Exercise training does not affect anthracycline antitumor efficacy while attenuating cardiac dysfunction

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Exercise training does not affect anthracycline antitumor efficacy while attenuating cardiac dysfunction. Am J Physiol Regul Integr Comp Physiol 309: R675–R683, 2015. First published August 5, 2015; doi:10.1152/ajpregu.00185.2015.—Highly effective anthracyclines, like doxorubicin (DOX), have limited clinical use due to protracted cardiotoxic effects. While exercise is known to be cardioprotective, it is unclear whether exercise compromises chemotherapy treatment efficacy. To determine the effect of exercise training on DOX antitumor efficacy as well as DOX-induced cardiotoxicity, female Fisher 344 rats were randomly assigned to sedentary + saline (SED+SAL), SED+DOX, wheel run exercise training + SAL (WR+SAL), or WR+DOX. On week 11, animals were inoculated with 1×10⁶ MatBIII tumor cells. Once tumors reached ~1 cm in diameter, animals were treated with 12 mg/kg of DOX or SAL. Animals were killed 1, 3, or 5 days following treatment. Tumor growth and cardiac function were measured at each interval. DOX accumulation and multidrug resistance protein (MRP) expression were quantified in tumor and heart tissue. No significant difference (P > 0.05) existed between DOX-treated SED and WR groups for tumor measurements. Exercise preserved cardiac function up to 5 days following DOX treatment. Exercise reduced ventricular DOX accumulation and upregulated ventricular MRP1 and MRP2. In contrast, no differences were observed in DOX accumulation or MRP expression in tumors of SED and WR animals. Endurance exercise had no effect on DOX antitumor efficacy as evidenced by a definitive DOX-induced reduction in tumor growth in both the SED and WR groups. Although exercise did not affect the antitumor efficacy of DOX, it still provided protection against cardiac dysfunction. These effects may be mediated by the degree of DOX tissue accumulation secondary to the regulation of MRP expression.

doxorubicin; MatBIII; cancer; physical activity; accumulation

Structured, exercise-based rehabilitation programs are becoming an important adjuvant therapy in the treatment of many cancers. Preliminary studies show these rehabilitation programs to be safe and effective whether conducted during or after cancer treatments. Regular exercise in these cancer populations significantly improves cardiopulmonary and musculoskeletal function, attenuates many common treatment-related side effects, and enhances quality of life (37, 38). However, there is still much that we do not know regarding the implementation of exercise rehabilitation in these individuals. Although multiple studies report that physical activity reduces the risk for recurrence and mortality in breast and colorectal cancer patients (19, 23, 34), others have shown that physical activity is not significantly associated with cancer-specific survival or relapse-free period (9). Conclusions are further clouded by cause virtually all reports on cancer and physical activity are observational studies as opposed to randomized controlled trials. The fact that exercise has not undergone a rigorous risk-to-benefit analysis in cancer populations has been described by others (5, 26) and further highlights the need to better understand possible adverse effects of exercise in cancer patients.

Although there are a number of possible adverse effects of exercise in cancer populations, one possibility could be reduced treatment efficacy if exercise is conducted during treatment. We sought to determine whether exercise training alters the efficacy of chemotherapeutic treatments while still providing protection against negative drug-induced side effects. To address this, we used a well-characterized rat model of exercise-induced protection against doxorubicin (DOX) toxicity that has been used by our laboratory (15, 22, 25, 36) and others (1, 39). DOX is a broad-spectrum chemotherapeutic agent that has been among the most widely utilized drugs ever developed. Despite its highly beneficial antitumor effects, clinical use of DOX is limited by serious, possibly life-threatening, cardiotoxicity. Exercise training has been shown to attenuate a number of DOX-induced side effects, such as cardiac dysfunction (18, 21, 36), skeletal muscle dysfunction (22), and vascular dysfunction (15), and protects against a number of deleterious cellular and molecular changes that are believed to contribute to this dysfunction (1, 39).

We have previously shown that exercise provides cardioprotection against DOX-induced cardiotoxicity and that this protection is associated with reduced accumulation of DOX within cardiac tissue (25). Reduced accumulation may be related to the expression of multidrug resistance proteins (MRPs) (16), which have been shown to be upregulated by exercise (13, 14, 31). MRPs are located in the cellular plasma membrane where they efflux a broad range of substrates, including chemotherapeutic agents, such as DOX. Tumor cells expressing high levels of MRPs are capable of extruding therapeutic drugs, thereby making them resistant to therapy. Overexpression of MRPs is linked to poor outcomes in multiple cancer types, making this a serious threat to the efficacy of chemotherapeutic treatments. While exercise clearly protects against DOX-induced cardiotoxicity and reduces accumulation of DOX in cardiac tissue, it is unclear whether this protection is mediated by an upregulation of MRPs. Furthermore, it is unclear whether exercise confers this protective effect on host tumors and whether it too is related to the expression of MRPs. The purpose of this investigation was to 1) examine whether exercise-mediated cardioprotection is related to the extent of DOX accumulation and MRP expression in the heart, and 2) determine whether chronic exercise training prior to cancer treatment impacts the therapeutic efficacy of DOX, and whether
this is related to the extent of DOX accumulation and MRP expression in host tumors.

METHODS

Experimental design. Twelve-week-old female Fisher 344 rats (Harlan, Indianapolis, IN; n = 132) were housed in a temperature-controlled facility with a 12:12-h light-dark schedule. Rats were given standard rat chow and distilled water ad libitum. All procedures were approved by the University of Northern Colorado’s Institutional Animal Care and Use Committee and complied with the Animal Welfare Act guidelines.

Rats were randomly assigned to sedentary (SED; n = 66) or wheel running (WR; n = 66) groups on day 1 of the study. SED animals were restricted to normal cage activity throughout the entire study. Animals in the WR group were housed in cages equipped with a commercially available running wheel, and activity was recorded using a Vital View data acquisition system (MiniMitter, Bend, OR). WR animals had access to wheels 24 h/day, 7 days/wk. After 11 wk of cage activity or wheel running, rats were inoculated with a rat mammary adenocarcinoma cell line. After inoculation SED animals continued their normal cage activity, while WR animals continued to voluntarily exercise. Once tumors reached a minimum of 1 cm in width, WR animals were removed from wheel cages, and all experimental animals (both SED and WR) were restricted to normal cage activity for the remainder of the study. The time interval between inoculation and the tumor reaching a minimum of 1 cm ranged between 1 and 2 wk. All animals were restricted to cage activity 24 h prior to treatment with DOX or SAL. After this 24-h period, animals were further randomly subdivided into DOX or SAL groups: SED+ SAL (n = 30), SED+DOX (n = 36), WR+ SAL (n = 30), and WR+DOX (n = 36). Animals in DOX groups received a bolus injection of 12 mg/kg ip of DOX (50 mg/ml; Bedford Labs, Bedford, OH). Animals in SAL groups received an equivalent volume of 0.9% saline. Subgroups of rats were killed at 1, 3, or 5 days postinjection.

Cell culture, tumor inoculation, and measurement. A rat mammary gland tumor cell line, 13762 MatBIII [American Type Culture Collection (ATCC); Manassas, VA], was used to grow a localized subcutaneous tumor in each animal. Cells were suspended in McCoy’s 5a modified medium (ATCC; Manassas, VA) containing 10% FBS (ATCC). Cells were grown in an incubator at 37°C with 60–80% humidity and 5% CO2. On week 11 of the study, rats were inoculated subcutaneously in the left leg flank with 1 x 10^6 cells (11, 30). Rats were weighed, tumors were measured, and body condition (45) was assessed twice during the tumor growth period. Tumor length, width, and thickness were measured with calipers and recorded, while the rat was sedated (40 mg/kg ip ketamine). These measurements were used to estimate tumor mass, relative tumor mass, and tumor volume. Tumor mass was estimated using the following formula: mass (g) = 0.79768 + (0.000456 × length × width × thickness of tumor in mm) (6). Relative tumor mass was calculated (in grams) as follows: estimated tumor mass/total body mass = estimated tumor mass. Tumor volume was calculated using the following formula: volume (mm^3) = (a × b × c)/2, where a was the longest diameter and b was the shortest diameter (43). Animals were to be euthanized if estimated tumor mass exceeded 25% of body mass, the percent loss of tumor-free body mass exceeded 25% of starting mass, or they received a body condition score (45) of 2 or below on two consecutive assessments, or the tumor became ulcerated. At the time of euthanasia, tumors were flash frozen in liquid N2, and stored at −80°C for subsequent biochemical analyses.

Assessment of cardiac function. In vivo cardiac function was analyzed 1, 3, or 5 days after DOX or SAL treatment using echocardiography, as previously described by our laboratory (18). Transthoracic echocardiography was conducted on sedated rats using a Toshiba Nemio 30 ultrasound with a 10-MHz pediatric transducer (Tustin, CA). Upon sedation (ketamine, 40 mg/kg ip), the anterior and left lateral thoracic regions were shaved. M-mode images of the left ventricle (LV) were obtained by short axis view to measure LV-end systolic and diastolic diameters and fractional shortening.

Flow images were obtained from an apical view using pulsed wave Doppler and used to measure maximal flow velocity through the mitral (MVmax) and aortic (AVmax) valves. For all echocardiographic measures, data were averaged from three consecutive cardiac cycles. All M-mode and Doppler measurements were made in accordance with guidelines established by the American Society of Echocardiography and were analyzed using UltraLing ultrasound management system (New York, NY).

Following echocardiography, cardiac function was analyzed ex vivo using an isolated working heart model (ADInstruments, Colorado Springs, CO). Each animal was anesthetized with an injection of heparinized (500 U ip) pentobarbital sodium (50 mg/kg), and the heart was quickly excised and placed into ice-cold Krebs-Henseleit buffer (in mM: 120 NaCl, 5.9 KCl, 2.5 CaCl2, 1.2 MgCl, 25 NaHCO3, 17 glucose, and 0.5 EDTA) aerated with 95% O2–5% CO2. The ascending aorta was cannulated; the heart was cleaned of all connective tissue and then subjected to retrograde perfusion until all blood was

Table 1. Animal characteristics at time of death

<table>
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<tr>
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<th>SED+ SAL</th>
<th>SED+ DOX</th>
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Values are expressed as means ± SD. SED, sedentary; SAL, saline; DOX, doxorubicin; WR, wheel run endurance exercise training. Estimated tumor mass (g) = 0.79768 + (0.000456 × length × width × thickness of tumor in mm) (6). Relative tumor mass (g) = estimated tumor mass/total body mass = estimated tumor mass. *P < 0.05 vs. SED+ SAL. $P < 0.05 vs. SED+ SAL, SED+ DOX. #P < 0.05 vs. all other groups.
cleared from the coronary vasculature. The pulmonary vein was then cannulated, and blood flow was redirected from the aorta to the left atrium to initiate the working heart mode. Once stabilized, preload and afterload were standardized at 10 cmH2O and 100 cmH2O, respectively. A microtip catheter pressure transducer (Millar, Houston, TX) was placed into the left ventricle via the apex for determination of LV developed pressure (LVDP), LV maximal rate of ventricular pressure development (\(\frac{dP}{dt}\)), and LV maximal rate of pressure decline (\(\frac{-dP}{dt}\)). Data collection began following a 10-min equilibration period. Hearts were paced at 240 beats per min using electrodes attached to the cannulas. Data were collected and analyzed using a PowerLab data acquisition system (ADInstruments). Following isolated working heart experiments, the left ventricle was isolated, flash frozen in liquid N2, and stored at −80°C for subsequent biochemical analyses.

**High-performance liquid chromatography.** Tissue samples were prepared and analyzed according to methods described by our laboratory (24). Frozen LV and tumor samples (~50 mg) were homogenized in 0.067 M phosphate buffer with a Virtishear homogenizer (Virtis, Gardner, NJ) at 8,000 rpm for 1 min. For protein precipitation, a 50:50 mixture of 40% ZnSO4 and HPLC-grade methanol (200 μl) was added to the homogenate (150 μl). For an internal standard, 50 μl of daunorubicin (500 ng/ml; Sigma, St. Louis, MO) was added to the sample, and then vortexed for 1 min and centrifuged at 1,500 g for 10 min. The supernatant was collected, filtered through a 0.2-μM syringe filter, and 20 μl of the sample was injected directly onto the column (initiating the analytical method).

The stationary phase featured a reverse-phase Zorbax C8 column (Agilent Technologies, Santa Clara, CA). Quantification of DOX and daunorubicin was performed using a Shimadzu HPLC system (Shimadzu, Japan) containing a degasser, dual-pump chromatograph, diode array detector, and fluorescence detector. The fluorescence detector was set to an excitation of 470 nm and emission of 550 nm, followed by analysis.

### Table 2. Mean weekly wheel-running distances before and after tumor inoculation

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<th>WR + SAL</th>
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<th>WR + DOX</th>
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<td>Mean distance after tumor inoculation, m</td>
<td>37,622</td>
<td>3,558</td>
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Tumor burden did not influence wheel run exercise capacity of tumor-bearing rats. SAL, control saline; DOX, doxorubicin. Data are expressed as means ± SD.

**Fig. 1.** Tumor characteristics. Endurance exercise did not influence the anti-tumor efficacy of DOX treatment, as measured by wet tumor mass (A) or tumor volume (B). Tumor volume (mm³) = \((a \times b^2)/2\), where a is the longest diameter, and b is the shortest diameter. SED, sedentary; SAL, saline; DOX, doxorubicin; WR, wheel run exercise training. Data are expressed as means ± SD. *P < 0.05 vs. WR+SAL, 3 day. +P < 0.05 vs. SED+SAL, 5 day.

**Fig. 2.** Tumor DOX accumulation and multidrug resistance protein expression at one, three, and five days post DOX injection. Exercise does not affect tumor DOX accumulation (A), tumor MRP1 expression (B), or tumor MRP2 expression (C). SED, sedentary; WR, wheel run exercise training. Data are means ± SD.
and flow was set to 1.0 ml/min. The mobile phase started with a 4-min phase that consisted of a 75:25 mixture of 10 mM phosphate buffer and acetonitrile, then took 4 min to change to a linear gradient of 50:50, which was held for an additional 4 min. Next, the mobile phase changed to 5:95 over a 6-min period, and then was changed back to initial concentrations by means of a linear gradient. DOX and daunorubicin eluted from the column within the 20-min method. All chemicals used were HPLC grade.

**Western blot analysis.** LV and tumor homogenates were analyzed for MRP1 and MRP2 expression by Western blot analysis. Approximately 100 mg of tissue was homogenized in RIPA buffer (1:100 wt:vol; Sigma-Aldrich, St. Louis, MO) with a Virtishear homogenizer (Virtis, Gardner, NJ). Homogenates were centrifuged for 10 min at 10,000 g, and the supernatant was collected for analysis. Protein concentration was determined using the Bradford method (4). Protein was loaded onto 12% polyacrylamide gels and separated by SDS-PAGE. Proteins were transferred to nitrocellulose membranes and incubated with primary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) for MRP1 and MRP2 (1:500 dilution, anti-rabbit). Membranes were developed using WesternBreeze Immunodetection kits (Invitrogen, Carlsbad, CA), and protein band intensity was analyzed using densitometry software (ImageJ, National Institutes of Health, Bethesda, MD).

**Statistical analysis.** All data are presented as means ± SD and were analyzed using GraphPad Prism statistical software (La Jolla, CA). A one-way ANOVA was performed for 1-day, 3-day, and 5-day variables to determine differences between groups (SED+SAL, SED+DOX, WR+SAL, WR+DOX). Additionally, for accumulation and MRP protein expression variables, a one-way ANOVA was performed for SED vs WR groups at each time point (1 day, 3 day, and 5 day) for LV and tumor samples to determine differences within groups (SED, WR). All analyses were two-tailed, and an alpha level of 0.05 was used to define statistical significance. If a significant difference (P < 0.05) was identified, Tukey’s post hoc testing was performed to identify where significant differences existed.

**RESULTS**

**General observations.** Following tumor inoculation, tumors reached at least 1 cm in diameter in 1–2 wk. Once tumors reached the 1-cm threshold, animals were treated with either SAL or DOX. At the time of SAL/DOX treatment, there were no significant differences (P > 0.05) between groups in terms of body mass or tumor volume (data not shown). At time of death (1, 3, and 5 days post-SAL/DOX treatment), there were no significant differences (P > 0.05) between group body masses. However, there was a significant difference between groups in both mean estimated tumor mass and heart mass at the time of death (Table 1). At 1 and 3 days posttreatment, SED+DOX heart mass was significantly lower (P < 0.05) compared with all other groups (SED+SAL, WR+SAL, WR+DOX). At 1 and 3 days post-DOX treatment, WR+SAL heart mass was significantly greater (P < 0.05) than SED groups (SAL and DOX). It should be noted that no animals had to be euthanized and removed from the study due to tumor sizes becoming too large or animal body conditioning dropping too low. No significant differences (P > 0.05) existed between groups for mean weekly wheel run distances (Table 2), indicating that weekly running volume was equivalent. This also indicates that no relationship could be determined between the amount of weekly physical activity and tumor size, tumor DOX accumulation, and tumor MRP1 or MRP2 expression.

**Tumor evaluation.** Endurance exercise training had no effect on DOX’s antitumor efficacy. At 1 day posttreatment, there were no significant differences (P > 0.05) between groups for any tumor measurements. At 3 days posttreatment, SED+DOX wet tumor mass (Fig. 1) was significantly less (P < 0.05) compared with WR+SAL and SED+SAL. At 5 days posttreatment, SED+SAL tumor volume and wet tumor mass were significantly greater (P < 0.05) compared with DOX-treated groups (SED and WR). These data clearly show that the antineoplastic effect of DOX was not influenced by chronic exercise training since there was no significant difference in tumor size between the sedentary and exercise-trained group at any time interval.

**Tumor doxorubicin accumulation and MRP expression.** To further elucidate the effect of exercise training on DOX’s antitumor efficacy, tumor tissue accumulation of DOX (Fig. 2A) was determined by high-performance liquid chromatography, and tumor expression of MRP1 and MRP2 was measured by Western blot analysis (Fig. 2B and C). At 1, 3, and 5 days posttreatment, there were no significant differences (P > 0.05) in the amount of DOX accumulating within the tumor tissue between SED and WR animals. There were also no differences

<p>| Table 3. Echocardiogram derived blood flow velocity and cardiac function measurements |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
|                                                | SED+SAL                                      | SED+DOX                                      | WR+SAL                                       | WR+DOX                                       |</p>
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Values are expressed as means ± SD. Cardiac function measured in vivo by echocardiography revealed that DOX does induce cardiac dysfunction and that endurance exercise training is able to preserve function. \( V_{\text{max}} \), maximal blood flow velocity. *P < 0.05 vs. SED+SAL. †P < 0.05 vs. WR+DOX.
(P > 0.05) between these groups (SED vs. WR) across time, indicating that exercise did not influence tumor DOX extrusion over time. Furthermore, there was no significant difference (P > 0.05) in tumor tissue MRP1 or MRP2 expression between either the DOX-treated group (SED vs. WR). Collectively, these data suggest that endurance exercise training does not influence the antitumor efficacy of DOX.

Cardiac function evaluation. Cardiac function measured in vivo by echocardiography (Table 3) revealed that while DOX did induce cardiac dysfunction, endurance exercise training was able to offset dysfunction up to 5 days post-DOX treatment. At 1, 3, and 5 days post-DOX treatment, SED+DOX had significantly slower aortic and mitral blood flow velocities, lower fractional shortening (FS), and longer heart rate intervals compared with SED+SAL, except for one measure. Furthermore, SED+DOX had significantly slower mitral V_max on day 1, significantly slower aortic V_max and lower FS on day 3, and significantly slower aortic V_max on day 5 compared with WR+DOX, indicating preserved cardiac function by endurance exercise. WR+DOX did not significantly differ from SED+SAL in mitral V_max until 3 days after DOX treatment or in heart rate interval until 5 days after DOX treatment.

Fig. 3. Ex vivo cardiac function. Endurance exercise preserves cardiac function up to 5 days post-DOX treatment. Top: left ventricular developed pressure; middle: maximal rate of pressure development; bottom: maximal rate of pressure decline. Column 1: 1 day post-DOX/SAL treatment; column 2: 3 days post-DOX/SAL treatment; column 3: 5 days post-DOX/SAL treatment. SED, sedentary; SAL, saline; DOX, doxorubicin; WR, wheel run endurance exercise training. Data are expressed as means ± SD. *P < 0.05 vs. SED+SAL, †P < 0.05 vs. WR+DOX.
The cardioprotective effect of exercise was further confirmed by ex vivo cardiac function analysis using the isolated perfused working heart, as summarized in Fig. 3. At 1 day posttreatment, SED/DOX LVDP and +dP/dt were significantly lower ($P < 0.05$) compared with SED+SAL. Three days posttreatment, DOX group −dP/dt was significantly lower ($P < 0.05$) compared with SED+SAL. Five days posttreatment, SED+DOX LVDP was significantly lower ($P < 0.05$) compared with SED+SAL and WR+DOX, and SED+DOX +dP/dt was significantly lower ($P < 0.05$) compared with SED+SAL. It should be noted that nontumor-bearing controls in our laboratory have LVDPs approximating 115 mmHg (unpublished data). Since all animals in this study were tumor-bearing, our control values were lower than what we typically observe. It is believed that the lower ventricular pressures observed in the present study are the result of a negative effect of tumor burden on cardiac function. Investigation of the effects of tumor burden on cardiac function in this model is a current area of study in our laboratory.

**Cardiac doxorubicin accumulation and MRP expression.**

Left ventricular DOX accumulation, as measured by HPLC, is presented in Fig. 4A. At 1 day posttreatment, significantly more ($P < 0.05$) DOX accumulated in the SED LV compared with the groups killed 3 or 5 days post-DOX treatment. The same pattern held true for the WR exercise-trained animals when compared across time (1 day vs. 3 days vs. 5 days post-DOX injection). Additionally, WR endurance exercise significantly reduced ($P < 0.05$) the amount of DOX accumulating within the LV at day 1, but was not significantly different at later time points (3 or 5 days post-DOX injection).

Left ventricular expression of MRP1 and MRP2 was determined by Western blot analysis (Fig. 4, B and C). Analysis revealed that WR exercise significantly upregulated ($P < 0.05$) LV MRP1 expression 1, 3, and 5 days post-DOX treatment compared with SED+DOX. WR exercise also significantly upregulated ($P < 0.05$) LV MRP2 expression 1, 3, and 5 days post-DOX treatment compared with SED+DOX. These data indicate that chronic endurance exercise training decreases the amount of DOX accumulating within the left ventricle and that this may be mediated, at least in part, by the upregulation of MRP1 and MRP2.

**DISCUSSION**

This is the first study to show that chronic endurance exercise training prior to cancer treatment initiation does not compromise anthracycline antitumor efficacy, while still protecting against its cardiotoxicity. Exercise attenuated DOX-induced declines in both in vivo and ex vivo measures of cardiac function, yet tumor growth was equally inhibited by DOX in sedentary and exercised groups. Furthermore, although less DOX accumulated in left ventricular tissue from exercised animals, no significant differences in tumor DOX accumulation were observed between sedentary and exercised animals. Although a number of different factors may contribute to this observation, we investigated the possibility that this was related to the expression of multidrug resistance proteins. Our results suggest that there may be a differential regulation of MRPs in the heart vs. tumors. Cardiac MRP1 and MRP2 upregulation was significantly higher in exercised animals compared with sedentary animals, yet there were no differ-
ences in tumor MRP regulation at any time point after exposure to DOX.

Previous studies have outlined the protective effects of exercise against anthracycline cardiotoxicity, but only two previous studies have been conducted in a tumor-bearing model. Data presented here suggest that the protective effects of exercise are observable even in tumor-bearing animals, which is particularly important considering that cancer alone can induce multiple cardiac abnormalities. Cancer has been shown to have a number of adverse effects on the heart, including decreased glucose metabolism (10), enhanced proteolysis (8), increased oxidative stress (3, 33), and cardiac atrophy, independent of energy intake (33, 42). Tian et al. (42) reported that tumor-bearing mice showed cardiac fibrosis, inflammation, disrupted sarcomere alignment, mitochondrial damage, unfavorable shifts in myosin heavy-chain (MHC) expression, and overt in vivo functional deficits. Thus, even in the face of these combined cardiac insults—cancer and DOX—exercise was still capable of providing protection against anthracycline cardiotoxicity. It should be noted that there was a trend toward reduced tumor growth in exercised animals, and the reduced tumor burden associated with wheel running may partially explain improved cardiac function independent of direct effects of the exercise intervention on the heart.

MatBIII cells utilized in this study originated from a transplantable rat ascites tumor from a primary 13762 mammary adenocarcinoma. MatBIII cells have a high metastatic potential and produce metastases in axillary lymph nodes, lungs, and liver within 18 days of implantation (17). DOX has been quantified in MatBIII tumors (43, 44), and DOX treatment reduces tumor size and extends survival in rat models (29), making this a relevant model to study the interaction of physical activity and resistance to chemotherapy. Multidrug resistance (MDR) by tumor cells is a significant obstacle to the success of first line chemotherapeutic regimens across a wide range of cancers. MDR is mediated by several mechanisms, one of which being the expression of ATP-binding cassette (ABC) transporters. The impact of increased expression of ABC transporters and, in particular, MRPs, on tumor resistance has been demonstrated utilizing cell culture and animal models (7, 20). Clinical studies suggest that increases in MRP expression correlate with decreases in response to various chemotherapeutic drugs, as well as reductions in recurrence-free survival and overall survival (12). Thus, the need to understand the interaction of exercise and MDR is highlighted. Only two other studies have investigated the effects of exercise on anthracycline efficacy. Jones et al. (27) observed median tumor growth delay in mice transplanted with MDA-MB-231 breast xenografts that received no treatment, received DOX only, or received DOX and engaged in an 8-wk forced treadmill exercise regimen. Median tumor growth delay was increased in both DOX-only and DOX + exercise groups compared with controls, suggesting that exercise did not attenuate DOX efficacy. Additionally, Sturgeon et al. (41) found that 2 wk of low-intensity exercise (10 m/min, 45 min/day, 5 days/wk) significantly decreased murine melanoma tumor size in DOX-treated animals compared with sedentary animals treated with DOX and exercise-trained controls, indicating exercise increased DOX’s antitumor efficacy. However, this low-intensity exercise model did not protect against DOX-induced cardio-
toxicity. To our knowledge, the present study is the first to investigate DOX accumulation and MRP expression in MatBIII tumors in vivo. Expression of MRPI and MRP2 were virtually identical in tumors from sedentary and exercised animals, and this was accompanied by equivalent levels of tumor DOX accumulation and similar tumor growth attenuation patterns.

While only MRPI and MRP2 expression was quantified in this study, there are at least six other ABC transporters known to mediate cellular efflux of DOX, including p-glycoprotein (ABCB1; also MDR1), MRP3 (ABCC3), MRP6 (ABCC6), MRP7 (ABCC10) BCRP (ABCG2), and ABCA8. There is extensive redundancy of function among MDR proteins, which adds to the complexity of teasing out which ones may be involved with the observed responses in the heart and in tumors. Furthermore, it is possible that other factors may be involved with controlling DOX accumulation in hearts and tumors unrelated to mechanisms controlling DOX efflux. We did not quantify DOX metabolites, which could possibly be altered by exercise. One of the lipid peroxidation byproducts of DOX is the lipid aldehyde 4-hydroxy-2-nonenal (HNE) (28, 32). HNE is an electrophile with high reactivity toward protein residues, which may initiate myocardial dysfunction in the heart (28) or increase cellular injury and apoptosis in tumor cells (40). Doxorubicinol (DOXol), the major metabolite of DOX, has been shown to be equally toxic to cells as DOX (35, 46) and has been shown to increase its cellular accumulation up to twofold in ABCB1 knockout mice (46). DOX accumulation is also regulated by factors controlling cellular influx. DOX enters the cell via passive diffusion, which can be affected by its concentration gradient, the presence of cellular structures that have a high affinity for DOX, including cardiolipin and DNA, as well as factors that affect the permeability of the cell and its organelles. To our knowledge, no studies have investigated the possible role exercise may play in regulating DOX entry into the cell, and it is possible that some of the changes observed in the current study may be related to exercise-induced adaptations in DOX influx. It is unclear whether exercise can alter cardiolipin species distribution or appreciably change membrane characteristics, or whether these changes could impact DOX accumulation in hearts or tumors. Chronic exercise training has been shown to improve tumor vascularity (2), which may serve to improve chemotherapy delivery to the tumor and possibly enhance cellular influx. Overall, many questions persist regarding mechanisms underlying the differential regulation of cardiac vs. tumor DOX accumulation and ABC transporter expression, but MRPI and MRP2 may be key contributors to this process.

**Perspectives and Significance**

As the implementation of exercise-based cancer rehabilitation programs increases, our need to understand the risks associated with exercise in this population grows. An increasing number of studies have been published that suggest chronic exercise facilitates recovery from cancer treatments and improves quality of life. While these benefits are clearly an important step in establishing the utility of exercise in the rehabilitation process, it is unclear whether exercise enhances, diminishes, or has no influence on the efficacy of traditional cancer treatments. If in fact exercise compromises the efficacy
of cancer treatments, standardized treatment regimens must be reevaluated for those patients with a history of regular physical activity, and its utilization in the rehabilitation of cancer patients must be called into question. It was our hypothesis that exercise-induced cardioprotection is mediated by a multidrug resistance phenotype in which specific ABC transporter proteins are upregulated, and that while exercise will protect against DOX cardiotoxicity, exercise will not confer a multidrug resistance phenotype on host tumors. Preliminary evidence from this study suggests that this may be the case. Findings from the present study demonstrate that exercise does not compromise the anti-tumor efficacy of anthracyclines while still providing protection against its negative side effects. These data contribute to a growing body of evidence suggesting that exercise is indeed safe as an adjuvant therapy for cancer survivors.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: T.L.P. and R.H. conception and design of research; T.L.P. and R.H. performed experiments; T.L.P. and R.H. analyzed data; T.L.P. and R.H. interpreted results of experiments; T.L.P. and R.H. drafted manuscript; T.L.P. and R.H. edited and revised manuscript; R.H. approved final version of manuscript.

REFERENCES
32. Luo X, Evrovske Y, Cole D, Trines J, Benson LN, Lehotay DC. Doxorubicin-induced acute changes in cytotoxic aldehyde, antioxidanr


