltage) resulted in a substantial increase in tumor perfusion (current). This explanation assumed the majority of the tumor arterial vasculature (and associated resistance) was arranged in parallel with the prostate, and thus flow to the tumor would be relatively independent of flow or changes in vascular resistance in the host-tissue (i.e., prostate for the orthotopic model). However, the tumor vasculature is inexorably intertwined with the host tissue such that the vascular organization of vessels perfusing the tumor are in both series and in parallel arrangement (73) with those of the host.

Exercise Oncology

Although tumor O_2 delivery is critical in understanding the mechanistic bases of aggressiveness and therapeutic outcomes (e.g., chemotherapy requires tumor perfusion), there are sparse data on tumor hemodynamics during alterations in physical activity (at any intensity). This is due predominately to the technical difficulty of measuring conscious blood flow during exercise. Exercise has been shown to produce many health benefits including but not limited to

improvements in aerobic capacity, decrease in body fat, an increased effectiveness in the body's immune system capabilities (62), as well as decreased depression (19). Despite the many benefits of adopting a regular exercising routine, only ~10% of adults in the United States have been shown to engage in appropriate levels of physical activity (79). The National Cancer Institute has put out a bulletin recommending that cancer patients and survivors exercise regularly (2), however, with the current research available there is no clear consensus on the effects of either a single bout or a sustained exercise program on tumor blood flow, oxygenation, or responses to anticancer therapies. By understanding how exercise affects tumor perfusion and hypoxia, better exercise prescriptions can be made to ensure that patients diagnosed with cancer, or cancer survivors, get the correct amount of exercise to help them more effectively fight the disease.

Several investigators have reported that cancer mortality in all sites was inversely related to the amount of physical activity performed (30,61). With respect to prostatic cancer, the available research on the effects of exercise on prostate cancer is equivocal. A direct (47,63,78), inverse (3,15) or no effect (30,48,65) of exercise on risk of prostate cancer have been reported. The effect of exercise, after development of prostatic cancer, on tumor growth and perfusion also remains controversial. In subcutaneous tumor models exercise had either no effect (69) or increased tumor growth (36), and resulted in earlier appearances of tumors compared to sedentary groups (85). These studies therefore suggest that exercise may actually have negative effects on tumor progression.

Tumor Models

In preclinical models, the growth of injected tumor cells or heterotransplantation of tumor biopsies (xenograft) in rodents is a common method for determining tumorigenic potential and/or

therapeutic intervention efficacy *in vivo* for a number of cancer types. Tumor growth and therapeutic success are dependent, in part, upon the transplantation or injection site (57). Most transplantable tumors are placed ectopically and tumor cell lines injected subcutaneously, because of accessibility and lack of stress to the animal (71). The majority of preclinical exercise oncology studies in rodents also use ectopic (subcutaneous injections) tumor models, as summarized by Betof et al. (11). However, rodent skin has a very low blood flow (8), and ectopic tumors implanted subcutaneously may have a reduction in blood flow during exercise compared to rest, as previously suggested (53,54). Given the low blood flow to the skin at rest and during exercise in the rodent, it is possible that ectopic tumor models using this host tissue may become more hypoxic during exercise, adopt a more aggressive phenotype (80) and enhance tumor progression (59). Indeed, a recent study suggests that voluntary wheel running does not affect tumor growth when implanted orthotopically (37).

Orthotopic Prostate Model during Exercise

Orthotopic models, where the tumor is grown in the organ from which tumor cells originate (e.g., prostate tumor in prostate, breast tumor in mammary gland), have been shown to be better predictors of clinical success than ectopic models (12,40). Evidence suggests that exercise, in the orthotopic model, does not promote tumor growth and may protect against metastasis, which could be related to the effects of exercise on tumor blood flow or oxygenation (38,51).

Given that the tumor vasculature cannot increase its vascular resistance because of its impaired vasoconstriction to alpha-adrenergic agonists, we believe that the increase in blood pressure during exercise allows for the blood to use the tumor as the path of least reistance. Therefore, in the face of an increasing pressure during exercise, the tumor arterioles, which have

a diminished myogenic constriction, would have an increased diameter and based upon Poiseuille's law of fluid dynamics, mandate a greater blood flow to the tumor.

With the increased cardiac output and the inability of the tumor arterioles to vasoconstrict, there is an increased blood flow to the tumor. However, it is not the total blood flow that determines oxygenation, but the distribution of perfusion. Likely due to a combination of the enhanced flow and greater arterial pressure (which would increase transmural pressure), there is an increase in the vasculature within the tumor compared to rest, suggesting a more homogenous spatial distribution of blood flow in the tumor. The combination of the increased blood flow and augmented functional tumor vasculature result in a significant reduction in tumor hypoxia. Based on these dramatic changes in tumor blood flow, functional vasculature, and reduced hypoxia of orthotopic prostate cancer (53,54), the logical extension of these findings is to investigate whether it is possible to utilize physical exercise as a tool to enhance radiosensitivity and hence improve outcomes of radiotherapy.

Chapter 2 - Hypothesis

This study investigates the location effects of exercise on tumor blood flow, vascular reactivity, and host-tissue interactions in two-commonly used models of prostate cancer, ectopic and orthotopic. Specifically, the following hypotheses were tested: **1)** ectopic tumors (flank model) will demonstrate an attenuated exercise-hyperemic response versus those located orthotopically, which will be related to host-tissue hemodynamics, **2)** blood flow during exercise in the host-tissue of the ectopic tumor (skin and subcutaneous adipose) will decrease compared to values at rest, and **3)** there will be an enhanced vasoconstriction to the alpha-adrenergic agonist, NE in the host tissue of the ectopic versus orthotopic tumors. Furthermore, as there is evidence that the tumor cells themselves can impair smooth muscle function of the host vessels (40), it is possible that the tumor itself alters local host-tissue perfusion at rest and during exercise. Therefore, we performed an additional study in a sham group to compare the same host-tissue of the tumor-bearing groups both at rest and during exercise. Given the advancing field of exercise-oncology and the prescription of aerobic-exercise therapy in cancer patients, this study will establish several key facets of the tumor environment at rest and during exercise.

Chapter 3 - Methods

Animals

All procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University and the University of Florida 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). A total of 66 Copenhagen rats (immunocompetent) (COP/CrCrl; Charles River, Wilmington, MA) were investigated at ∼6 months of age for all three studies detailed below. The parental tumor from which the cell line is derived is the original Dunning R-3327 discovered in Copenhagen rats (35). The rats were housed at 23°C and maintained on a 12:12-h light-dark cycle and provided rat chow and water ad libitum.

Models of Prostate Cancer

The Dunning R3327-MatLyLu (MLL) rat prostate adenocarcinoma cell line (Sigma Aldrich, European Collection of Cell Cultures) was utilized in this study for both tumor locations in tumor-bearing (TB) groups. This cell line is a well-established model of prostate cancer (39) with a high metastatic potential, fast growth rate, and characteristics similar to progressive human prostate cancers (35). MLL cells were cultured in RPMI 1640 medium (supplemented with 2 mM glutamine, 250 nM dexamethasone, 10% fetal bovine serum, and 1% penicillin/streptomyocin; Sigma Aldrich) and maintained in a humidified incubator at 5% CO_2 at 37°C. At ∼80-90% confluence, viable cells were counted, and a tumor cell stock solution was prepared with physiological saline (PSS) and separated into aliquots of 0.1 ml containing ∼10^4 MLL cells each.

Under anesthesia (isoflurane, 2%/O_2 balance), cells were injected either ectopically or orthotopically. For the ectopic injection, using sterile insulin syringes (26 gauge), 0.1 ml of cell

stock solution (or 0.1 ml of PSS for sham rats) was injected subcutaneously into the left rear flank of the rat, superficial to the posterior (dorsal) end of the latissimus dorsi muscle, and the animal was then allowed to recover. For the orthotopic injection, the bladder and prostate complex were exposed and isolated through a small abdominal incision (<2 cm) lateral to the midline of the abdomen. Using sterile insulin syringes (26 gauge), 0.1 ml of cell stock solution (or 0.1 ml of PSS for sham rats) was injected into the ventral lobe of the prostate. Following the injection, closure of the abdominal wall (4-0, polyglycolic acid coated; DemeTECH, Miami Lakes, FL) and overlying skin/fascia (4-0 nylon monofilament; DemeTECH, Miami Lakes, FL) incisions were performed, and the animal was allowed to recover. All procedures were performed under aseptic conditions and buprenorphine (0.05 mg/kg, S.C.) was administered to control for postoperative pain. Postoperative monitoring of the animals was performed daily until experimental protocols were performed ~21 days post injection.

Protocol I: Tumor blood flow and vascular resistance during acute exercise.

Blood flow and vascular resistance in the ectopic tumor and surrounding tissue (i.e, skin and subcutaneous tissue) and in the orthotopic tumor and surrounding tissue (i.e., prostate and bladder) was determined in ectopic tumor-bearing (Ectopic TB; n=10) and orthotopic tumor-bearing (Ortho TB; n=10) rats at rest and during exercise using the radionuclide-tagged microsphere technique (46). Prior to surgical procedures, all rats were familiarized with treadmill exercise, during which they walked for 5 min/day for 5 days at a speed of 15 meters/min with no incline. These parameters provide a mild-exercise intensity for rats of this body mass and age (28). At least 24 hr after the last bout of the familiarization period, animals were anesthetized with isoflurane (2%/O_2 balance), and a catheter (Dow Corning, Silastic; inner diameter 0.6 mm, outer diameter 1.0 mm) filled with heparinized saline solution (100 U/ml,

Elkins-Sinn) was advanced into the ascending aorta via the right carotid artery. This catheter was used for infusion of radiolabeled microspheres for tissue blood flow measurements and for monitoring mean arterial pressure. The carotid catheter was externalized dorsally at the base of the neck and secured to the skin. A second polyurethane catheter (Braintree Scientific; inner diameter 0.36 mm; outer diameter 0.84 mm) was implanted in the caudal tail artery and externalized at the tail. The caudal artery catheter was used to obtain a reference blood sample, which serves as an artificial organ for calculating tissue flows. After the closure of incisions, the animals were given 2-4 hr to recover; circulatory dynamics, regional blood flow, arterial blood gases, and acid-base status are stable in the awake rat 1–6 h after anesthesia (25).

After the recovery period, the rat was placed on the treadmill, and the tail artery catheter was connected to a 1-ml plastic syringe that was connected to a Harvard infusion/withdrawal pump (model 907, Cambridge, MA). The carotid artery catheter was connected to a blood pressure transducer (BP100, ADInstruments). Exercise was initiated at 15 meters/min (no incline) which would correspond to an intensity of <50-60% of maximal aerobic capacity (28). We chose a mild intensity exercise vs. a more energetically demanding intensity as 1) the animals do not display any distress at this intensity and 2) high-intensity exercise has been suggested to enhance tumor metastases (18). After 5 min of total exercise time, blood withdrawal from the caudal artery at a rate of 0.25 ml/min was begun. The right carotid artery catheter was disconnected from the pressure transducer, and a specified radiolabeled (^{113}Sn or ^{57}Co) microsphere (15-µm diameter; PerkinElmer/NEN, Boston, MA) was infused (2.5–5.0 x 10^5 in number) into the ascending aorta and flushed with warmed saline to ensure clearance of the beads. Blood withdrawal from the caudal artery continued for 45 s after microsphere infusion. After a 60-min recovery period, a second microsphere infusion was performed with the same

procedures as described above for the resting condition. This strategy was utilized to minimize the pre-exercise anticipatory response (7) and facilitates an accurate "resting" measurement. Following the microsphere infusion, animals were euthanized with pentobarbital sodium (100 mg/kg ip), and the heart was removed to verify correct placement of the carotid catheter into the ascending aorta. The tumors and host tissues, as listed above, were removed as well as the kidneys (for determination of microsphere mixing; see below), visceral adipose (representing another adipose depot), and soleus muscle (to demonstrate locomotory exercise hyperemia). The radioactivity level of the tissues was determined by a gamma scintillation counter (Cobra II Auto Gamma Counter; Packard, Downer's Grove, IL) set to record the peak energy activity of each isotope for 5 min. Total blood flow to each tissue was calculated by the reference sample method (46) and expressed in milliliters per 100 g of tissue per minute. Vascular resistance was calculated (i.e., mean arterial pressure/blood flow) and expressed in millimeters of mercury per milliliter per 100 g per minute. To account for potential elevations in tumor flow during exercise that may be due to the exercise pressor response, vascular conductance was calculated as blood flow/mean arterial pressure and expressed in milliliters per 100 g per minute per millimeters of mercury. Tumor flow was divided by the host tissue flow under each condition to determine the relative perfusion ratio. Adequate mixing of the microspheres was verified by demonstrating a $\leq$ 15% difference in blood flows to the right and left kidneys. To be included in data analysis for paired comparison, adequate mixing of the microspheres had to be evidenced after infusions of microspheres both at rest and during exercise. Of the data collected in the 10 animals from each group, this a priori criterion in the same animal was met in six rats from the ectopic and four from the orthotopic groups.

Protocol II: Healthy-host tissue blood flow during acute exercise.

After studies measuring blood flow to the different tumor models and host-tissue of the tumor-bearing animals, a second study was undertaken to determine if host-tissue blood flow is influenced by the local tumor. Blood flow in anatomically similar tissues as used for implantation of the ectopic (skin and subcutaneous adipose) and orthotopic (prostate and bladder) tumor models was measured in sham-operated animals (Ectopic-Sham, n=10; Ortho-Sham, n=10) at rest and during exercise. The same methods (microsphere technique), procedures (acclimation period and exercise paradigm) and criteria (for adequate distribution) as detailed above for Protocol #1 were employed for Protocol #2. Of the data collected in the animals from each group, this a priori criterion in the same animal was met in five rats from the Ectopic-Sham and five from the Ortho- Sham groups.

Protocol III: Tumor and host-tissue vascular reactivity

In separate groups of rats, alpha-adrenergic vasoconstrictor responsiveness using the isolated microvessel technique (21,52,54) was measured in ectopic and orthotopic tumors of the tumor bearing rats (Ectopic-TB, n=6; Ortho-TB, n=6) and in the prostate, skin, and subcutaneous adipose tissue from a separate group of non-tumor bearing rats (n=14). As there were no differences in host-tissue blood flow between the TB and Sham animals (see Results from Protocol I and II, respectively), we chose to isolate microvessels from site-specific host-tissue of healthy rats to ensure data representative of host-tissue was analyzed. Animals were euthanized with pentobarbital sodium (>100 mg/kg i.p.) and the heart was removed. For the orthotopic tumors the prostate tumor tissue was excised and for the ectopic tumors the tumor and surrounding tissue were excised and placed in cold (4°C) physiological saline solution (PSS) containing the following (mM): 145.0 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 1.17 $MgSO_4$, 1.2 NaH_2PO_4, 5.0

glucose, 2.0 pyruvate, 0.02 EDTA, 3.0 MOPS buffer, and 1 g/100 ml BSA at pH 7.4. Host

tissues (prostate, skin and subcutaneous adipose adjacent to tumor cell injection site) for the two

tumor models were excised and placed in cold PSS as described above. Resistance arterioles

($<200\,\mu m$; defined as the first branch of the feed artery perforating the tumors or host tissues)

were isolated with the aid of a dissecting microscope (Olympus SVH12), cleared of surrounding

tissue and placed in Lucite chambers containing cold PSS equilibrated to room air. The arterioles

were cannulated on both ends to glass micropipettes and secured with ophthalmic nylon suture

 (Alcon 11–0). After cannulation, the chambers were transferred to the stage of an inverted

microscope (Olympus IX70), equipped with a video camera (Panasonic BP310) and video

caliper (Colorado Video) for recording luminal diameter. Given no significant hydrostatic

gradients would be expected based upon the location of the tissues used herein, intraluminal

pressure was set at 90 cmH$_2$O with two independent hydrostatic pressure reservoirs in all

arterioles. This intraluminal pressure is equivalent to that used in previous in vitro studies using

arterioles from these sites (52,54). Leaks were detected by pressurizing the vessel and

determining whether vessel diameter was maintained. Vessels that exhibited leaks were

discarded. Vessels free of leaks were warmed to 37°C and allowed to develop spontaneous tone

during a 60 min equilibration period. To evaluate vasoconstrictor responsiveness, arterioles were

exposed to cumulative additions of the alpha-adrenoreceptor agonist NE (10^{-9} to 10^{-4} M).

Diameter was continuously recorded for 5 min at each dose of NE. After the final dose of NE,

the vessels were incubated at 37°C for 60 min in Ca^{2+} -free PSS containing 100 μM sodium

nitroprusside to determine maximal diameter and wall thickness.

Intraluminal diameter was measured in response to NE and expressed as a percentage of

vasoconstrictor response according to the following equation:

Vasoconstriction (% maximal response) = $(D_b – D_s) / D_b * 100$

Where D_s is the steady-state inner diameter recorded after addition of agonist, D_b is the initial baseline inner diameter before the first addition of a NE. Spontaneous tone was expressed as a percentage of maximal diameter as follows:

Spontaneous tone (%) = $(D_{max} – D_b) / D_{max} * 100$

Where D_{max} is the maximal intraluminal diameter obtained in Ca^{2+}-free PSS Comparison of data as a percentage of the maximal response normalizes for potential differences in maximal diameter or spontaneous tone among vessels. Sensitivity: The concentration that produced 50% of the maximal vasoconstriction to the agonist was designated as the EC_{50}.

Data Analysis

Dose-response curves and blood flow responses were analyzed by two-way ANOVA with repeated measures to detect differences between (experimental groups) and within (concentration, blood flow, arterial pressure, oxygen delivery) factors. Post hoc analyses were performed using Duncan's multiple range test. Vascular sensitivity, the concentration of NE exhibiting EC_{50}, was determined by logarithmic curve-fitting equations. A one-way ANOVA was performed to determine the significance of differences among groups in vessel characteristics, body mass, tumor mass and tumor mass/body mass ratio. A one-sample t-test was used to determine if the change in blood flow (delta) from rest to exercise for a given tissue was different from zero. All values are presented as means ± SE. $P \leq 0.05$ was required for significance. When a trend was observed (i.e., $P<0.1$ but greater than 0.05) the exact P-value was reported.

Chapter 4 - Results

There was no significant difference in body mass between the Ortho-TB (320 ± 11 g),
Ectopic-TB (329 ± 11 g), Ortho-Sham (338 ± 19 g) and Ectopic-Sham (336 ± 19 g) groups. The
tumors from the Ectopic-TB group (3.49 ± 0.32 g) were larger than those from the Ortho-TB
group (2.49 ± 0.23 g; P≤0.05). The tumor mass (mg):body mass (g) ratio was greater in the
Ectopic-TB (10.6 ± 0.9 mg/g) compared to the Ortho-TB group (7.8 ± 0.7 mg/g; P≤0.05).

Study I. Tumor and host-tissue hemodynamics at rest and during exercise

There were no differences in blood pressure measured at rest between tumor-bearing
groups (Table 1). Mean arterial pressure during exercise was significantly increased above
resting values in all animals, with no differences between groups (Table 1). Exercise nearly
doubled soleus muscle blood flow (Table 1) and reduced kidney blood flow by ~ 25% (Table 1)
in all groups, with no differences in these variables between groups. Mass specific blood flow
was lower to the ectopic tumor both at rest and during exercise (Figure 1A) versus the orthotopic
tumor (P≤0.05). In response to exercise, there was a ~24% reduction in blood flow to the ectopic
tumors versus rest, whereas flow increased by ~180% in the orthotopic tumors from rest to
exercise (Figure 1B; P≤0.05). Vascular conductance tended to be lower at rest (trend, P=0.06)
and during exercise (P≤0.05) in the ectopic versus orthotopic tumors (Figure 2). In response to
exercise, vascular conductance decreased in the ectopic tumors and increased significantly in the
orthotopic tumors compared to that at rest (Figure 2; P≤0.05).

Host-Tissue and regional perfusion in tumor bearing groups

From our dissections it was apparent that the ectopic tumor was located within the
subcutaneous adipose tissue with no focal attachments to the skin, and the orthotopic tumor was
located within and on the prostate with no adhesions to the bladder. Therefore, the skin and

bladder are reported to reflect regional organ perfusion and not the specific host-tissue for the tumor. The prostate had the highest blood flow at rest of all tissues (Figure 3A), which was over double that of the subcutaneous adipose tissue (Figure 3B). The skin had the lowest flow at rest of all tissues (Figure 3B). In response to exercise, there was not a significant change in blood flow to the prostate or bladder (Figure 3A and Figure 4) versus that measured at rest. Conversely, during exercise blood flow was reduced significantly in both the subcutaneous adipose tissue and skin versus rest (Figures 3B and 4; P≤0.05); however, the reduction in flow with exercise was significantly greater in the subcutaneous adipose versus the skin (Figure 4). When comparing the relative perfusion ratio (i.e., tumor flow/host-tissue flow), even with a greater perfusion to the orthotopic versus ectopic tumor at rest (Figure 1), due to the much higher prostate versus subcutaneous adipose flows (Figure 3A and 3B) the tumor: host-tissue flow ratio was lower in the orthotopic (0.50 ± 0.05) versus ectopic (0.88 ± 0.07; P≤0.05) model. During exercise, in the ectopic model the ratio increased to 1.93 ± 0.30, which was due to the greater decrease in flow to the host-tissue (i.e., subcutaneous adipose; Figure 4) versus the reduction in flow to the ectopic tumor (Figure 1B). A similar increase in the perfusion ratio was observed in the orthotopic model during exercise (1.43 ± 0.17; no significant between tumor effects found), which was due to the substantial increase in flow to the orthotopic tumor (Figure 1B), with no significant change in flow to the prostate (Figure 3A and 4). To determine changes in active vasoconstriction to exercise, vascular resistance (arterial pressure/ blood flow) was calculated in host-tissue of the tumor bearing animals. Both the host- tissue for the ectopic (subcutaneous adipose) and orthotopic (prostate) tumors showed an active vasoconstriction in response to exercise with significant elevations in vascular resistance. Specifically, in response to exercise, vascular resistance in subcutaneous adipose increased ~ 270% (rest, 8.2 ± 0.6 vs. exercise, 28.2 ± 4.2

mmHg/ml/100 g/min; P≤0.05) whereas there was a 20% increase in the prostate (rest, 3.6 ± 0.2 vs. exercise, 4.3 ± 0.1 mmHg/ml/100 g/min; P≤0.05). Vascular resistance was higher both at rest and during exercise in the subcutaneous adipose tissue compared to the prostate tissue (P≤0.05).

Study II. Influence of the tumor on host-tissue hemodynamics

To determine if the host- tissue flows from the tumor-bearing animals at rest and during exercise were an artifact of the tumor itself, blood flow measures were repeated in sham-operated groups of tissue representing both the ectopic and orthotopic tumor locations. There were no differences in blood flow to anatomically similar tissue of the sham-operated groups compared to the host tissue of tumor- bearing groups at rest or during exercise as shown in Table 1. There were also no differences in mean arterial pressure, or in renal and visceral adipose tissue flow between sham and tumor- bearing groups at rest or during exercise (Table 1). Surprisingly, despite no difference in body weight or exercise regimen, there was a trend for a greater mass-specific soleus muscle blood flow during exercise in the Sham groups (P=0.07 for Ortho-Sham; P=0.06 for Ectopic-Sham) versus corresponding tumor-bearing groups (Table 1).

Study III. Tumor and host-tissue vasoreactivity

Blood vessel characteristics are reported in Table 2. Spontaneous tone and the wall: lumen ratio was lower in arterioles from both the ectopic and orthotopic tumor versus that in all other tissue measured herein (Table 2). To determine vasoactive responsiveness in tumor and host-tissue arterioles, vasoconstriction was assessed in isolated arterioles from the tumor-bearing animals and from host-tissue in healthy animals. There were no differences in vasoconstriction to cumulative doses of the alpha-adrenergic receptor agonist NE in arterioles from the ectopic versus orthotopic tumors (Figure 5A), though the dose response was severely diminished versus host-tissue (Figure 5B). Maximal constriction to NE was $8.6 \pm 1.5\%$ and $13.2 \pm 3.9\%$ in the

arterioles from the orthotopic and ectopic tumors, respectively (P>0.05 between groups). In host tissue, there was a significantly greater vasoconstriction to NE in the arterioles from the subcutaneous adipose tissue than in those from the skin and prostate (Figure 5B). There was no difference in vasoconstriction between the skin and the prostate (Figure 5B). All host-tissue demonstrated a significantly enhanced (P≤0.05) vasoconstriction to NE versus the tumors from either location (Figure 5A versus 5B). There were no differences in sensitivity to NE between groups (Table 2).

Blood Flow	Ortho-Sham (n=5)		Ortho-TB (n=4)	
(ml/100 g/min	Rest	Exercise	Rest	Exercise
Bladder	17.8 ± 1.2	17.2 ± 1.2	18.3 ± 2.0	17.8 ± 1.1
Prostate	29.4 ± 2.4	28.2 ± 2.5	33.0 ± 2.1	31.8 ± 1.3
Soleus	102.2 ± 14.7	190.3 ± 14*	100.5 ± 15.1	176.4 ± 10.3*
Kidneys	615 ± 25	449 ± 25*	597 ± 32	488 ± 29*
MAP (mmHg)	114 ± 6	137 ± 6*	118 ± 9	136 ± 13*
	Ectopic-Sham (n=5)		**Ectopic-TB (n=6)**	
Blood Flow	Rest	Exercise	Rest	Exercise
(ml/100 g/min)				
Skin	9.3 ± 0.7	7.6 ± 0.4*	10.2 ± 0.5	7.9 ± 0.4*
Subcutaneous Adipose	13.2 ± 1.1	4.7 ± 0.4*	14.6 ± 1.2	5.1 ± 0.7*
Visceral Adipose	11.4 ± 0.6	5.3 ± 0.9*	12.2 ± 1.2	4.4 ± 0.7*
Soleus	92.4 ± 6.2	186.2 ± 7.8*	91.5 ± 8.6	171.8 ± 4.0*
Kidneys	566 ± 22	431 ± 23*	550 ± 48	389 ± 38*
MAP (mmHg)	111 ± 4.2	130 ± 7*	116 ± 4	129 ± 6*

Table 1. Host-tissue, renal, skeletal muscle blood flow and mean arterial pressure at rest and during exercise in tumor bearing and sham-operated animals

*P ≤ 0.05 versus rest for same tissue. There was a trend for a greater mass-specific soleus muscle blood flow during exercise in the Sham groups (P=0.07 for Ortho-Sham; P=0.06 for Ectopic-Sham) versus corresponding tumor-bearing groups

	TB-Groups	Sham-Operated Groups	
	Ectopic Tumor (n= 6)	Subcutaneous Adipose (n= 6)	Skin (n=6)
Maximal Diameter (μm)	165 + 12	180 + 12	130 + 10 *
Wall Thickness (μm)	12.7 ± 0.9	27.4 ± 1.3 *	24.0 ± 2.0*
Wall-to-Lumen Ratio	0.08 ± 0.01	0.15 ± 0.01*	0.19± 0.02*#
Tone (%)	11.2 ± 1.3	24.7 ± 1.3*	29.0 ± 6.2*
$EC_{50}([M])$	4.7 ± 1.9(e^{-7})	5.9 ± 2.6(e^{-7})	4.5 ± 3.5(e^{-6})
	Orthotopic Tumor (n=6)	Prostate (n=6)	
Maximal Diameter (μm)	158 ± 13	181 ± 12	
Wall Thickness (μm)	12.0 ± 1.0	18.8 ± 1.0*	
Wall-to-Lumen Ratio	0.07 ± 0.01	0.11 ± 0.01*	
Tone (%)	9.1 ± 0.8	23.9 ± 4.9*	
$EC_{50}([M])$	8.0 ± 5.0(e^{-7})	1.3 ± 1.1(e^{-6})	

Table 2. Isolated Arteriole Characteristics

*$P \leq 0.05$ versus measurement in corresponding tumor (i.e., orthotopic tumor for prostate and ectopic tumor for subcutaneous adipose and skin).
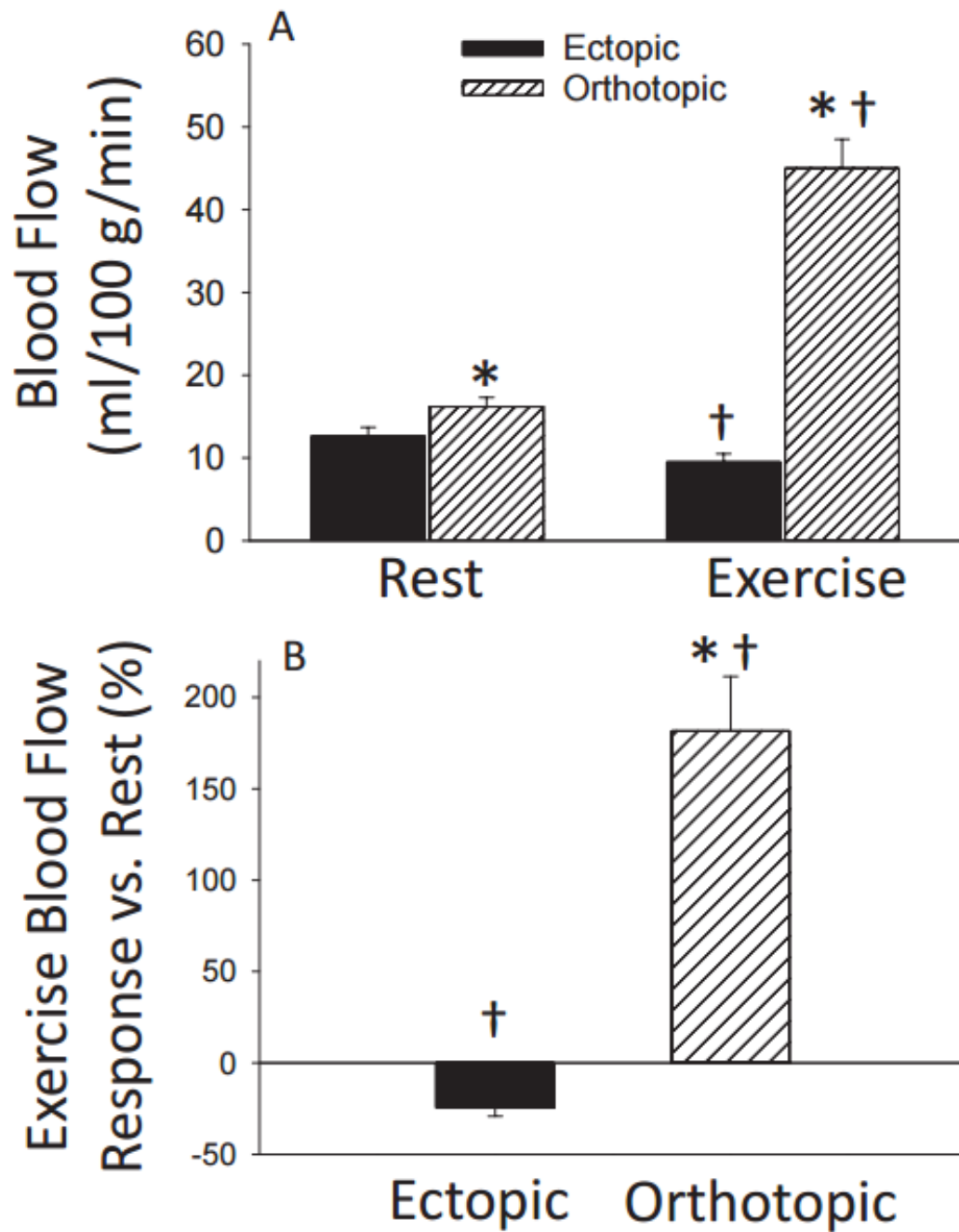#$P=0.07$ versus measurement in subcutaneous adipose.

Figure 1. Ectopic and orthotopic tumor blood flow measured at rest and during exercise (A) and the change in tumor flow during exercise compared to rest (B).

*$P \leq 0.05$ versus ectopic tumor for same condition.
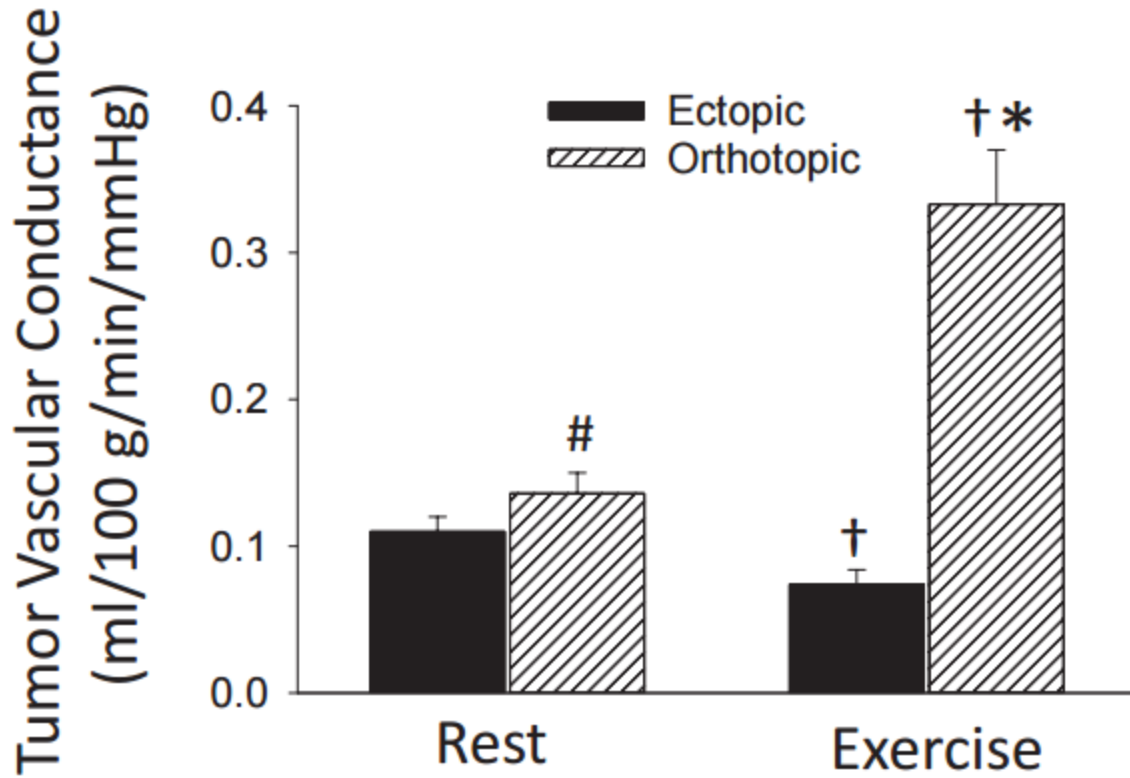†$P \leq 0.05$ versus resting value in same tumor.

Figure 2. Vascular conductance (blood flow normalized to the change in blood pressure) in the ectopic and orthotopic tumor at rest and during exercise.

*$P \leq 0.05$ between tumors during exercise.
†$P \leq 0.05$ versus rest for the same tumor.
#$P=0.06$ between tumors at rest.
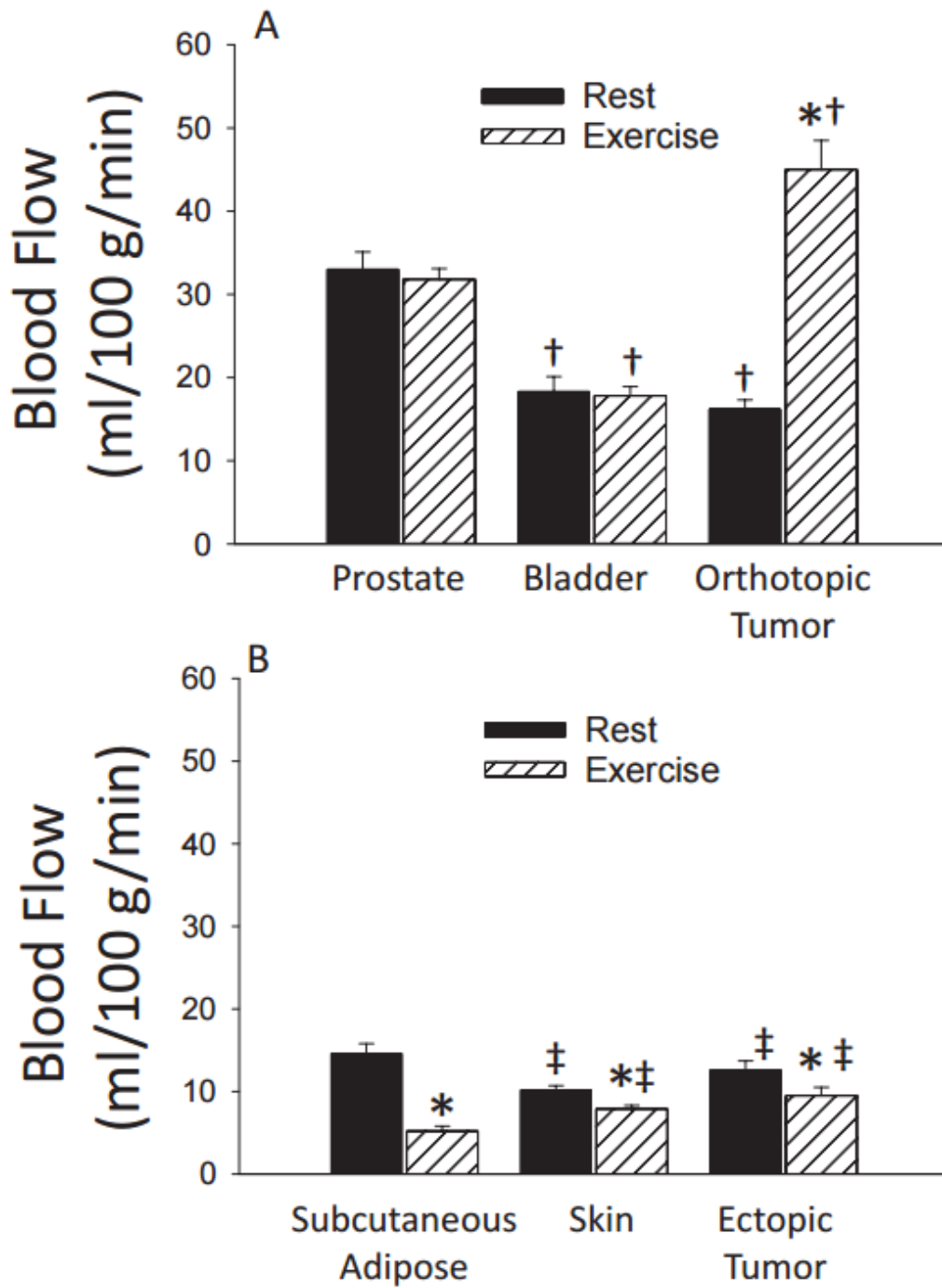
Figure 3. Blood flow measured at rest and during exercise to the host-tissue and regional organs of orthotopic (A) and ectopic (B) tumor-bearing animals.

*$P \leq 0.05$ rest for same tissue.
†$P \leq 0.05$ versus prostate for same condition (i.e., rest or exercise).
‡$P \leq 0.05$ versus subcutaneous adipose for same condition (i.e., rest or exercise).
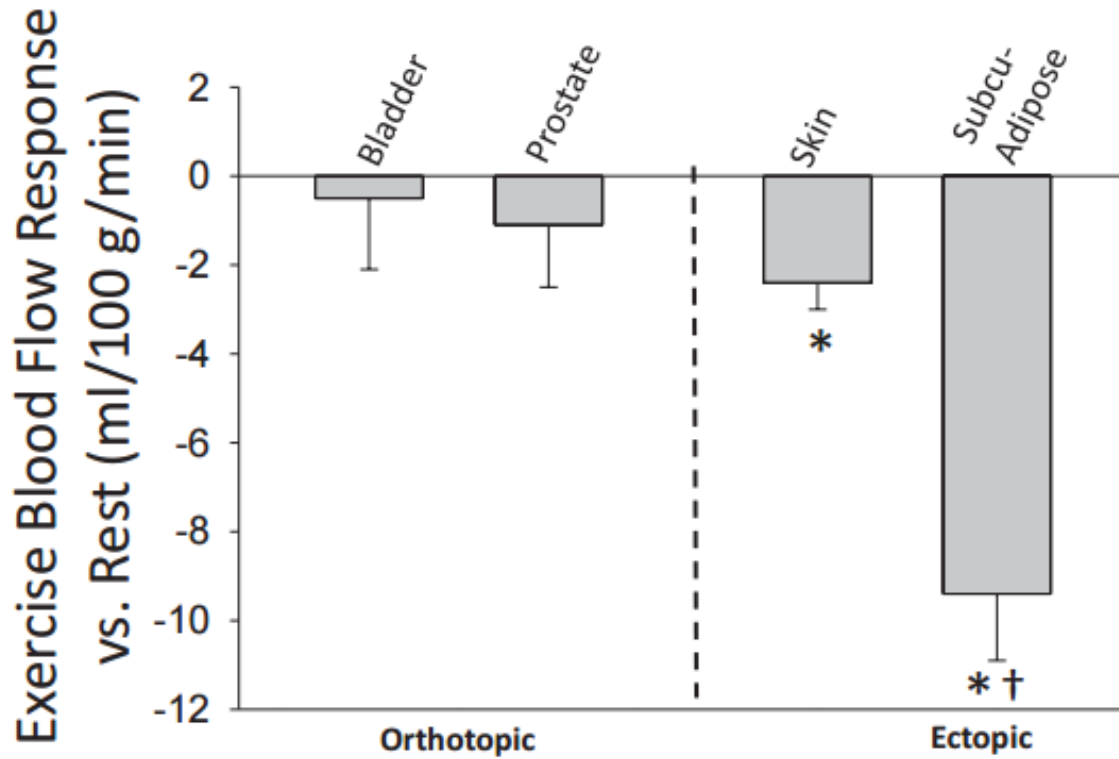
Figure 4. Changes in host-tissue and regional organ blood flow during exercise compared to rest (i.e., value at exercise minus value at rest).

*P≤0.05 versus rest (i.e., horizontal asymptote on figure).
† P≤0.05 versus skin.
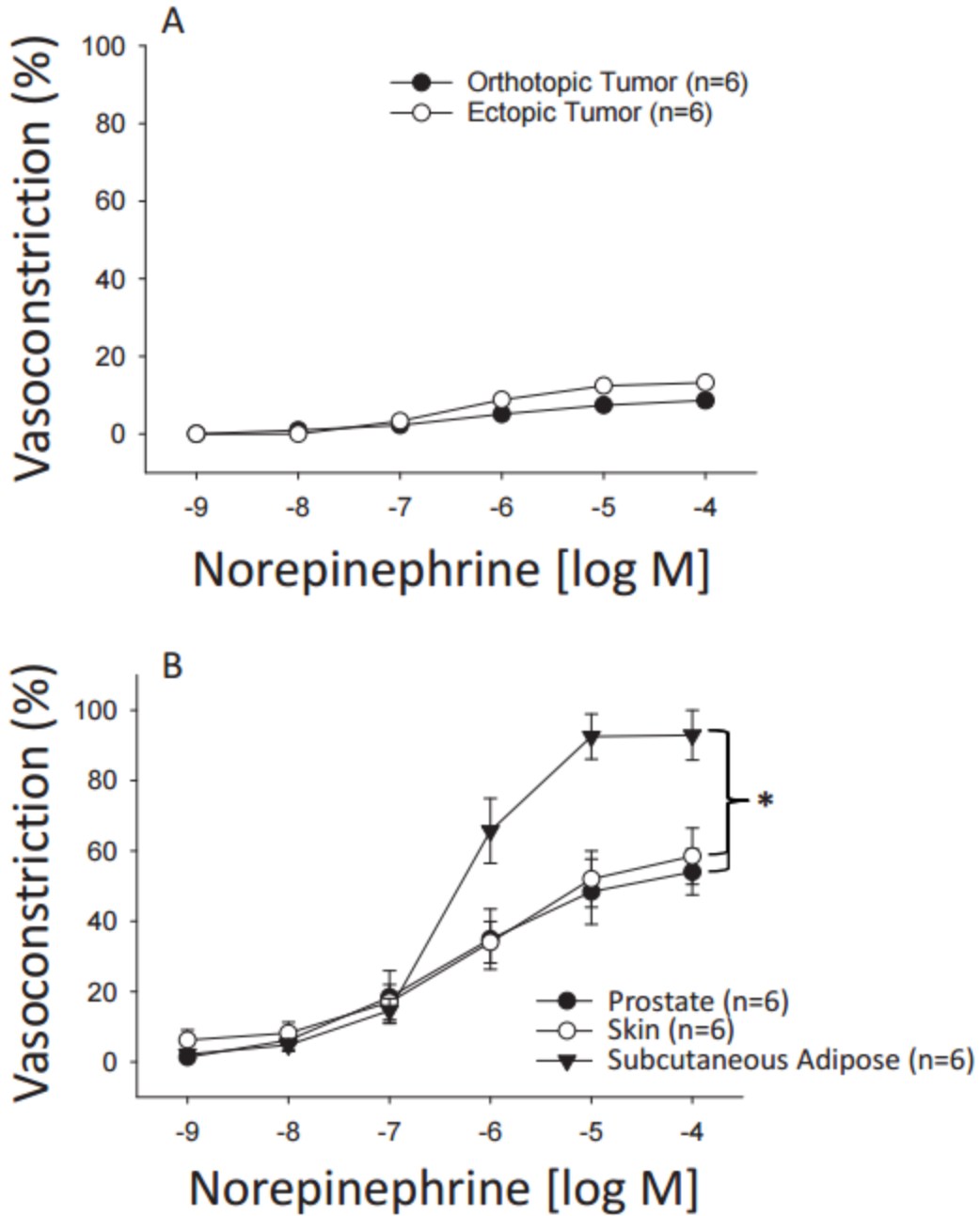
Figure 5. Dose-response relations to alpha-adrenergic receptor agonist NE in arterioles from (A) ectopic and orthotopic tumors and (B) prostate, skin, and subcutaneous adipose tissue from healthy animals (Sham).

*P≤0.05 versus subcutaneous adipose.

Chapter 5 - Discussion

There were several novel findings from these sets of studies including, 1) in response to mild exercise, there was a directionally opposed blood flow response in the orthotopic versus ectopic tumors; 2) during the steady-state of exercise, blood flow was ~4-fold greater in the orthotopic vs. ectopic tumors; 3) with exercise, host-tissue blood flow remained unchanged in the prostate (orthotopic host) but was significantly reduced in the subcutaneous adipose (ectopic host) relative to resting values; 4) in the tumor-bearing groups, there was no difference in tumor host-tissue blood flow at rest or during exercise compared to anatomically equivalent tissue in healthy, non-tumor bearing (sham) animals; 5) in tumor arterioles from both tumor groups, vasoconstriction to NE was severely diminished; and 6) there was a more robust vasoconstriction to NE in arterioles from the ectopic tumor host-tissue (i.e., subcutaneous adipose) versus orthotopic tumor host-tissue (prostate). Collectively, these data demonstrate a profound location-dependent difference in tumor perfusion and oxygen delivery both at rest and during exercise. Two other important findings include a larger ectopic versus orthotopic tumor size (despite the same number of cells injected) and the trend for a lower soleus muscle blood flow at the same exercise intensity in the tumor-bearing groups compared to that found in healthy animals.

Tumor Blood Flow Responses at Rest and During Exercise

After doing extensive literature searches, there are no other studies investigating how tumor location or host- tissue affects tumor flow to any type, duration, or intensity of exercise in any cancer type. Despite a lower intensity of exercise used herein, the current data extend those of McCullough et. al. (54) by demonstrating a net hyperemic response (increased flow above resting values) to exercise in the orthotopic prostate tumor (Figure 1A). Contrary to the hypothesis, there was no net hyperemic response in the ectopic tumors. In fact, there was not

only a lower flow at rest but also a directionally opposed flow response to exercise in the ectopic versus orthotopic tumor. With respect to O_2 delivery, these data suggest that the tumor in the ectopic location and tissue (subcutaneous adipose) will experience a substantially lower O_2 delivery at rest and during exercise.

Tumor Host-Tissue Blood Flow Relationship

The majority of perforating arteries to the tumor branch from large arteries (i.e., conduit and large feed arteries) that also supply the host-tissue. Although the majority of vascular resistance occurs within the arterioles of $\leq$ 150 μm luminal diameter (17) (i.e., resistance vasculature), larger feed arteries can contribute a significant portion of vascular resistance (84). If tumor flow was determined solely from tumor arteriolar properties, one would expect a similar flow response to exercise regardless of tumor location as arterioles from both tumor models displayed similar vascular dysfunction (Figure 5A) and structural properties (i.e., diminished wall-to-lumen ratio; Table 2). However, a directionally opposite blood flow response to exercise occurred between tumors (Figure 1B), suggesting tumor perfusion must be regulated, in part, by the vascular reactivity of the host-tissue. Indeed, systemic infusions of norepinephrine elicit large changes in the relative perfusion ratio (i.e., tumor/host-tissue flow; which varies across host-location (66,72,87), suggesting a substantial portion of tumor flow is dependent upon host-tissue vascular reactivity. It is important to note that, even though a large percentage of tumor flow at rest and during exercise must be dependent upon the host tissue, both tumors did display some independent control of blood flow relative to the host. Specifically, despite substantial differences in the magnitude of blood flow, both tumors displayed a relative under- and over-perfusion at rest and during exercise, respectively, versus their host (Figure 1B versus Figure 4). Even though flow decreased to the ectopic tumor during exercise compared to rest (Figure

1A&B), the reduction in subcutaneous adipose was much greater resulting a relative perfusion ratio quantitatively similar to that of the orthotopic tumor. This demonstrates that, even in the face of large regional changes in blood flow during exercise, the tumor displays some independent regulation of blood flow, although this is likely due to vascular dysfunction (Figure 5A) versus a coordinated change in vascular tone occurring in healthy tissue.

Tumor blood flow during exercise was clearly linked to that of the host in which the tumor was located. Despite some evidence that tumor cells can inhibit host vascular smooth muscle function (55), we did not see any differences in host-tissue blood flow at rest or during exercise in the tumor-bearing versus sham-operated groups (Table 1). Therefore, the function and vasoactive properties of the tumor host appear to be important variables in determining how tumor perfusion may change with exercise. The physiological functions of the prostate are primarily controlled through the autonomic nervous system, with innervation from both the parasympathetic and sympathetic branches (83). There are few data on prostate perfusion responses to exercise, but none that demonstrate it increases above rest. Our results demonstrate an unchanged prostate blood flow during mild (Table 1) or moderate-intensity (54) exercise. We are unaware of any data on prostate blood flow during strenuous exercise, but there is evidence of reduced blood flow to some reproductive tissue under such conditions (e.g., testes (26)). Therefore, it is possible that a more intense exercise regimen may induce a regional vasoconstriction to the prostate, potentially reducing flow to the orthotopic tumor, although this is currently unknown. In stark contrast to the prostate, during mild-exercise there is a reduction in blood flow to subcutaneous adipose tissue (Figure 3B and 4). This is consistent with other studies showing an initial decrease in adipose tissue blood flow to exercise in rats (21,45), thought to contribute to the redistribution of cardiac output to supply the working skeletal muscle

(21). During shorter- duration exercise (i.e., 30 min) low-to- moderate intensity exercise in human adipose tissue blood flow generally increases 1-4 fold over resting values, although this increase can take several hours (16). In contrast, inguinal adipose tissue blood flow in the rat was lower after ~ 1 hr of mild-intensity exercise versus pre-exercise values (44). Therefore, adipose tissue blood flow responses to exercise appear to be location-, time-, and species-dependent. Similar to the prostate, there is significant sympathetic innervation of white adipose tissue (31,75) with adipose tissue blood flow being regulated predominately by beta-adrenergic vasodilation (5) and alpha-adrenergic vasoconstriction (29). In response to NE, we observed a robust vasoconstriction in adipose tissue arterioles, with a maximal constriction significantly greater than size-matched arterioles from the prostate. Therefore, for a given sympathetic nervous outflow, a greater vasoconstriction and reduction in blood flow would be expected in the adipose versus prostate tissue, consistent with the much greater increase in vascular resistance and change in blood flow what occurs in the subcutaneous adipose versus prostate with exercise (Figure 3A&3B), demonstrating substantial differences in the tumor host perfusion to these conditions.

Blood Flow: Rodent Versus Human Prostate Cancer

Blood flow was measured at rest and during exercise with the radiolabeled microsphere technique as it remains the standard for quantifying blood flow in vivo (64). Healthy prostate tissue flows found herein (Table 1) are similar to those found in human prostate (mean flow 21 ml/100 g/min (81)). The data demonstrates a lower tumor flow versus the prostate at rest (Figure 4). However, in human prostate tumors, blood flow (quantified predominately by MRI and CT) is higher, and tumor oxygenation lower, than healthy prostate (host) (81). The likely explanation for this paradox (i.e., higher bulk blood flow yet lower oxygenation in the tumors vs. host) is the

large anastomoses present in many solid tumors (77) that would effectively shunt oxygenated blood past the tumor microcirculation where gas exchange occurs. As for the discrepancy between blood flow to the human prostate tumor and the orthotopic tumor reported currently, it is likely due to the techniques employed. Specifically, 15 µm radiolabeled microspheres, which are slightly larger in diameter than red blood cells, to measure blood flow. Thus, these microspheres would only lodge in the terminal branches of the microcirculation (i.e., terminal arterioles and capillaries), and would flow through anastomoses within the tumor and would not be counted. Therefore, flows reported herein reflect that to functionally essential (with respect to gas exchange) blood vessels, and would underestimate total blood flow (i.e., micro- plus macro-circulation) to a tumor with significant anastomoses. Support for this reasoning comes from tumor oxygenation studies between human and rat orthotopic prostate tumors in which, despite potential differences in total flow (human) and microcirculatory flow (rat), similar oxygenation values are found. Specifically, the partial pressure of oxygen (P_{O2}) measured at rest is ~ 6 mmHg in the rat orthotopic tumor (52), which is identical to the mean value in human prostate tumors (81), despite different measurement techniques. Collectively, these data support the ability of this orthotopic prostate cancer model in the rat to recapitulate the perfusion and oxygenation environment found within human prostate tumors.

Chapter 6 - Conclusions

Exercise-oncology is a rapidly developing field with many studies using preclinical animal models to characterize treatment outcomes and alterations in the tumor microenvironment, with the majority of studies using ectopic models (11). In addition to the potential inability of ectopic models to recapitulate tumor/stroma interactions of the original host, the present results clearly demonstrate altered hemodynamics and rest and during mild-intensity exercise, with directionally opposed results between the same tumor type grown at different anatomical sites. Specifically, we observed a net hyperemic response in the orthotopic tumor and a reduction in blood flow to the ectopic tumor with exercise. These differences in flow were not dependent upon the tumor arteriolar responses to NE as this was severely diminished in tumor vessels from either location. Such substantial changes in blood flow during exercise (and daily O_2 delivery from differences at rest) may partly explain why orthotopic models, in general, are better predictors of clinical success with experimental treatments than ectopic models (12,40).

Limitations

There are well over a 100 types of cancer, many of which are investigated in ectopic and orthotopic pre-clinical models, with the data reported herein from prostate cancer. In the current study we chose to investigate blood flow to the tumor and host tissue of prostate cancer for several reasons, including, 1) this is the most commonly diagnosed malignancy in males in the United States (and central Europe), and 2) prostate tumors contain areas of hypoxia making them difficult to treat with conventional therapies (81). Therefore, our findings are valuable in determining potential models to generate clinically relevant data. To our knowledge, blood flow during exercise has not been measured in any other tumor type. Most tumors contain a lower

percentage of vessels that display vasoactive properties than surrounding tissue (76), which was found herein in tumors from both locations (Figure 5A). Therefore, it is likely that host-tissue hemodynamics to exercise will dictate the directional change in tumor blood flow, regardless of the tumor type, though this remains to be determined. We chose to measure vasoconstrictor responses to NE as the literature suggests this is one of the main mechanisms regulating flow in these host-tissues (see above), and all host-tissue displayed an active vasoconstriction to exercise (i.e., vascular resistance was increased). Within the tumors, a blunted vasodilation in arterioles from the ectopic vs. orthotopic model could have also contributed to the different flow patterns between tumors. In the tumor arterioles used herein the level of tone achieved (~10%; Table 2) was not sufficient for us to reliably measure vasodilation to any agonist. Furthermore, due in large part to the dysfunctional endothelium and smooth muscle present (9,42), tumor arterioles display little to no vasodilation to a number of different agonists (77). Therefore, it is unlikely that changes in vasodilation between tumor arterioles contributed to the altered flow patterns, although this remains to be determined.

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