Resistance Exercise Counteracts Tumor Growth in Two Carcinoma Rodent Models

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ABSTRACT

PADILHA, C. S., M. T. TESTA, P. C. MARINELLO, P. S. CELLA, F. A. VOLTARELLI, F. T. FRAJACOMO, R. CECHINI, J. A. R. DUARTE, F. A. GUARNIER, and R. DEMINICE. Resistance Exercise Counteracts Tumor Growth in Two Carcinoma Rodent Models. *Med. Sci. Sports Exerc.*, Vol. 51, No. 10, pp. 2003–2011, 2019. **Purpose:** Although resistance exercise (RE) is now recognized as an adjuvant in cancer treatment because of its capacity to prevent muscle wasting, weakness, and cachexia, it is unknown whether RE can mitigate tumor development. Two solid adenocarcinoma models (Walker-256 and Ehrlich) were used to investigate the effects of RE on tumor cell proliferation, growth, and aggressiveness parameters in tumor-bearing animals' life span. **Methods:** Walker-256 tumor-bearing rats and Ehrlich tumor-bearing mice were subjected to RE, which consisted of climbing a ladder apparatus with loads tied to their tails. After 4 wk, animals were euthanized, and tumors were excised and assessed for tumor microenvironment evaluation such as cell proliferation and apoptosis determination, collagen deposit, and presence of malignant tumor morphology. **Results:** Our data demonstrate that RE mitigated tumor growth and favored tumor end points such as lower Scarff–Bloom–Richardson histological grade tumor, denoting slow cell aberrant form and division, decreased tumor cell proliferation (evaluated by nucleus marked with antigen ki-67), and lower viable tumor area in both types of tumors studied. In addition, RE stimulated tumor microvessel density in Walker-256 tumor-bearing rats, but there was no change in their life span. **Conclusion:** RE may mitigate tumor growth and tumor malignancy parameters such as lower histopathological grade, assuming less nuclear pleomorphism and mitotic cells, smaller viable tumor area, and decreased tumor cell proliferation in both adenocarcinomas. In addition, RE induced tumor vascularization. **Key Words:** WALKER-256, EHRLICH, TUMOR CELL PROLIFERATION, CARCINOMA, MALIGNANCY

During the last decade, the use of physical exercise as an adjuvant in conventional cancer treatment and its potential benefits for cancer survivors have been discussed (1). In 2010, the American College of Sports Medicine convened an expert roundtable to issue exercise guidelines for cancer survivors (2), concluding that resistance exercise (RE) is a safe and important tool for clinical management, attenuating susceptibility to cancer itself and therapy side effects, such as fatigue, diminished physical functioning, and health-related quality of life (1,3). Recently, a meta-analysis performed by our group demonstrated that RE offers a myriad of benefits for cancer subjects undergoing adjuvant and neoadjuvant therapies, including improvements in muscle strength and muscle mass and reduction in body fat regardless of the type of treatment (4). These outcomes are relevant because both cancer itself and chemotherapy and radiotherapy promote muscle wasting and, consequently, weakness (5). Even with the evolution of the role of exercise in oncology, mainly on neuromuscular and body composition parameters, little is known about its effects on tumor microenvironment, physiology, and development. Few preclinical studies have demonstrated that moderate endurance exercise can mitigate or even delay the development of some types of tumors, including breast (6–8), colon (9,10), skin (11), and prostate (6). Indeed, despite research progression and

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international recognition, there are no preclinical studies testing if RE is able to change tumor development and aggressiveness.

The difficulties of examining exercise effects on the development of cancers in humans are obvious. Therefore, animal models, which reflect important aspects of tumorigenesis in humans, have been used. Walker-256 and Ehrlich are both murine mammary adenocarcinomas that were adapted to an ascites form by intraperitoneal serial passages (12) to solid tumors by subcutaneously cell implantation. Both Walker-256 and Ehrlich solid tumor models are widely used in experimental cancer because of its higher efficiency in producing free neoplastic cells and survival time length (13,14). In addition, these two tumors are also well explored for chemotherapeutic studies because they are easy to establish and maintain (12,14) as well as for easy tumor microenvironment and physiology assessment (15). Thus, our study is relevant because RE has been considered an important adjuvant in cancer treatment because of its potential to attenuate neuromuscular susceptibility to cancer itself, cancer therapies and complications such as muscle loss, cachexia, and weakness. However, the effects of RE on tumor microenvironment are unclear. The aim of the present study was to investigate the effects of RE on tumor cell proliferation, growth, and aggressiveness parameters of Ehrlich tumor-bearing mice and Walker-256 tumor-bearing rats, as well as tumor-bearing resistance-exercised life span. We hypothesize that RE may mitigate tumor growth and aggressiveness parameters as previously shown in endurance-exercised rodents.

METHODS

Animals. Thirty-two male Wistar rats 8–10 wk old and 32 male Swiss mice 6–8 wk old were obtained from the facilities of the State University of Londrina Animal Care Unit. The animals were housed under standardized conditions (22°C ± 1°C, 12-h light–12-h dark cycle, and 45%–65% air humidity), with free access to food and water. Both rats and mice were randomly divided into four groups (eight animals per group) as follows: sham inoculated (S), tumor bearing (TB), exercised (Ex), and tumor-bearing exercised (TBEx). Body weight was recorded weekly. The study was performed according to the national guidelines on animal experimentation and was approved by the Ethical Committee for National Experimentation of the State University of Londrina (nos. 28336.2014.38 and 9647.2015.12).

Study design. Both rats and mice from Ex and TBEx groups were subjected to a progressive RE protocol as described below. After 5 d of RE apparatus acclimatization, Walker-256 and Ehrlich tumor cells were inoculated subcutaneously at the right flank of rats and mice, respectively; the animals from sham groups received vehicle (phosphate-buffered saline [PBS]). Two days after inoculation, the exercised groups started the 4-wk RE protocol. Forty-eight hours after the last RE session, the animals were anesthetized with isoflurane (5%) and euthanized by exsanguination, with subsequent removal of tissues. Euthanasia was performed between 9:00 AM and 1:00 PM. Tumors were carefully excised and weighted and then histologically and immunohistochemically analyzed, as detailed below.

Walker-256 and Ehrlich tumor cell inoculation. Walker-256 and Ehrlich carcinoma cells were obtained from ascitic intraperitoneal tumor in host animals and inoculated as previously described by Padilha et al. (3) and Frajacomo et al. (13), respectively. Walker-256 and Ehrlich tumor cells were obtained from ascitic intraperitoneal tumor in host animals. Cell viability percentage was determined by trypan blue dye exclusion method (nonviable cells stained blue), using a Neubauer chamber. Rats from TB and TBEx groups received a Walker-256 cell suspension (4.0×10^7 cells) in 0.5 mL of PBS injected subcutaneously into the right flank. Mice from TB and TBEx groups received an Ehrlich carcinoma cell suspension (1×10^6) in 100 μL of PBS injected subcutaneously into the right flank. In both cases, sham animals were inoculated in the same region with PBS only.

RE protocol. RE consisted of a ladder climbing protocol, as described by Padilha et al. (3). The ladder climbing apparatus length was adapted for rats (1.1 × 0.18 m, 2-cm grid, 90° incline) and mice (0.55 × 0.18 m, 0.7-cm grid, 80° incline); hence, they favored 8–12 dynamic movements per climb. At the top of the ladder, a dark covered chamber was constructed for interval resting between climbing bouts. Animals from Ex and TBEx groups were subjected to four to eight ladder climbs with loads attached to their tails, which were progressively increased according to their daily performance. At the first training day, animals were submitted to carrying loads equivalent to 50%, 75%, 90%, and 100% of their body mass. After climbing successfully with 100% load (fourth climb), 30 g for rats and 3 g for mice were added to be lifted for each next climbing, until a total of eight climbs or a load that incapacitates the animal to climb the complete ladder length during three consecutive attempts is achieved. In the consecutive days, the maximum load achieved in the previous training session was used as a parameter to determine training load. This procedure was successively repeated three times per week over 4 wk, for a total of 12 training sessions.

The animals from S and TB groups remained sedentary during all experimental protocols because their movements were restricted to their cage space. The exceptions were the first and last days of experiment when they submitted to RE protocol for maximal carrying load determination. The maximal carrying load was measured weekly for Ex and TBEx rats and mice.

Tumor assessment. Tumor growth was recorded with a digital caliper (Sagyma Plus, 0–150 mm) every 2 d, measuring the higher and lower diameters of tumors. Tumor volume was calculated using the standard solid tumor formula: $V = 1 / 2 \times (D \times d2)$ (16), where V is the volume, D is the tumor's higher diameter, and d is the lower diameter. All measures were taken from the same examiner to minimize bias.

Histopathological analysis. For optical microscopy analysis, one portion of Walker-256 and Ehrlich tumors was fixed in 4% of formaldehyde for 24 h, dehydrated with graded ethanol, and embedded in paraffin blocks. Tumor sections (5 μm) were used for histological characterization and determination of fibrous tissue accumulation through hematoxylin and eosin stain and picrosirius red stain, respectively, as

previously described by Fonseca et al. (17). We used the Scarff–Bloom–Richardson histological grade, modified by Elston and Ellis (18), to evaluate the frequency of tubule formation (percentage of tumor composed by tubular structures), nuclear pleomorphism (change in nucleus cell size and uniformity), and cell mitosis (1 indicating few cells in division and 3 indicating several cells in division). Each of these features was assigned a score ranging from 1 (slower cell growth) to 3 (faster cell growth). Two representative hematoxylin and eosin–stained slide pictures of each animal (~48 pictures total) were graded in a blinded way by an experienced pathologist. The scores of each of the cellular features were then added together for a final sum that ranged between 3 and 9, as follows: grade 1 (low grade), score from 3 to 5; grade 2 (intermediate grade), score from 6 and 7; and grade 3 (high grade), score from 8 to 9. Picrosirius red–stained sections were used to determine fibrous tissue accumulation using a total of 30 images per group. The tumor tissue area occupied by collagen (stained red) was quantified for each visual field (19). The viable tumor area was calculated excluding collagen-rich/necrotic tumor area and expressed in percent of tumor tissue. All histological images were processed using ImageJ software (National Institutes of Health, Bethesda, MD).

Immunohistochemistry. Immunohistochemistry was performed on formalin-fixed paraffin-embedded Walker-256 and Ehrlich tumor sections mounted on silane-treated microscope slides. After deparaffinization and rehydration, the antigen-retrieval procedure was performed in an electric pressure cooker containing 10 mM citrate buffer (pH 6) for 5 min. After the tumor sections were cooled and washed with PBS, they were incubated with a solution containing 30% H_2O_2, methanol, and PBS containing 0.05% Tween 20 (v/v) (PBS-T) (1:1:8), at room temperature for 30 min in a humidified chamber to block the endogenous peroxidase activity. To suppress nonspecific binding, the slides were further rinsed with distilled water, washed with PBS for 5 min, and incubated with 3% bovine serum albumin (w/v) in PBS-T at room temperature for 30 min in a humidified chamber. After washing, Walker-256 and Ehrlich tumor sections were incubated with primary antibodies: antiproliferating nuclear cell antigen ki67 (Abcam no. ab16667 polyclonal antirabbit antibody, 1:50 in PBS-T) and anticluster of differentiation 31 (CD31, Cell Signaling no. 77699 monoclonal rabbit IgG antibody, 1:50 in PBS-T) for 2 h at 37°C, followed by a PBS washing and incubation with the secondary antibody (Abcam no. ab6721 goat antirabbit IgG HRP, 1:200 in PBS-T) for 1 h, at 37°C. The sections were then incubated with SIGMAFAST™ DAB with metal enhancer kit for 5 to 10 min, counterstained with hematoxylin, and mounted with slide mounting medium (DPX Sigma no. 06522) following routine procedures. Negative controls were obtained substituting the primary antibody for PBS-T. *In situ* Cell Death Detection Kit (Roche Applied Science, Indianapolis, IN) was used for TUNEL staining according to manufacturer's instructions, in which apoptotic cells could be visualized in paraffin-embedded tissue sections calorimetrically. All preparations were analyzed in a light microscope, and images were recorded with a coupled camera. A number between 10 and 15 photos were taken for each tumor section at 200× magnification. The images were analyzed using the image processing software ImageJ (originated at the National Institutes of Health). We colored the stained area from the initial image, and then the software quantified the percentage of the stained area. The percentage of the stained area corresponds to the percentage of the sample area that is specifically stained by the respective antibody. The percentage of the stained area was compared between the different groups.

Life span. For life span measurement, additional 24 Wistar rats (8–10 wk of age) were randomly allocated into two groups (12 rats per group): TB and TBEx. The rats were inoculated with Walker-256 tumor and were engaged in an RE routine, as described before. The end of the experiment for a given rat was defined as spontaneous death when the life span was measured. Once the inability to move, drink water, and eat or bad health signals such as nose bleeding, ulcers, and creepy hair have been detected, the rats were anesthetized and euthanized and their life span measured. Rats were checked daily for their health status and were engaged in RE program until the end of the experiment.

Statistical analysis. Data were reported as mean ± SD. A two-way ANOVA followed by Bonferroni's *post hoc* test was used to compare within- and between-group differences of tumor volume. For variables comparing TB with TBEx animals, Student's *t*-test was used. When the sham condition was compared with TB and TBEx animals, a one-way ANOVA followed by Tukey's *post hoc* test was used. Histological grade demonstrated nonnormal distribution and was tested using the Kruskal–Wallis test followed by the Dunn *post hoc* test. Life span data were analyzed for significant differences by the log-rank test. A two-sided Mann–Whitney *U*-test was used to test for differences in maximal life spans between TB and TBEx rats using the Statistical Package for the Social Sciences (version 18.0). A P value of <0.05 was significant in all cases.

RESULTS

General characteristics and RE. Body weight gain was significantly ($P < 0.05$) lower after 4 wk of inoculation with Walker-256 and Ehrlich tumors in both rodents; RE did not alter the body weight gain in any of the models (Fig. 1A and B). Training load increased significantly from the first to the fourth week for both RE-trained rats and mice when compared with sedentary animals (S and TB groups). Training load evolution demonstrated no differences between Ex and TBEx groups of Walker-256 tumor-bearing rats (Fig. 1C). RE-trained Ehrlich tumor-bearing mice presented significantly smaller training load than non-tumor-bearing exercised mice, only on the fourth week of RE protocol (Fig. 1D). Therefore, in both cases, load was one- and twofold higher in TBEx mice and rats, respectively, compared with sedentary controls and tumor-bearing mice and rats (S and TB groups).

The daily food intake was not different between groups (Walker-256: S 15.6 ± 2.3 $g \cdot d^{-1}$, TB 14.3 ± 2.9 $g \cdot d^{-1}$, and TBEx 14.6 ± 2.7 $g \cdot d^{-1}$; Ehrlich: S 6.6 ± 0.2 $g \cdot d^{-1}$, TB 6.0 ± 0.5 $g \cdot d^{-1}$, and TBEx 5.8 ± 0.6 $g \cdot d^{-1}$).

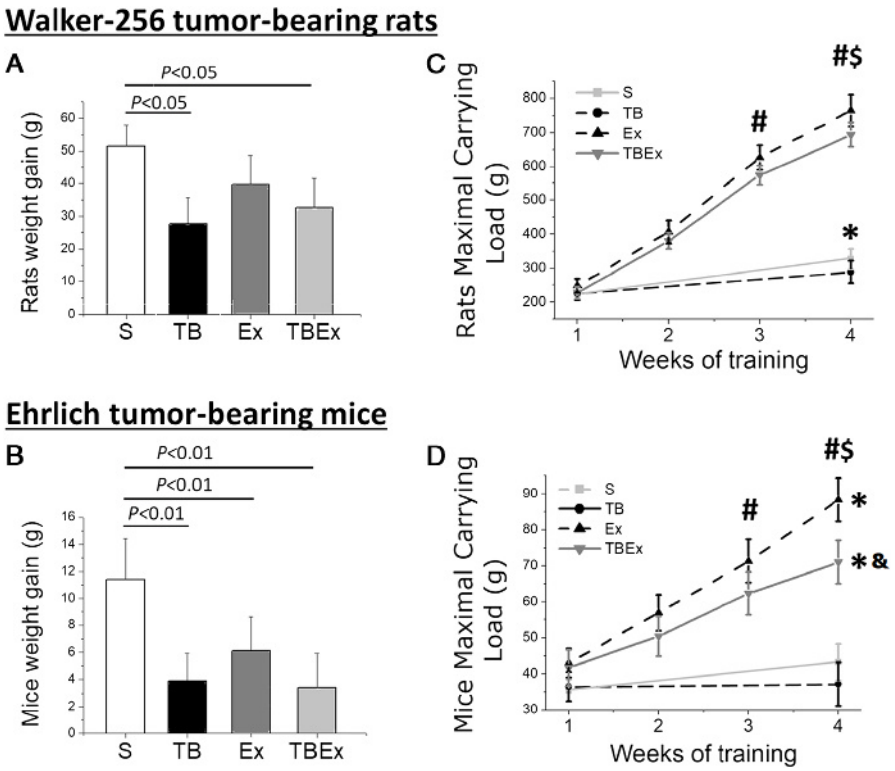

FIGURE 1—Walker-256 tumor-bearing rats (A) and Ehrlich tumor-bearing mice (B) body weight gain after 4 wk of experimentation. Rats (C) and mice (D) maximal carrying load evolution for 4 wk. Values are presented as mean ± SD. One-way ANOVA followed by Tukey's *post hoc* test was used in panels A and B. #Different from week 1. $Different from week 2. *Different from the S group. &Different from the TB group. £Different from the TBEx group ($P < 0.05$ by two-way ANOVA followed by Bonferroni *post hoc* test; C and D).

Effects of RE on tumor growth. Both Walker-256 and Ehrlich tumors grew progressively and significantly ($P < 0.05$) from the first to the third week and from the second to the fourth week, respectively, after tumor cell inoculation (Fig. 2A and B). The mean tumor weight for nonexercised groups were 13.8 ± 6.7 and 8.1 ± 3.1 g, 4 wk after the implantation of Walker-256

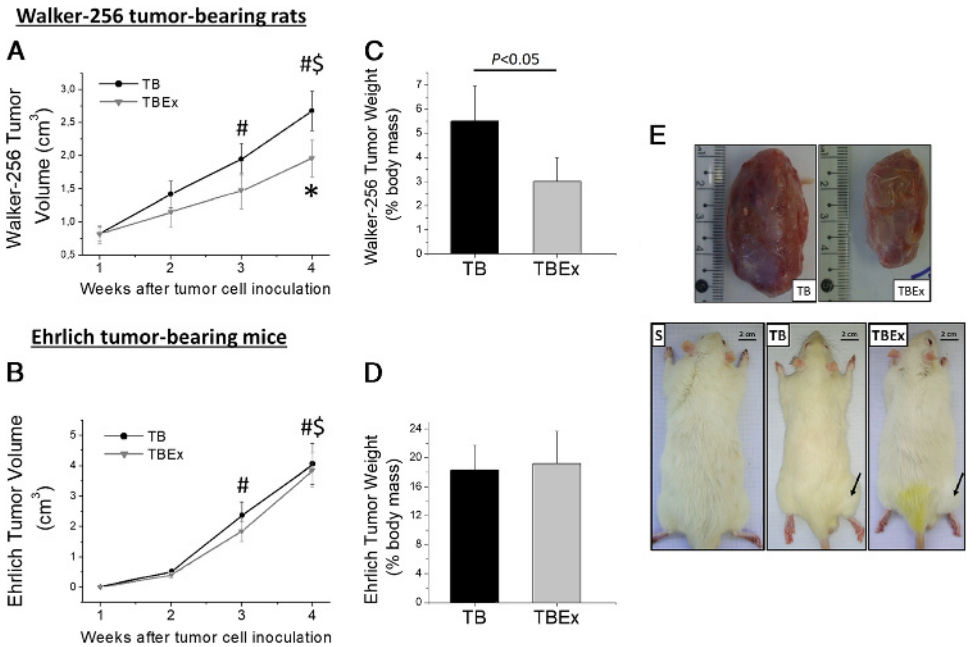

FIGURE 2—Walker-256 (A) and Ehrlich (B) tumor volume for 4 wk; final Walker-256 (C) and Ehrlich (D) tumor weight; *in situ* representative pictures (E) of Walker-256 tumor and inoculated rats after 4 wk of cell inoculation. Values are presented as mean ± SD. # Different from week 1. $Different from week 2. *Different from TB group ($P < 0.05$ by two-way ANOVA followed by Bonferroni *post hoc* test; A and B). Student's *t*-test was used in panels C and D. One-way ANOVA followed by Tukey's *post hoc* test was used in panels E and F.

and Ehrlich tumor cells, respectively. At this time point, the tumor mass corresponded to 5.5% ± 2.1% and 18.9% ± 6.2% of the body weight of rats and mice, respectively (Fig. 2C and D). The exercised Walker-256 tumor-bearing rats showed lower tumor volume compared with Walker-256 tumor-bearing sedentary rats (Fig. 2A, C, and G); the same did not occur in the exercised Ehrlich tumor-bearing mice (Fig. 2B and D). The apparent focus of metastasis in the abdomen and chest cavity was not observed.

Effects of RE on tumor histopathology. The histopathology demonstrated that both Walker-256 and Ehrlich tumors presented a fibrous rounded encapsulated solid tumor with great heterogeneity in cell composition and numerous necrotic zones predominantly in the central areas and enclosed by inflammatory cells (Fig. 3A and B). Both tumors presented nontubular structure and regular presence of neoplastic cells with frequent mitotic characteristic. The Scarff–Bloom–Richardson histological grade revealed that RE-trained animals presented lower nuclear pleomorphism, mitotic cell count, and overall score in both tumor-bearing Walker-256 and Ehrlich animals compared with sedentary tumor-bearing animals (Table 1). Sixty-seven percent of the tumors from sedentary animals (including Walker-256 and Ehrlich) were classified as grade 3, against 7% in TBEx group (TB: 16% grade 1, 17% grade 2, 67% grade 3 vs TBEx: 42% grade 1, 50% grade 2, 7% grade 3). The number of multinucleated and meganucleus cells as well as mitotic figures was also lower in TBEx compared with TB group (Fig. 3D and E).

Projections of connective tissue from the capsule to the interior of the tumor mass were common in both Walker-256 and Ehrlich tumors. In some areas, predominantly at the central ones, the deposit of connective tissue is more present. The RE increased the connective tissues of Walker-256 and Ehrlich tumors and decreased viable tumor areas when compared with tumor-bearing sedentary animals (Fig. 3B and C).

Effects of RE on tumor malignancy. Immunohistochemistry evaluation demonstrated that 4 wk of RE reduced the tumor cell proliferation of both Walker-256 and Ehrlich, demonstrated by reduced Ki67-marked cells (Fig. 4A). TUNEL-marked nucleus, however, did not demonstrate significant differences for tumor cell apoptosis in any of the models studied (Fig. 4B). TBEx rats (Walker-256) showed increased

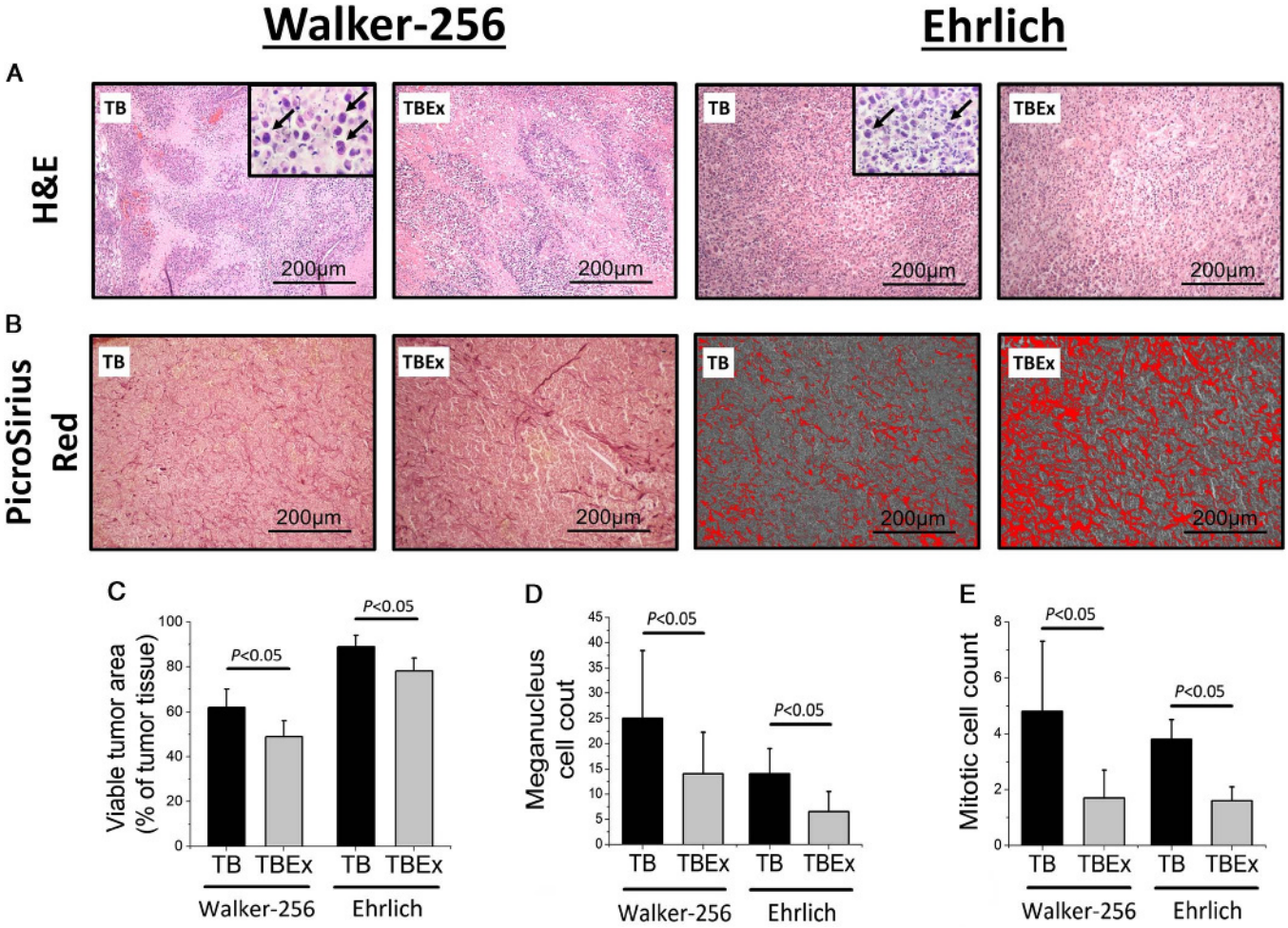

FIGURE 3—Representative light micrographs from the Walker-256 and Ehrlich tumors stained with hematoxylin and eosin (A), demonstrating tumor structural heterogeneity, with no folic formation and numerous meganucleus and mitotic cells (*arrow*). Picrosirius red demonstrated viable areas interspaced by necrotic zones infiltrated (B). Counting number of meganucleus (C) and mitotic cells (D) as well as viable tumor area (E). Values in panels C, D, and E are presented as mean ± SD. $P < 0.05$ by Student's *t*-test.

TABLE 1. Scarff–Bloom–Richardson histological grade determined in Walker-256 and Ehrlich tumors of sedentary (TB) and exercised (TBEx) animals.

	Walker-256		Ehrlich	
	TB	TBEx	TB	TBEx
Tubule formation	3 (0)	3 (0)	3 (0)	3 (0)
Nuclear pleomorphism	3 (1)	1 (1)*	3 (1)	2 (1)
Mitotic cell	2 (1)	1 (1)**	3 (1)	1 (1)*
Overall score	8 (2)	5 (1)*	9 (3)	6 (1)*

Values are presented as median (interquartile range). Intergroup differences were assessed by the Kruskal–Wallis test using the Dunn *post hoc* test.
*$P < 0.05$ vs TB.
**$P = 0.06$ vs TB.

angiogenesis, verified by a higher-density CD31-marked area (Fig. 4C).

Effects of RE on life span. The life span was not different between Walker-256 tumor-bearing sedentary and exercised rats (TB 38.5 ± 8.9 d vs TBEx 39.5 ± 10.6 d) (Fig. 5).

DISCUSSION

Evidence showing that RE can be a safe and effective adjuvant for cancer treatment has been emerging (4,20). However, the molecular mechanisms underlying this effect are unknown. Our data demonstrated that RE mitigated tumor growth and favored tumor end points, such as lower histopathological grade, assuming less nuclear pleomorphism and mitotic cells, smaller viable tumor area, and decreased tumor cell proliferation in both Walker-256 and Ehrlich adenocarcinomas. Also, RE stimulated tumor vessel density in Walker-256 tumor-bearing rats, but there was no change in their life span. These results follow several preclinical data using endurance exercise that demonstrated mitigating effects in different types of cancer, including breast, colon, and prostate carcinomas (8,10,21,22). Therefore, RE has been recently demonstrated to attenuate cancer morbidity and therapy side effects, such as muscle loss, cancer-associated cachexia, weakness, and health-related quality of life (1,3,4). To the best of our knowledge, this is the first study which demonstrated that RE can mitigate the development of tumor itself by decreasing aggressiveness parameters in the murine models of cancer. In this sense, these data stimulate further studies focusing on RE and its effects in cancer survivors.

Notably, RE counteracted both Walker-256 and Ehrlich tumor cell proliferation and decreased tumor viable area. Indeed, RE decreased the Scarff–Bloom–Richardson histological grade tumor assuming slow cell aberrant form and division. This is particularly relevant because histological grade has been an important prognostic indicator that can predict overall and metastasis-free survival in breast cancer (23). RE also decreased nuclear antigen Ki-67-marked tumor proliferating area, recognized to be associated with poor prognosis of patients with different types of cancer (24). Also, exercised animals presented higher conjunctive tissue deposition, smaller active tumor cells per total tumor volume, and smaller Walker-256 tumor size and weight. Taking together, these data provide the first evidence that RE may regulate tumor physiology and microenvironment to a less aggressive phenotype.

The molecular mechanisms underlying the effects of exercise on tumorigenesis remain to be elucidated; some hypotheses, therefore, have been explored in the literature. Probably one of the first ideas proposed was that increasing energy expenditure caused by exercise training could decreases energy supply

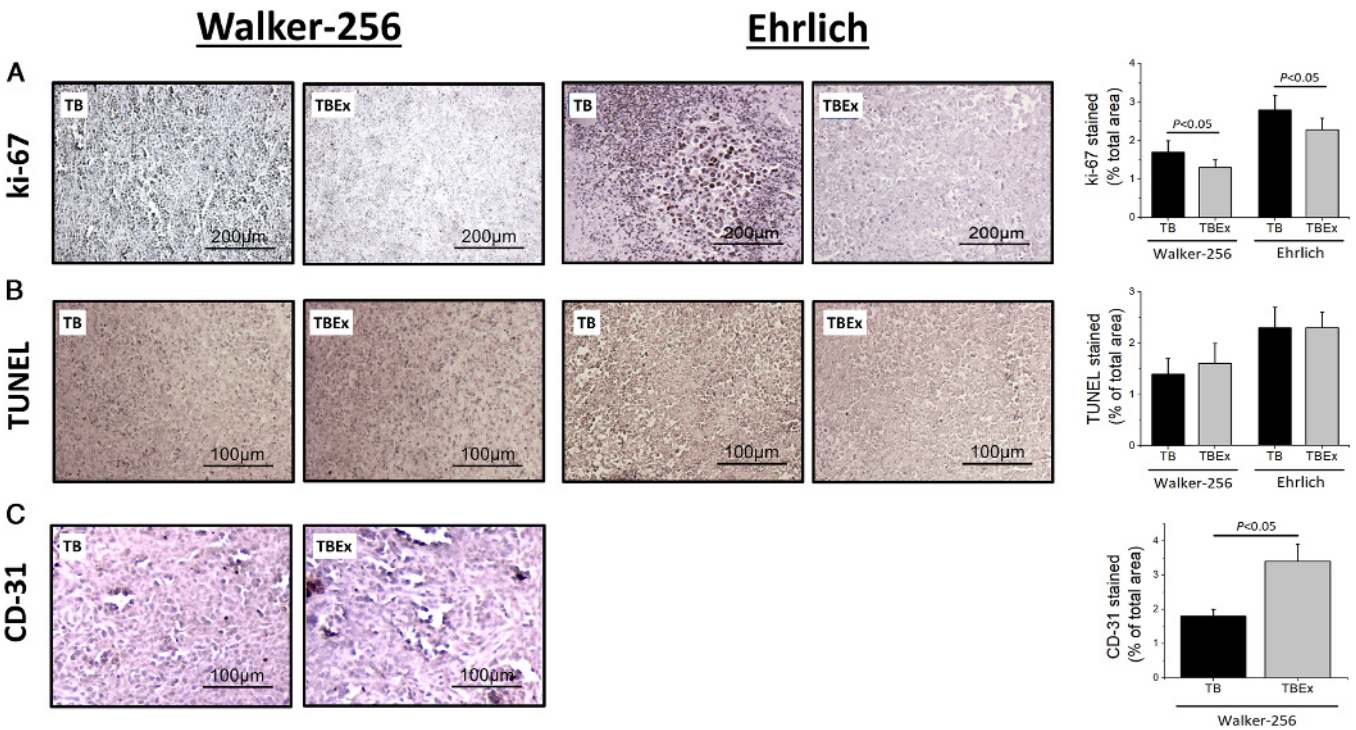

FIGURE 4—Representative light micrographs from Walker-256 and Ehrlich tumor immunohistochemistry and % of stained area for ki-67 (A), TUNEL (B), and CD-31 (C) detection. Values are presented as mean ± SD. $P < 0.05$; difference is from Student's *t*-test.

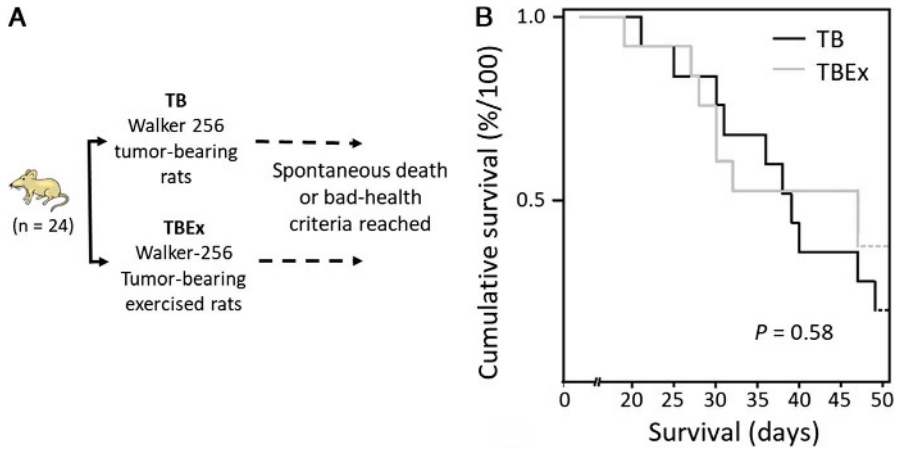

FIGURE 5—Life span experimental design (A) and Kaplan–Meyer survival curve for Walker-256 tumor-bearing rats (TB) vs Walker-256 tumor-bearing exercised rats.

for tumor survival. Briefly, tumor cells are less expected to develop if the energy available is scarce, especially from glycolytic pathways (25). This theory was proposed based on results demonstrating that caloric restriction caused tumor reduction effects (26,27). Studies have demonstrated that negative energy balance, induced by voluntary wheel running exercise, reduced the polyp number in the Apc^{Min} model of intestinal polyp development (28), although not in the same magnitude as with caloric restriction (29). Few years later, however, the same group of researchers demonstrated that a negative energy balance induced by treadmill exercise had no effect on intestine or breast cancer model's tumorigenesis. Furthermore, studies have shown decreased tumorigenesis imposed by exercise without caloric restriction (10,22). In fact, our data demonstrated that RE did not change both body weight gain and food intake, regardless of the cancer model analyzed. Thus, it is reasonable to affirm that energetic deficit imposed by exercise does not explain the antitumorigenesis effects of RE in the present study.

The theory that exercise can induce vascular tumor "normalization" was depicted in the previous years (6,22) and can better explain our results. Tumor normalization hypothesis came from the last decade, and some drugs (i.e., Bevacizumab) approved by the U.S. Food and Drug Administration focused on this mechanism (for a comprehensive review, see Andia and Maffulli [(30)]). In brief, solid tumors present deregulated vascularization, hypoxia, and acidic microenvironment that endorse a belligerent tumor phenotype characterized by invasion and metastasis (30). Abnormal vascularization in solid tumors favored a metabolic shift through glycolysis under normoxia (31). By contrast, increased vascularization/angiogenesis imposed by exercise may prevent tumor expansion and metastasis to leech vascularized tissues with greater presence of oxygen, facilitating the removal of glycolysis by-products such as lactate (22). Indeed, studies have demonstrated that exercise increases angiogenesis marker in different types of solid tumors (21). Faustino-Rocha and colleagues (21) demonstrated that rats submitted to a long-term endurance exercise training demonstrated increased expression of vascular endothelial growth factor A, leading to enhanced tumor vascularization and reduced tumor aggressiveness in *N*-methyl-*N*-nitrosourea–induced breast cancer. Jones et al. (6,22) showed that aerobic exercise promoted proangiogenic phenotype and increased intratumoral vascularization in an orthotopic model of murine prostate cancer and in human breast xenografts implanted in animals, respectively. Interestingly, the data of the present study demonstrated that RE increased CD31-marked tumor in Walker-256 tumor-bearing rats. CD31 covers up a large portion of endothelial cells, and the RE-increasing effects can be interpreted as an endothelial differentiation attempt resulting from tumor angiogenesis (32), demonstrating a preliminary evidence that RE may enhance tumor vascularization as it occurs in the preclinical models of aerobic exercise (6,21,22).

Some caution, however, must be used when interpreting all these data. Certainly, not all preclinical studies have reported increases in the tumor vascularization or tumor-elevated markers of angiogenesis in response to RE (33,34). In addition, some studies have shown that exercise did not decrease tumor volume, although increased intratumoral vascularization was verified (22). It is also important to consider that there are several angiogenic markers to interpret intratumoral vascularization/perfusion in rodent models. As an example, the colocalization of CD31/desmin demonstrated vascular maturity in murine models (35). Vascular maturity is more efficient to transport oxygen and maybe a more reliable marker to investigate hypoxia zones (36). Thus, a comprehensive analysis of tumor profile and microenvironment is necessary to unveil how exercise-induced vascularization may have different favorable tumor responses.

On the other hand, there seems to be a consensus: exercise-induced increases in angiogenesis and vascularization may enhance the delivery and efficacy of anticancer therapies (22). The first preclinical study performed with orthotopic breast cancer model showed that aerobic exercise regulated tumor vascularization and also improved chemotherapy efficacy (35). These data were followed by additional studies (37) and stimulate a new era of adjuvancy with regard to exercise

and chemotherapy drugs. Noteworthy, the exercise-induced tumor vascularization is evident from endurance exercise models. Our study is the first to demonstrate that RE may increase tumor CD31 and probably endothelial vascular density to tumor angiogenesis. A potential role of RE in stimulate chemotherapy delivery should be further investigated.

Intriguingly, despite smaller and less aggressive tumors compared with tumor-bearing sedentary rats, Walker-256 TBEx rats did not demonstrate increased survival rates than sedentary ones. Another study also demonstrated no increase in the survival of tumor-bearing rats in response to RE (38). It seems paradoxical because elevated proliferating cell nuclear antigen Ki-67, histological grade, and in some cases angiogenic (CD31) markers have been associated with poor prognosis of patients with different types of cancer (24). Certainly, not all preclinical studies have reported an ineffective action of exercise in increasing life span (39). The reasons for such distinct results are unknown but probably associated, at least in part, with a wide variety of tumor models studied, different exercise training protocols, rodent species, and diet and drug control (22).

In conclusion, our results demonstrated that RE may mitigate tumor growth and belligerence parameters, such as decreased tumor grade, viable tumor area, and tumor cell proliferation of both Walker-256 and Solid Ehrlich adenocarcinomas models. Also, our findings indicated a partial role of RE to induce tumor vascularization. Although RE was able to decrease tumor growth and aggressiveness, it did not increase the life span of tumor-bearing rats.

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The authors declare that they have no competing interests. The authors also declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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