Blood flow responses to mild-intensity exercise in ectopic vs. orthotopic prostate tumors; dependence upon host tissue hemodynamics and vascular reactivity

Emmanuel Garcia,2✉ Veronika G. C. Becker,2,3✉ Danielle J. McCullough,4 John N. Stablye,5 Elizabeth M. Gittemeier,2 Alexander B. Opoku-Acheampong,2 Dietmar W. Sieman,6 and Bradley J. Behnke1,2

1Johnson Cancer Research Center, Kansas State University, Manhattan, Kansas; 2Department of Kinesiology, Kansas State University, Manhattan, Kansas; 3Department of Sports Science, Leipzig University, Leipzig, Germany; 4Department of Anatomy & Physiology, Edward Via College of Osteopathic Medicine, Auburn Campus, Auburn, Alabama; 5Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas; and 6Department of Radiation Oncology, University of Florida Health Cancer Center, Gainesville, Florida

Submitted 24 March 2016; accepted in final form 27 April 2016

Garcia E, Becker VG, McCullough DJ, Stablye JN, Gittemeier EM, Opoku-Acheampong AB, Sieman DW, Behnke BJ. Blood flow responses to mild-intensity exercise in ectopic vs. orthotopic prostate tumors; dependence upon host tissue hemodynamics and vascular reactivity. J Appl Physiol 121: 15–24, 2016. First published April 28, 2016; doi:10.1152/japplphysiol.00266.2016.—Given the critical role of tumor O2 delivery in 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. In male COP/CrCl 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. α-Adrenergic vasoconstriction to norepinephrine (NE: 10−3 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. Blood flow was lower at rest and during exercise in ectopic tumors and host tissue (subcutaneous adipose) vs. the orthotopic tumor and host tissue (prostate). During exercise, blood flow to the ectopic tumor significantly decreased by 25 ± 5% (SE), whereas flow to the orthotopic tumor increased by 181 ± 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 ± 7%) vs. prostate arterioles (55 ± 7%). Establishment of the tumor did not alter host tissue blood flow from either location at rest or during exercise. These data demonstrate that 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.

NEW & NOTEWORTHY

Exercise oncology is a rapidly developing field; however, there is a paucity in our understanding of tumor blood flow responses during exercise and the potential dependence of tumor perfusion and outcomes upon host tissue type and location. This study is the first to demonstrate that blood flow responses to exercise are directionally opposed when the same tumor is grown in an ectopic or orthotopic manner and that host tissue vascular reactivity differs substantially in resistance vessels from different host tissues.

SOLID TUMORS CONTAIN aberrant vascular networks (33, 57), which lead to impaired blood flow and inadequate O2 delivery and result in regions of hypoxia, acidosis, and general nutrient depletion (55, 56). The development of tumor hypoxia modifies the composition and key signaling components of the tumor microenvironment (7), promotes the adoption of an aggressive tumor phenotype (23, 53), affects tumor progression (43), and enhances tumor cell dissemination (17, 62), all of which culminate in poor patient prognosis (26, 27). 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 (27, 49).

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 (42). As discussed by Schuh (47), most transplantable tumors are placed ectopically and tumor cell lines injected subcutaneously, because of accessibility and lack of stress to the animal. The majority of preclinical exercise oncology studies in rodents also use ectopic (subcutaneous injections) tumor models, as summarized by Betof et al. (8). However, 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), are better predictors of clinical success than ectopic models (10, 31). Furthermore, regardless of cancer origin, local host-tumor interactions are critical mediators of tumor growth and drug delivery (e.g., blood flow), yet how these may be affected by exercise is largely overlooked.

It is well known that vascular function is organ specific; however, much less is known regarding such function amongst tumors. With respect to tumor blood vessel growth and morphology, tumor vessels show limited tissue specificity and largely generate their own blood supply [for a review, see Nagy et al. (41)]. Therefore tumor blood flow during exercise may be independent of location. However, tumor blood vessels

* E. Garcia and V. G. C. Becker contributed equally to this work.

Address for reprint requests and other correspondence: B. J. Behnke, Johnson Cancer Research Center, Dept. of Kinesiology, Kansas State University, Manhattan, KS 66506 (e-mail: bjbehnke@ksu.edu).

http://www.jappl.org 8750-7587/16 Copyright © 2016 the American Physiological Society

First published April 28, 2016; doi:10.1152/japplphysiol.00266.2016.
are abundant at the host interface (41), and the tumor would be expected to show some dependency upon host tissue perfusion, especially during conditions involving exercise-induced changes in autonomic nervous system activity and subsequent vasconstriction. Currently, it is unknown whether exercise alters tumor blood flow and $O_2$ delivery depending upon location. The effects of exercise on tumor growth and metastasis in preclinical models are controversial with exercise having an increased (1), a decreased (9), or no effect (11) on tumor growth as well as earlier appearances (60), suppressed development (14), and reduced (37) or increased (14) metastases of tumors compared with sedentary groups. A confounding factor in this controversy in preclinical investigations may be choice of tumor transplantation site. For example, given the low blood flow to the skin and subcutaneous adipose at rest (5) and exercise (35) in the rodent, it is possible that ectopic tumor models implanted subcutaneously may have less perfusion during exercise. A lower perfusion may result in the adoption of a more aggressive phenotype (53) and enhance tumor progression (43). This may not be the case in the orthotopic model if the hyperemic response to exercise differs. Indeed, recent data suggest that, when using an orthotopic prostate tumor model, voluntary wheel running (29) or treadmill exercise (38) does not affect tumor growth, a finding which may be related to the effects of exercise on tumor blood flow or oxygenation.

In this study, we investigated 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, we tested the following hypotheses: 1) ectopic tumors (flank model) will demonstrate an attenuated exercise-hyperemic response vs. 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 with values at rest; and 3) there will be an enhanced vasconstriction to the $\alpha$-adrenergic agonist norepinephrine in the host tissue of the ectopic vs. 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.

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/CrCr; Charles River, Wilmington, MA) were investigated at $\sim$6 mo 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 (28). 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 (30) with a high metastatic potential, fast growth rate, and characteristics that are characteristic of progressive human prostate cancers (28). MLL cells were cultured in RPMI 1640 medium (supplemented with 2 mM glutamine, 250 nM dexamethasone, 10% fetal bovine serum, and 1% penicillin/streptomycin; 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$^6$ 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. The animal was then allowed to recover. All procedures were performed under aseptic conditions. Postoperative monitoring of the animals was performed daily until experimental protocols were performed 21 days postinjection.

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) were 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 radioisotope-tagged microsphere technique (36). 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 m/min with no incline. These parameters provide a mild exercise intensity for rats of this body mass and age (21). At least 24 h 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 0.6 mm, outer diameter 1.0 mm) filled with heparinized saline solution (100 U/ml, Elkins-Sinn) was advanced into 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 h 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 (20).

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 m/min (no incline), which would correspond to an intensity of <50–60% of maximal aerobic capacity (21). We chose a mild-intensity exercise vs. a more energetically demanding intensity as it the animals do not display any distress at this intensity and 2) high-intensity exercise has been suggested to enhance tumor metastases (14). 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/NEU, Boston, MA) was infused (2.5–5.0 $\times$ 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 (4) 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 (36) 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 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 tumor. Blood flow in anatomically similar tissues as used for implantation of the microsphere technique (15, 38, 39) was measured in ectopic (prostate and bladder) tumor models was measured in sham-operated (Protocol II) animals (Ectopic-Sham, 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 protocols I and II, respectively), we chose to isolate microvessels from site-specific host tissue of healthy rats to ensure data representative of host tissue were analyzed. Animals were euthanized with pentobarbital sodium (>100 mg/kg ip), 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$_2$PO$_4$, 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 μm; 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) and 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 (38, 39).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 α-adrenoceptor agonist norepinephrine (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:

$$\text{Vasoconstriction} \% = \left( \frac{D_e - D_b}{D_b} \right) \times 100$$

where $D_b$ is the steady-state inner diameter recorded after addition of agonist and $D_e$ is the initial baseline inner diameter before the first addition of a norepinephrine. Spontaneous tone was expressed as a percentage of maximal diameter as follows:

$$\text{Spontaneous tone} \% = \left( \frac{D_{max} - D_b}{D_{max}} \right) \times 100$$

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. 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...
Exercise, Tumor Perfusion, and Host Tissue Responses • Garcia E et al.

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

<table>
<thead>
<tr>
<th></th>
<th>Ortho-Sham (n = 5)</th>
<th>Ortho-TB (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Flow, ml·100 g⁻¹·min⁻¹</td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>Bladder</td>
<td>17.8 ± 1.2</td>
<td>17.2 ± 1.2</td>
</tr>
<tr>
<td>Prostate</td>
<td>29.4 ± 2.4</td>
<td>28.2 ± 2.5</td>
</tr>
<tr>
<td>Soleus</td>
<td>102.2 ± 14.7</td>
<td>190.3 ± 14*</td>
</tr>
<tr>
<td>Kidneys</td>
<td>615 ± 25</td>
<td>449 ± 25*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>114 ± 6</td>
<td>137 ± 6*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood Flow, ml·100 g⁻¹·min⁻¹</th>
<th>Ectopic-Sham (n = 5)</th>
<th>Ectopic-TB (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>9.3 ± 0.7</td>
<td>10.2 ± 0.5</td>
</tr>
<tr>
<td>Subcutaneous adipose</td>
<td>13.2 ± 1.1</td>
<td>14.6 ± 1.2</td>
</tr>
<tr>
<td>Visceral adipose</td>
<td>11.4 ± 0.6</td>
<td>12.2 ± 1.2</td>
</tr>
<tr>
<td>Soleus</td>
<td>92.4 ± 6.2</td>
<td>91.5 ± 8.6</td>
</tr>
<tr>
<td>Kidneys</td>
<td>566 ± 22</td>
<td>431 ± 73*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>111 ± 4.2</td>
<td>130 ± 7*</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure. *P ≤ 0.05 vs. 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) vs. corresponding tumor-bearing groups.

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 ≤ 0.05 was required for significance. When a trend was observed (i.e., P < 0.1 but >0.05), the exact P value was reported.

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 with 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 (Fig. 1A) vs. the orthotopic tumor (P ≤ 0.05). In response to exercise, there was a ∼24% reduction in blood flow to the ectopic tumors vs. rest, whereas flow increased by ∼180% in the orthotopic tumors to exercise (Fig. 1B; P ≤ 0.05). Vascular conductance tended to be lower at rest (trend, P = 0.066) and during exercise (P ≤ 0.05) in the ectopic vs. orthotopic tumors (Fig. 2). In response to exercise, vascular conductance decreased in the ectopic tumors and increased significantly in the orthotopic tumors compared with that at rest (Fig. 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

Fig. 1. Ectopic and orthotopic tumor blood flow measured at rest during exercise (A) and the change in tumor flow during exercise compared with rest (B). *P ≤ 0.05 vs. ectopic tumor for same condition. †P ≤ 0.05 vs. resting value in same tumor.
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 (Fig. 3A), which was over double that of the subcutaneous adipose tissue (Fig. 3B). The skin had the lowest flow at rest of all tissues (Fig. 3B). In response to exercise, there was not a significant change in blood flow to the prostate or bladder (Figs. 3A and 4) vs. that measured at rest. Conversely, during exercise, blood flow was reduced significantly in both the subcutaneous adipose tissue and skin vs. rest (Figs. 3B and 4; \( P \leq 0.05 \)); however, the reduction in flow with exercise was significantly greater in the subcutaneous adipose vs. the skin (Fig. 4). When comparing the relative perfusion ratio (i.e., tumor flow/host tissue flow), even with a greater perfusion to the orthotopic vs. ectopic tumor at rest (Fig. 1), because of the much higher prostate vs. subcutaneous adipose flow ratios (Fig. 3, A and B) the tumor: host tissue flow ratio was lower in the orthotopic (0.50 ± 0.05) vs. ectopic (0.88 ± 0.07; \( P \leq 0.05 \)) 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; Fig. 4) vs. the reduction in flow to the ectopic tumor (Fig. 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 (Fig. 1B), with no significant change in flow to the prostate (Figs. 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\(^{-1}\)·100 g·min\(^{-1}\); \( P \leq 0.05 \)) whereas there was a 20% increase in the prostate (rest 3.6 ± 0.2 vs. exercise 4.3 ± 0.1 mmHg·ml\(^{-1}\)·100 g·min; \( P \leq 0.05 \)). Vascular resistance was higher both at rest and during exercise in the subcutaneous adipose tissue compared with the prostate tissue (\( P \leq 0.05 \)).
There were several novel findings from these sets of studies including the following: 1) in response to mild exercise, there was a directionally opposed blood flow response in the orthotopic vs. ectopic tumors; 2) during the steady state of exercise, blood flow was approximately fourfold 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 with anatomically equivalent tissue in healthy, non-tumor-bearing (sham) animals; 5) in tumor arterioles from both tumor groups, vasoconstriction to norepinephrine (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) vs. 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 vs. orthotopic tumor size (despite the same number of cells injected) and the trend for a lower soleus muscle blood flow at the onset of exercise (18). Thus the extent of vasoconstriction is tissue specific [i.e., different responses in skin, muscle, and viscera (3, 18)] and collectively “shunts” 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.

The tumor vasculature often possesses a poorly developed medial layer lacking in functional smooth muscle (32) and contractile wall components (61). 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 (51). We have previously demonstrated dysfunctional vasoconstriction within the arteries perfusing orthotopic prostate tumors (39), which was associated with an enhanced blood flow during exercise (39). Analogous to Ohm’s law, we reasoned that 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 volume) resulted in a substantial increase in tumor perfusion (current). This explanation assumed that 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 is in both series and in parallel arrangement (51) with that of the host. This was also verified in the current study through visualization of the vascular network upon isolation of the tumor(s). Specifically, 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 ≤150-µm luminal diameter (13) (i.e., resistance vasculature), larger feed arteries can contribute a significant portion of vascular resistance (59). 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 (Fig. 5A) and structural properties (i.e., diminished wall-to-lumen ratio; Table 2). However, a directionally opposed blood flow response to exercise occurred between tumors (Fig. 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 (45, 48, 63)], 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 overperfusion at rest during exercise, respectively, vs. their host (Fig. 1B vs. Fig. 4). Even though flow decreased to the ectopic tumor during exercise compared with rest (Fig. 1, A and B), the reduction in subcutaneous adipose was much greater resulting in 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 (Fig. 5A) vs. a coordinated change in vascular tone occurring in healthy tissue.

**Host Tissue Blood Flow Responses**

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 (40), we did not see any differences in host tissue blood flow at rest or during exercise in the tumor-bearing vs. 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 (58). 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 (39) 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 (19)]. 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 (Figs. 3B and 4). This is consistent with other studies showing an initial decrease in adipose tissue blood flow to exercise in rats (16, 34), thought to contribute to the redistribution of cardiac output to supply the working skeletal muscle (16). During shorter-duration exercise (i.e., <30 min) in pigs (3) and humans (25), adipose tissue blood flow remains unchanged vs. rest, although the subcutaneous adipose directly above the working muscle has been shown to increase flow during this time period (25). During prolonged (>30 min) low-to-moderate-intensity exercise in humans, adipose tissue blood flow generally increases onefold to fourfold over resting values, although this increase can take several hours (12). In contrast, inguinal adipose tissue blood flow in the rat was lower after ~1 h of mild-intensity exercise vs. pre-exercise values (35). 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 (24, 50) with adipose tissue blood flow being regulated predominately by β-adrenergic vasodilation (2) and α-adrenergic vasoconstriction (22). 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 vs. prostate tissue, consistent with the much greater increase in vascular resistance and change in blood flow that occurs in the subcutaneous adipose vs. prostate with exercise (Fig. 3, A and B), demonstrating substantial differences in the tumor host perfusion to these conditions.

**Blood Flow: Rodent vs. Human Prostate Cancer**

Hypoxia is a critical mediator of tumor aggressiveness and is associated with poor patient prognosis (53, 54). In the current study, we chose to measure tumor blood flow at rest and during exercise with the radiolabeled microsphere technique as it remains the standard for quantifying blood flow in vivo (44). Healthy prostate tissue flow values found herein (Table 1) are similar to those found in human prostate [mean flow 21 ml·100 g−1·min−1 (56)]. Our data demonstrate a lower tumor flow vs. the prostate at rest (Fig. 4). However, in human prostate tumors, blood flow (quantified predominately by MRI and computed tomography) is higher, and tumor oxygenation lower, than healthy prostate (host) (56). 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 (51) that would effectively shunt oxygenated blood past the tumor microcirculation. 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, we used 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., microcirculation plus macrocirculation) 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 (P02) measured at rest is ~6 mmHg in the rat orthotopic tumor (38), which is identical to the mean value in human prostate tumors (56), 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.

**Limitations**

There are well over 100 types of cancer, many of which are investigated in ectopic and orthotopic preclinical 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 that 1) this is the most commonly diagnosed malignancy in men in the United States (and central Europe), and 2) prostate tumors contain areas of hypoxia making them difficult to treat with conventional therapies (56). 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 (51), which was found
herein in tumors from both locations (Fig. 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, in large part because of the dysfunctional endothelium and smooth muscle present (6, 32), tumor arterioles display little to no vasodilation to a number of different agonists (52). Therefore it is unlikely that changes in vasodilation between tumor arterioles contributed to the altered flow patterns, although this remains to be determined.

Conclusion

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 (8). 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 during rest and 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 O2 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 (10, 31).

GRANTS

American Cancer Society (B. J. Behnke; D. W. Sieman) (RSG-14-150-01-CCE), American Physiological Society, and the National Institutes of Health (B. J. Behnke; E. Garcia) (National Heart, Lung, and Blood Institute; 1 R25 HL115473-01) supported this study.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


