

Original Article

Tumor bearing in untreated breast cancer decreases exercise tolerance without lowering maximal oxygen uptake in rats

Ramona E Weber¹, Kiana M Schulze¹, Nathan J Kenney¹, Britton C Scheuermann¹, Olivia N Kunkel¹, Carl J Ade¹, Timothy I Musch^{1,2}, Brad J Behnke¹, David C Poole^{1,2}

¹Department of Kinesiology, Kansas State University, Manhattan, KS 66506, USA; ²Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506, USA

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Abstract: Breast cancer patients' maximal O₂ uptake (VO₂max) values average 60-80% of age-predicted values which is often attributed to adjuvant therapy rather than risk factors, comorbidities, or the tumor and associated factors (e.g., pro-inflammatory cytokines). It is crucial to understand the physiological mechanisms behind exercise intolerance in breast cancer patients to enhance targeted interventions; however, the effect of breast cancer, as an isolated condition on VO₂max, exercise tolerance, and resting cardiac function has not been investigated. We hypothesized that breast cancer, in the absence of underlying conditions or chemotherapy, would lower VO₂max, exercise tolerance, and cardiac function in proportion to tumor mass. Female Fischer-344 rats (~6-8 months, n = 8) were acclimatized to treadmill running for 5 days at 25 m/min for 5 min/day. To measure VO₂max, rats were placed within a plexiglass metabolic chamber connected to CO₂ and O₂ analyzers. Tests began at 25 m/min and increased (5 m/min) until exhaustion. Cardiac function was determined by echocardiography before rats received a mammary intraductal injection of rat adenocarcinoma cells (MATBIII, 6 × 10³ in 50 µl saline). Tumor growth was monitored daily and ~7 days following palpation (~24 days post-injection), VO₂max and echocardiography measurements were repeated. Tumor mass and volume were 2.1 ± 0.6 g and 1685 ± 428 (range 256-3749) mm³, respectively. Body mass (217 ± 6 vs 218 ± 6 g), VO₂max (72.1 ± 2.7 vs 70.0 ± 2.8 ml/kg·min; P > 0.05), and all measures of cardiac function were unchanged following tumor formation, with no significant correlation between tumor mass and VO₂max (P > 0.05). However, time to exhaustion (376 ± 20 vs 297 ± 25 s), final treadmill speed (48 ± 1 vs 42 ± 2 m/s), distance run (209 ± 16 vs 152 ± 18 m), and total work (45 ± 3 vs 32 ± 4 m·kg) were significantly reduced with tumor bearing. Contrary to our hypothesis, breast cancer did not affect VO₂max or cardiac function, but reduced exercise tolerance.

Keywords: Cardio-oncology, exercise capacity, oxygen transport, skeletal muscle, blood flow, muscle dysfunction, exercise oncology, cardiac function, adenocarcinoma

Introduction

Maximal oxygen uptake (VO₂max), measured during maximal cycle ergometry or treadmill exercise, assesses the coordinated capacity of the pulmonary, cardiovascular, and muscle systems to transport and utilize O₂. A high VO₂max and physical activity level are associated with greater exercise tolerance and lowered risk of breast cancer [1-5], which is the most diagnosed malignancy in U.S. women with 272,454 new cases in 2021 [6].

A cardinal phenotype of breast cancer patients is a low VO₂max [7-9] with values typically rang-

ing from 60-80% of age-predicted values [7, 10, 11]. It is assumed that this low VO₂max results primarily from adjuvant anticancer therapies [7, 9, 10, 12]. Monoclonal antibodies and anthracyclines, such as trastuzumab and doxorubicin, are cardiotoxic and increase the risk and prevalence of cardiomyopathy, arrhythmia, heart failure, and skeletal muscle dysfunction [13-16]. Chemotherapy-induced cardiomyocyte injury decreases left ventricular (LV) ejection fraction in breast cancer patients [17, 18] and contributes to a ~10% or more reduction in VO₂max [7, 19-21]. However, this is belied by the absence of requisite VO₂max validation tests [22] and

reliance on relative rather than absolute VO₂ values; especially where therapy results in altered body mass. An exemplar of this is seen in Peel et al. [23] where, despite no change in absolute VO₂max (1.6 L/min pre- and post-chemotherapy), the ~10% increase in body mass across treatment lowered the relative VO₂max proportionally and in the absence of cardiovascular dysfunction. This observation raises the question: how much of the low VO₂max post-therapy in individuals with breast cancer is 1) present pre-therapy, 2) pre-existed the cancer or 3) resulted from the pro-inflammatory impact of tumor-bearing?

Pre-treatment cancer patients have an extraordinarily low VO₂max compared with the predicted VO₂max for 50-59-year-old women (i.e., 30.4 ml/kg·min) [24]. Specifically, pre-treatment breast cancer patients may have a VO₂max below 20 ml/kg·min with 32% falling below the 15.4 ml/kg·min [7] that is considered necessary for functional independence [25]. We propose that the reductions in VO₂max before anticancer therapy are either characteristic of individuals at greater risk for cancer or due to the impact of the tumor itself, which is profoundly inflammatory [26, 27]. In the rat, 8 weeks of untreated prostate tumor-bearing significantly reduced LV and skeletal muscle(s) mass and running endurance compared to sham-operated animals [28]. LV mass was negatively correlated to tumor mass in these prostate cancer rats [28], which suggests that tumor-bearing itself can decrease cardiovascular and skeletal muscle function even in the absence of decreased muscle oxidative enzymes. Similarly, in the transgenic polyomavirus middle T-antigen oncogene (PyMT) model of breast cancer in mice, breast cancer induces reductions in endurance capacity and concomitant increases in muscular weakness in the absence of changes in skeletal muscle mass or citrate synthase activity [29]. Unfortunately, VO₂max was not measured in those investigations.

VO₂max measurements are a useful tool in exercise oncology to assess the feasibility or preliminary effectiveness of a pharmacological or exercise intervention. Higher VO₂max and activity levels are associated with less solid tumor hypoxia and reduce tumor growth rates [4, 30] as well as a lower incidence of treatment-related toxicities, exercise intolerance,

and cardiovascular risk [7, 9, 31]. One imperative here is that a higher VO₂max not only supports the effective implementation of exercise therapy but is also pivotal in further enhancements in VO₂max which has been demonstrated to decrease immune suppression and tumor progression [32], lessen fatigue [33], and improve clinical outcomes for individuals with breast cancer. For example, a 3.5 ml/kg·min increase in VO₂peak is associated with an 18% lower cardiovascular mortality risk in cancer patients [1].

There is a pressing urgency to characterize the physiological mechanisms underpinning exercise intolerance in breast cancer patients to improve risk stratification and guide targeted interventions [34, 35]. As such, preclinical animal cancer models are essential to investigate tumor pathophysiology and resolve the mechanisms by which exercise is protective and anti-tumorigenic [4]. These models have significant advantages given that genetic, nutritional, and exercise backgrounds are invariant, and the type and duration of cancer are controlled with the presence/absence of treatment protocols dictated by the investigators.

Herein the 13762 MAT B III orthotopic model of mammary intraductal breast cancer in the rat was utilized to test the hypothesis that breast cancer itself, in the absence of underlying conditions and chemotherapy, would lower VO₂max and exercise tolerance in proportion to tumor mass.

Materials and methods

Animals

Female retired breeder Fischer-344 rats (~6-8 months, n = 10) were obtained from Charles River Laboratories (Boston, MA). Two rats were excluded from this study due to tumor ulceration. A subsample of healthy rats (n = 5) were used for morphometric comparisons. Upon arrival at Kansas State University, rats were housed in facilities approved by the Association for the Assessment and Accreditation of Laboratory Animal Care. Rats were maintained on a 12:12 hour light-dark cycle in a thermo-neutral environment and food and water were provided ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University (Protocol

VO₂max during treadmill running in breast cancer rats

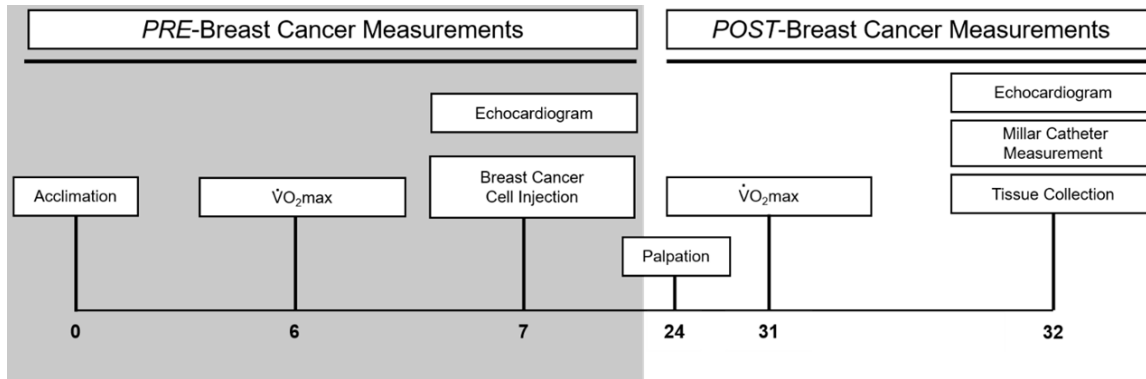


Figure 1. Schematic representation of the experimental protocol. Day 0-5, acclimation; day 6, pre-VO₂max; day 7, pre-echocardiography and breast cancer cell injection; day 24, first palpation; day 24-31, tumor growth; day 31, post-VO₂max; day 32, post-echocardiography, Millar catheter measurements, and tissue collection.

#4684) and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Orthotopic breast cancer model

The 13762 MAT B III adenocarcinoma cell line (CRL-1666; ATCC, Manassas, VA) originated from rat mammary glands. The cells were cultured in McCoy's 5A (modified) medium, supplemented with 10% fetal bovine serum, and stored in a 5% CO₂ incubator until reaching confluency. Cell counts and viability were tested with Trypan blue staining prior to breast cancer cell injection procedures. While anesthetized under 2.5% isoflurane-O₂ and positioned on a heating pad to maintain core temperature at ~37°C (measured via rectal thermometer), using a 20-G needle, 6 × 10³ cells in 50 μL of saline were injected into a left abdominal mammary duct [36]. Rats were monitored closely for ~2 hours for signs of distress.

Experimental protocol

The experimental protocol is shown in **Figure 1**. All rats were acclimated to treadmill running on a custom-built motor treadmill for 5 min/day at 25 m/min up a 5% incline. Importantly, this acclimation protocol does not elicit training adaptations in skeletal muscle [37]. Within 5 days of completing acclimation, VO₂max was measured using previously established methods from our laboratory [38]. Following 24 hours of recovery, pre-breast cancer echocardiography determined baseline cardiac characteristics, and intraductal breast cancer cell injections were performed while under 2.5% isoflurane anesthesia (see "Orthotopic Breast

Cancer Model"). Animals were monitored daily for signs of tumor growth. Upon tumor palpation, daily measurements of tumor volume were made using calipers. Tumor volume was measured in two orthogonal dimensions with the greatest dimension of the tumor being recorded as length, and the other as width. Tumor volume was calculated as $(l \times w^2)/2$, which is the standard formula for tumor mass estimation in breast cancer rats [39]. We monitored the time course of tumor growth and based on this experience, the final (post) measurement protocols and experiments were conducted ~1 week following tumor palpation to avoid ulceration. Following tumor growth (~24 days post-injection) rats performed a post-VO₂max test and 24 hours afterwards post-echocardiographic measurements were made. Animals were anesthetized (~2.5% isoflurane anesthesia) and echocardiograms measured LV function and then the right carotid artery was isolated, cannulated, and a 2-French catheter-tip pressure transducer (Millar Instruments, Houston, TX, USA) was advanced into the LV for measurements of systolic and diastolic pressures and changes in LV pressure over time (LV $\Delta P/\Delta T$). Following the experimental protocol and while under isoflurane anesthesia (~2.5%), rats were euthanized with an overdose of pentobarbital sodium (> 100 mg/kg) and the tumor, heart, and skeletal muscle samples were removed for morphometric evaluation.

Determination of VO₂max and exercise tolerance

Following acclimation, VO₂max was determined utilizing previously established methods that

VO₂max during treadmill running in breast cancer rats

Table 1. Morphometric, performance and VO₂max measurements

	Pre-Breast Cancer (n = 8)	Post-Breast Cancer (n = 8)
Body weight (g)	217 ± 6	218 ± 6
Tumor weight (g)		2.1 ± 0.6
Non-tumor body weight (g)		215 ± 5
VO ₂ max (ml/kg·min)	72.0 ± 2.7	70.0 ± 2.8
Time to max (s)	376 ± 20	297 ± 25*
Final speed (m/s)	48 ± 1	42 ± 2*
Total distance (m)	209 ± 16	152 ± 18*
Work (m·kg)	45 ± 3	32 ± 4*

Data are means ± SE. n, number of rats; *P < 0.05 vs pre-breast cancer.

have been used extensively in our laboratory [40]. Before each test, CO₂ and O₂ analyzers (models CD-3A and S-3A/I, respectively, AEI Technologies, Pittsburgh, PA) were calibrated with precision-mixed gases that span the expected range of gas concentrations. Each rat was weighed and placed within a plexiglass metabolic chamber designed to fit within one treadmill lane. Initiation of the maximal exercise test consisted of 1-minute intervals beginning at 25 m/min and progressively increasing by 5 m/min every minute until the rat was unable to keep pace with the treadmill belt. Time to exhaustion was recorded to the nearest second and the test was terminated to avoid injury. Exhaustion was confirmed by a slowing of the righting reflex. Each rat was reweighed following VO₂max measurements, and the results reflect the post-exercise test weight (**Table 1**). This protocol has been demonstrated to yield reproducible measurements of VO₂max and time to exhaustion in rats in our laboratory [37] and elsewhere [41]. To determine the O₂ cost of exercise, we calculated the ΔVO₂ for both pre- and post-measurements (Maximal O₂ value - baseline O₂ value, ml/kg·min) and divided this value by the final speed run(s). We then subtracted the post-breast cancer ΔVO₂/final speed from the pre-breast cancer ΔVO₂/final speed to assess the degree of change between pre- and post-breast cancer measurements.

Transthoracic echocardiography

Before breast cancer cell injections, rats were initially anesthetized with a 5% isoflurane-O₂ mixture and subsequently maintained on 2.5% isoflurane-O₂ while positioned on a heating pad

to maintain core temperature at ~37°C (measured via rectal thermometer). Transthoracic echocardiography was performed using a commercially available system (Logiq S8; GE Health Care, Milwaukee, WI) with an 18 MHz linear transducer (L8-18i) as previously described [42]. Standard 2-dimensional and M-mode images from the midpapillary level were obtained with frame rates of > 50 frames/s. M-mode measurements over 4 consecutive cardiac cycles determined ventricular dimensions and wall thicknesses. The measurements presented are the

average of the 4 cardiac cycles. LV internal dimensions (ID, cm) and posterior wall (PW, cm) thicknesses were measured at end systole (LVIDs; LVPWs) and end diastole (LVIDd; LVPWd). Fractional shortening (FS, %) was calculated from the measurements of LV chamber diameters: FS = [(LVIDd - LVIDs)/LVIDd] × 100. LV end-systolic (LVESv, ml) and end-diastolic (LVEDv, ml) volumes were calculated using the Teicholz formula: LV volume = (7.0/2.4 + LV dimension) × LV dimension³. Stroke volume (SV, ml) was calculated as: SV = LVEDv - LVESv. Ejection fraction (EF, %) was calculated as EF = [LVEDv - LVESv/LVEDv] × 100. Post-breast cancer echocardiography was performed 24 hours following VO₂max measurements and before euthanasia.

Statistical analyses

Student's paired t-tests were performed to determine differences in pre- and post-breast cancer VO₂max, speed, distance, time to max, work, echocardiography, and LV pressures. Pearson's product-moment correlation was used to determine relationships among variables. Data are presented as means ± SE. Significance was accepted at P < 0.05.

Results

Morphometry and tumor burden

Body mass did not change during the protocol and non-tumor mass (total minus tumor weight) was not significantly different compared to the pre-tumor body mass (**Table 1**). Moreover, soleus muscle mass was not correlated with tumor burden (r = 0.385, P = 0.272). The final tumor

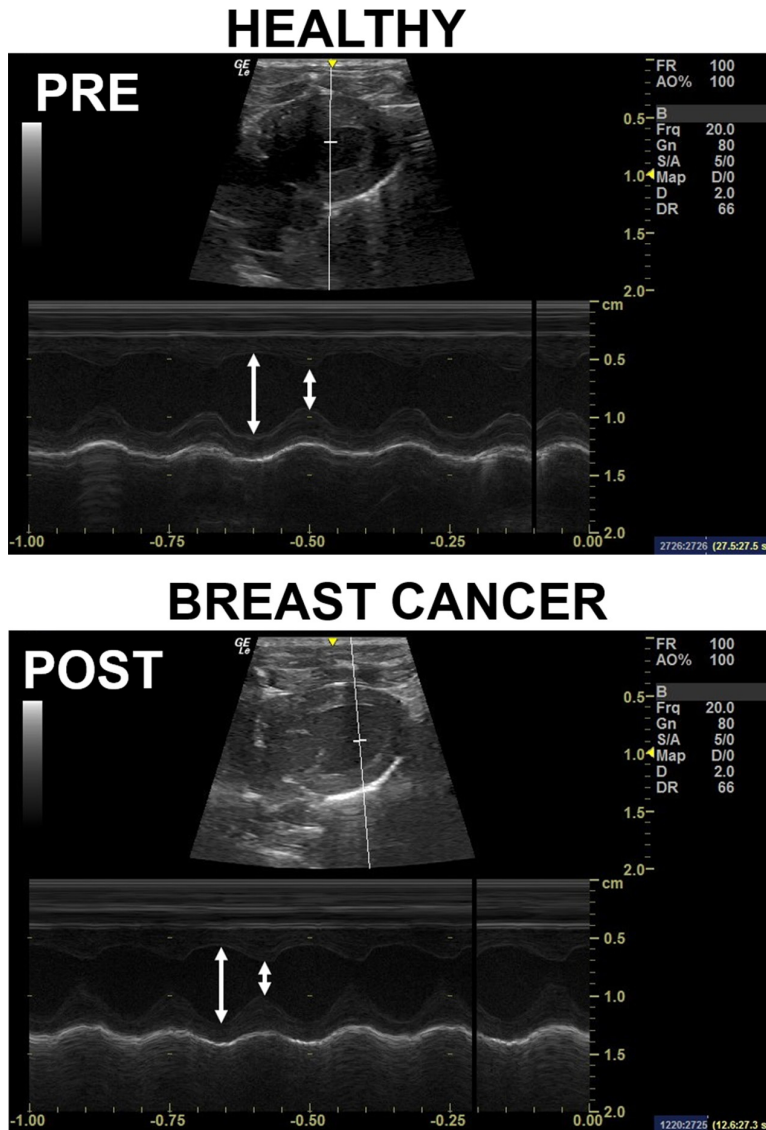


Figure 2. Representative images of pre- and post-breast cancer echocardiography measurements. No changes were seen in cardiac function over time (~25 days between measurements). Arrows represent the LV during diastole (widened 1st arrow) and during systole (narrow 2nd arrow).

burden was 2.1 ± 0.6 g (**Table 1**) and tumor volume was 1685 ± 428 (range 256-3749) mm³. The healthy animals used to compare invasive hemodynamic measurements weighed 224 ± 4 g.

Cardiac function

All animals displayed normal resting LV function before and following tumor establishment (**Table 1**; **Figure 2**). There were no differences in heart rate or cardiac output. Millar catheter

measurements suggest no change in LV $\Delta P/\Delta T$; however, breast cancer rats had a significantly higher LVEDP in comparison to a subsample of healthy control rats ($P < 0.05$, **Table 2**). Cardiac output was not different pre- vs post-breast cancer. No significant correlations between LV measurements and tumor mass were present ($r = 0.094$, $P = 0.825$, **Figure 3**).

Maximal O₂ uptake

VO₂max was not different pre- vs post-tumor growth (**Figure 4**; **Table 1**, $P = 0.534$). The respiratory exchange ratio at the end of exercise was not different between pre- and post-VO₂max tests, respectively (1.12 ± 0.03 vs 1.07 ± 0.03 ; $P = 0.261$). Δ VO₂ uptake (maximal minus baseline) was not different between pre- and post-tumor growth VO₂max tests, (pre: 46.1 ± 2.3 vs post: 43.3 ± 4.6 ml/kg·min; $P = 0.537$). In addition, tumor burden was not significantly associated with post-VO₂max ($r = 0.6726$, $P = 0.073$).

Exercise tolerance

Exercise tolerance, as measured during the VO₂max test, demonstrated that endurance time was significantly reduced (~21%) following tumor growth ($P < 0.05$, **Table 1**). Thus, with the incremental protocol these rats reached VO₂max at a slower speed ($P < 0.05$, **Table 1**; **Figure 5**). The total distance run was ~27% lower following tumor growth and there was a ~29% reduced work capacity ($P < 0.05$, **Table 1**). There was a strong positive correlation between tumor size and O₂ cost of exercise ($r = 0.923$, $P = 0.003$, **Figure 6**), which suggests that, as tumor size increases, economy decreases.

VO₂max during treadmill running in breast cancer rats

Table 2. Echocardiography measurements

	Pre-Breast Cancer (n = 8)	Post-Breast Cancer (n = 8)
LVIDd, cm	0.67 ± 0.01	0.63 ± 0.03
LVIDs, cm	0.36 ± 0.01	0.33 ± 0.02
LVPWd, cm	0.18 ± 0.10	0.18 ± 0.02
LVPWs, cm	0.28 ± 0.01	0.31 ± 0.09
FS, %	46 ± 1	54 ± 4
LVEDV, ml	0.67 ± 0.05	0.60 ± 0.07
LVESV, ml	0.12 ± 0.01	0.10 ± 0.03
SV, ml	0.55 ± 0.04	0.52 ± 0.05
EF, %	82 ± 1	87 ± 3
Heart rate (bpm)	304 ± 6	314 ± 12
Cardiac output (mL/min)	167 ± 12	163 ± 17
LV ΔP/ΔT (mmHg/s)	8024 ± 191 ‡	8585 ± 717
LVEDP (mmHg)	2 ± 0 ‡	5 ± 1*

Data are means ± SE. n, number of rats; LV, left ventricular; LVIDd, LV internal diameter in diastole; LVIDs, LV internal diameter in systole; LVPWd, LV posterior wall in diastole; LVPWs, LV posterior wall in systole; FS, fractional shortening; LVEDV, LV end diastolic volume; LVESV, LV end systolic volume; SV, stroke volume; EF, ejection fraction; bpm, beats per minute; LV ΔP/ΔT, LV change in pressure over change in time; LVEDP, LV end diastolic pressure; *P < 0.05 vs pre-breast cancer; ‡measured from subsample of healthy control rats (n = 5).

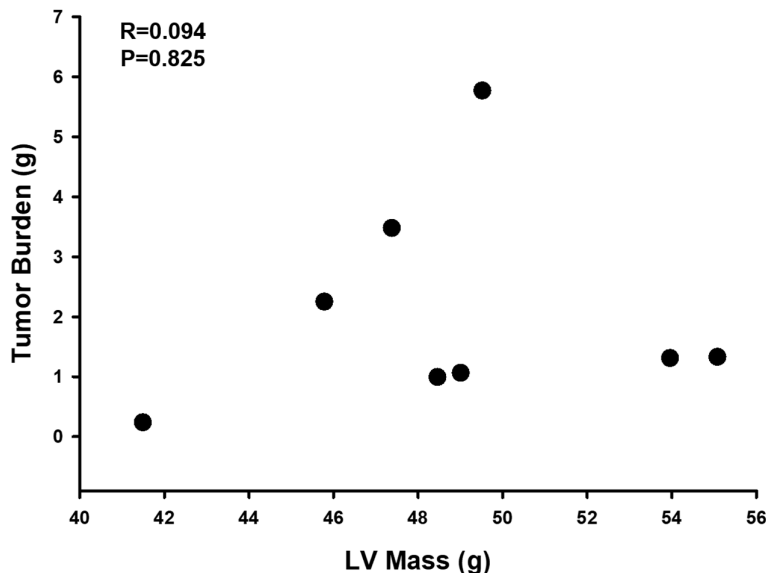


Figure 3. Total tumor burden vs left ventricular (LV) mass (n = 8). There is no significant correlation between tumor burden and LV mass.

Discussion

To our knowledge, this is the first investigation specifically examining how breast cancer impacts VO₂max in the absence of anticancer treatment and in an animal model where activ-

ity, diet and genetic make-up are homogeneous. Our results demonstrate that despite exercise performance being reduced, VO₂max during treadmill running remained unaffected after breast cancer tumor establishment. Importantly, no signs of cardiac dysfunction, except for the elevated LVEDP, were observed concomitant with tumor bearing. Thus, without cardiovascular dysfunction, the reduced exercise tolerance may have resulted from intrinsic skeletal muscle contractile dysfunction. These results support that, while VO₂max is extremely low in individuals with breast cancer, the tumor itself and the associated pro-inflammatory state do not ipso facto lower VO₂max. Accordingly, a low VO₂max observed in individuals with breast cancer may primarily result from comorbid conditions and the adverse effects of cancer treatments on cardiac function and cardiopulmonary O₂ transport.

VO₂max reflects the integrated function of cardiopulmonary, vascular, and skeletal muscle O₂ transport and is an independent predictor of cardiovascular mortality and morbidity in the general population [43-45] as well as in individuals with cancer [46]. Higher VO₂max and activity levels are associated with reduced tumor hypoxia and tumor growth rates [4, 30] as well as a lower incidence of treatment-related toxicities,

exercise intolerance and cardiovascular risk [9, 46, 47]. However, VO₂max is not always a reliable predictor of exercise tolerance. For instance, while exercise training may increase VO₂max, this does not always translate to significant improvements in exercise tolerance

VO₂max during treadmill running in breast cancer rats

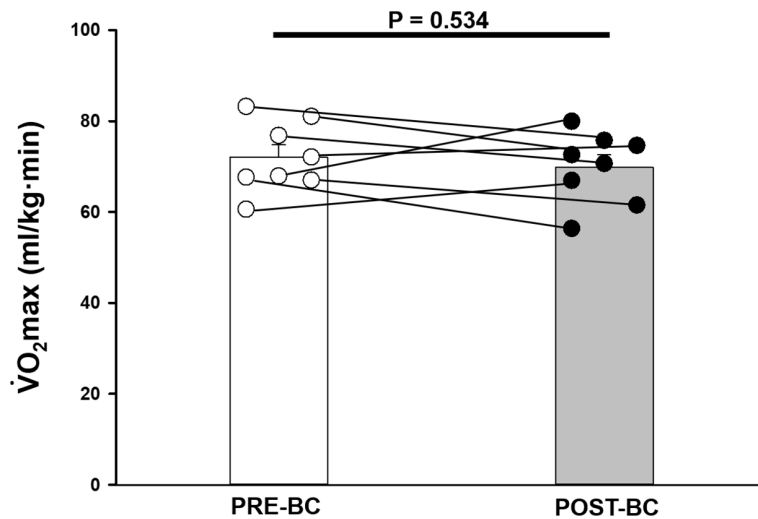


Figure 4. Pre-breast cancer (BC, $n = 8$, left) vs post-breast cancer ($n = 8$, right) relative $\dot{V}O_2$ max. There was no difference in pre- vs post- $\dot{V}O_2$ max measurements ($P = 0.534$). Data are mean \pm SE with individual datum points plotted.

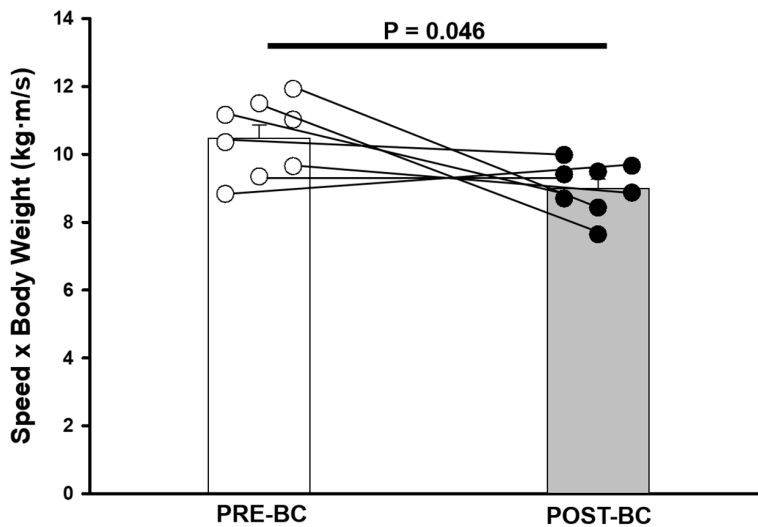


Figure 5. Pre-breast cancer (BC, $n = 8$, left) vs post-breast cancer ($n = 8$, right) final speed x body weight. There was a significant difference in pre- versus post-measurements. Data are mean \pm SE and individual datum points.

[48]. Functional measures of physical performance, such as critical speed, may provide greater evidence for: 1) how cancer treatment compromises lifestyle activities, and 2) the efficacy of therapeutic/exercise interventions [49]. In this context, for running, exercise tolerance refers to the total duration of exercise that can be sustained at a given speed or as speed increases, which provides valuable insights into intramuscular contractile function and metabolism. Indeed, the National Accredita-

tion Program for Breast Cancer Standards [50] requires functional assessment through the Patient-Reported Outcomes Measurement Information System (for) Physical Fitness (PROMIS PF) survey which, "measures the outcome of patients or clients with musculoskeletal disorders by assessing physical function through a grading scale of activities of daily living" [51]. Flores et al. [52] recently observed that physical fitness is impaired similarly across tumor types independent of treatment.

Tumor model

In preclinical research there are several different methods to induce breast cancer including: transgenic, syngeneic allograft, human cell lines and patient-derived xenograft (PDX) or patient derived orthotopic xenograft (PDOX), chemical carcinogens (1-methyl-1-nitrosourea (MNU) and 7,12-dimethylbenz(a)anthracene (DMBA)), as well as orthotopic implantation. Each model presents varied factors that must be considered (presence of estrogen, rodent strain and age, immune function, location of implantation/tumor growth) and other features which may complicate data interpretation [3, 53-56]. In this investigation, we cultured rat mammary adenocarcino-

ma cells and performed injections directly in the mammary duct to recapitulate the host tissue environment. Studies suggest that such orthotopic models are greater predictors of translational success than ectopic models [53, 57, 58]. Blood flow distribution in tumors is dependent mostly on where the tumor is grown [54] and overlooking this factor could significantly compromise the validity of preclinical exercise oncology research. The rats used in this study were ~6-8 months old and the time

VO₂max during treadmill running in breast cancer rats

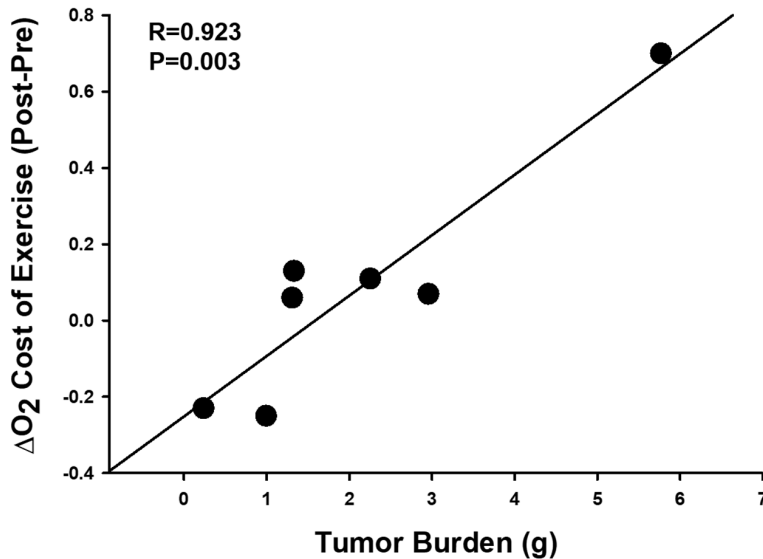


Figure 6. Change in O₂ cost of exercise (ΔVO_2 (ml/kg·min)/final speed (m/s)) between post- and pre-breast cancer ($n = 7$). This graph displays that an increase in O₂ cost of exercise is attributable, in part, to increases in tumor burden. One rat was not included due to error in collecting baseline O₂ uptake measurement.

from injection to final testing was ~24 days. In humans, a tumor may grow within the breast tissue for 1-5 years without noticeable palpation and regarding human age, the rat model herein reflects young adult women (~30 years old) bearing a tumor for ~2-3 years [59].

Maximal O₂ uptake in breast cancer

Using a standardized protocol that displays high reproducibility [37], we found that VO₂max was unchanged from pre- to post-breast cancer tumor development. Whilst contrary to our hypothesis, this finding strengthens the proposition that pre-existing low cardiorespiratory fitness combined with comorbidities/chemotoxicities, especially in those with higher cardiovascular risk [2, 11, 20, 47], may be responsible for the low VO₂max in breast cancer patients rather than the cancer itself. These results highlight the urgency to define reliable, valid, and translatable exercise measurements in breast cancer models. Unlike other forms of cancer (i.e., lung, gastric, esophageal [60]), individuals with breast cancer exhibit a low prevalence of cachexia [61] and are more likely to gain weight during chemotherapy [62]. This is important, because while breast cancer patients exhibit a reduction in VO₂max after adjuvant therapy [23], in some studies this

reduction only appears when VO₂max is calculated in relative terms (e.g., per kilogram of body weight) but not when measured in absolute terms (e.g., total oxygen uptake without considering body weight). Like our findings, Kirkham et al. [63] found no differences in VO₂peak between pre-chemotherapy breast cancer patients and body mass index (BMI)-matched non-cancer controls. As discussed above, VO₂max does decrease with breast cancer treatment [19] and remains lower than healthy sedentary controls [7]. Accordingly, targeted exercise and/or pharmaceutical approaches to improve VO₂max can potentially offset the detrimental cardiovascular dysfunction in-

curring by chemotherapy as well as improving the efficacy of that treatment [1, 9] (see below).

Exercise training benefits cancer patients by increasing VO₂max and is related to reduced solid tumor hypoxia and growth rates and a lower incidence of treatment-related toxicities, cardiovascular risk, and all-cause mortality [1, 9]. This capacity for exercise training to reduce tumor hypoxia is important because hypoxia itself is associated with greater histological grade, increased risk of metastasis, reduced responses to anticancer therapy, and worse outcomes [64]. During exercise, in a rat model of prostate cancer, tumor blood flow increased by ~200% compared to basal conditions, inducing a ~50% reduction in tumor hypoxia [65] and following exercise training, prostate cancer tumors had greater microvascular O₂ pressures than those in sedentary rats [56]. Collectively, this suggests a relationship between increases in VO₂max and tumor blood flow. Numerous lines of evidence support the benefits of exercise interventions applied along the continuum of cancer diagnosis and treatment. For example, cancer prehabilitation improved quality of life and augmented adherence to adjuvant therapy [66]. Completing 1 hour/week of moderate intensity exercise during treatment allowed for the maintenance of breast cancer

patient's pre-therapy VO₂ max while control subjects experienced a ~30% decrease [67], and weight lifting increased muscular strength and reduced the progressive lymphedema in breast cancer survivors [68].

Exercise intolerance in breast cancer

Breast cancer patients experience muscular weakness and fatigue before chemotherapy and independent of muscle wasting [60], yet the mechanisms precipitating muscle dysfunction in cancer remain elusive [69]. Tumor-derived inflammatory secretions are associated with diminished time to exhaustion [29] and reduced mitochondrial biogenesis [70] in rodent breast cancer models. Likewise, we found that tumor-bearing breast cancer rats had a reduced time and speed at which they reached VO₂max (**Table 1**), which suggests intrinsic skeletal muscle dysfunction as a potential generative mechanism. Breast cancer mice have a reduced endurance capacity that is associated with 1) impaired muscular force development and 2) increased intramuscular and tumor-derived tumor necrosis factor alpha (TNF- α) [29]. TNF- α has been demonstrated to compromise contractile function before cachexia [69] and impairs peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1 α)-mediated mitochondrial biogenesis [71]. Reduced PGC-1 α is apparent in both human and rodent breast cancer skeletal muscle biopsies across all cancer subtypes and independent of treatment status [72]. Interestingly, four weeks of voluntary wheel running in PyMT breast cancer mice: 1) reversed the breast cancer-induced muscle weakness observed in a sedentary PyMT group and 2) normalized downstream TNF- α target gene expression, however there was no improvement evident in PGC-1 α [29]. These data warrant future studies addressing exercise and pharmaceutical approaches to reduce cancer-induced chronic inflammation and improving skeletal muscle function in breast cancer patients.

Cardiac measurements

Echocardiography identifies cardiovascular complications from chemotoxicity in breast cancer patients, which is a rapidly growing concern [16, 17, 73]. Beaudry et al. [74], found that - before adjuvant treatment - breast cancer may impair heart function via reduced maximal car-

diac output and that these breast cancer-induced increases in LV stiffness are a potential culprit for the reduced aerobic capacity. Similarly, we found significantly increased LVEDP in breast cancer rats in comparison to healthy controls which is indicative of reduced ventricular compliance and LV stiffening [75] and is associated with exercise intolerance and skeletal muscle contractile dysfunction [31, 76]. In the study herein, we measured cardiac morphology and function before and following tumor growth and saw no significant decline in key measures of cardiac function (EF, FS, SV, cardiac output; **Figure 2**; **Table 2**). This finding is important, because circulating tumor-secreted cytokines such as TNF- α [77] could potentially increase myocardial injury [78] or induce cardiac cachexia [79]. Also, there is evidence for cardiac cachexia in prostate cancer rats [28]. We found no correlation between tumor burden and LV mass (**Figure 3**), which highlights that there may be distinct, pro-cachectic pathways in prostate cancer [28] and not in breast cancer. Variations in tumor phenotype, duration of tumor bearing, and/or the inoculation site may accelerate the development of cardiac and skeletal muscle cachexia [80] which may explain differences between prostate [28] and breast cancer herein.

Clinical insights

Our findings suggest that exercise intolerance in breast cancer patients is not solely a consequence of treatment or reduced VO₂max but may originate from underlying physiological changes associated with the disease itself. Therefore, future studies must explore the specific molecular pathways that contribute to muscle dysfunction in this population, as identifying these pathways could lead to targeted exercise and/or pharmacological interventions. Results from Clayton et al. [81] suggest that Pioglitazone, a treatment option for patients with diabetes mellitus, is partially effective in treating breast cancer-associated muscle dysfunction by increasing mitochondrial bioenergetic pathways.

Experimental considerations

The preclinical model herein is considered the gold standard for modeling breast cancer tumor effects on systemic O₂ transport in rodents [53]. This allows for tight control over diet, phys-

ical activity, and genetic variability in the absence of comorbidities or treatment effects. Alternative models, such as the PDOX/PDX, which use human cancer cell lines, are more often studied, and most require immunocompromised recipient rodents. Immunodeficiencies are not typically present early in breast cancer diagnosis [82], therefore we argue that the model used herein better recapitulates changes in exercise capacity solely due to breast cancer. Nevertheless, as with any animal model, translation to humans with breast cancer should be made with caution. The animals used in this study were middle aged (~30-year-old woman equivalent) and therefore may not appropriately reflect the distinct cardiovascular and metabolic responses of breast cancer development that are seen in older women. It has been shown that older rodents develop more aggressive breast cancer than younger rodents [82], and that there is a linear decline in cardiovascular fitness across the lifespan [24], therefore greater tumor growth and decrements in exercise tolerance may be seen in aged breast cancer rats. Younger rodents were specifically selected for this investigation because we wanted to unveil the effects of tumor bearing alone on exercise capacity versus other potential confounding factors (i.e., age, body weight, cardiovascular disease, diabetes). Lastly, we did not use a separate group of non-cancerous control animals to compare exercising measurements. However, the primary focus was to effectively demonstrate changes in VO₂max attributable to breast cancer and longitudinal measurements allowed us to analyze the extent of change within the same animals and minimize the number of animals consistent with best research practices.

Conclusion

This study provides evidence that 1) resting cardiac function and VO₂max during treadmill running remains unchanged between pre- and post-breast cancer assessments and 2) breast cancer contributes to exercise intolerance by reducing economy and elevating the O₂ cost of running. Collectively, these findings indicate that intrinsic muscle dysfunction may be present in breast cancer patients before the initiation of neoadjuvant or adjuvant therapies and molecular targets, such as PGC-1 α , may serve as a promising approach to improve skeletal

muscle function in individuals with breast cancer prior to adjuvant care. Exercise can reduce mortality by ~40% in breast cancer patients [83] and future research focused on understanding the mechanisms by which breast cancer affects muscle function could enhance the development of tailored exercise programs to improve physical fitness and overall health outcomes for individuals with breast cancer.

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Disclosure of conflict of interest

None.

Address correspondence to: Ramona E Weber, Department of Kinesiology, Kansas State University, Manhattan, KS 66506, USA. E-mail: monaw@ksu.edu

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