Resistance Training during Chemotherapy with Doxorubicin

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ABSTRACT

BREDAHL, E. C., S. SHARIF, J. A. SIEDLIK, M. K. WAGNER, M. D. TWADDELL, A. T. TIGNER, M. D. DOVGAN, W. O. NAJDAWI, D. S. HYDOCK, J. M. ECKERSON, and K. M. DRESCHER. Resistance Training during Chemotherapy with Doxorubicin. *Med. Sci. Sports Exerc.*, Vol. 52, No. 12, pp. 2529–2537, 2020. Previous research has shown that resistance training (RT) before doxorubicin (DOX) treatment attenuates the decline in muscle dysfunction; however, the effect of RT during DOX treatment is less known. **Purpose:** Investigate the effects of RT before and during a 4-wk course of incremental DOX treatment on skeletal muscle function. **Methods:** Male, Sprague-Dawley rats (N = 36) were randomly assigned to the following groups: sedentary+saline (SED + SAL), sedentary+DOX (SED + DOX), RT + SAL, or RT + DOX. The RT protocol utilized a raised cage model, which provided progressive hindlimb loading throughout the 14-wk study, whereas SED animals were kept in normal housing. Starting at week 10, DOX-treated animals received 3 $mg \cdot kg^{-1}$ DOX weekly for 4 wk (12 $mg \cdot kg^{-1}$ cumulative); whereas SAL-treated groups received 0.9% NaCl as a placebo. Grip strength was recorded at 0, 10, 12, and 14 wk. *Ex vivo* muscle function was performed on excised soleus (SOL) and extensor digitorum longus (EDL) from the right hind limb 5 d after the last injection and were analyzed for expression of creatine kinase (CK) and creatine transporters. **Results:** SED + DOX–treated animals had significantly lower EDL mass compared with SED + SAL– and RT + DOX–treated animals. Grip strength, EDL maximal force, and EDL force development were significantly lower in SED + DOX–treated animals compared with RT + SAL and SED + SAL. No significant differences in EDL function were found between RT + DOX and RT + SAL animals. DOX treatment reduced expression of CK in the SOL, which abated with RT. **Conclusions:** Low-intensity RT may attenuate the decline in skeletal muscle function during incremental DOX treatment. **Key Words:** CREATINE, EXERCISE, CANCER, MUSCLE DYSFUNCTION

Doxorubicin (DOX) is used in the treatment of a wide variety of cancers ranging from solid tumors to systemic malignancies. Although effective, treatment with DOX is associated with a number of adverse side effects including nausea, vomiting, neutropenia, alopecia, arrhythmias, heart failure, and skeletal muscle dysfunction (1). Skeletal muscle dysfunction is of particular concern, because it is associated with decreased physical function and a lower quality of life (QOL) (2). Patients receiving DOX often experience lower limb weakness, weight loss, muscle loss, a reduced tolerance to exercise, and excessive fatigue (2), all of which affects their ability to perform activities of daily living (ADLs). It has also been reported that patients with lymphocytic leukemia demonstrate a reduced exercise capacity for 1 to 5 yr after chemotherapy with DOX (3), suggesting that the adverse effects of DOX treatment on skeletal muscle function continues long after cessation of treatment.

Doxorubicin-induced muscle dysfunction is attributed to deficits in energy metabolism, which negatively affects mitochondrial function (4), reduces ATP generation (5), alters anaerobic energy production (6), and impairs proper coupling of mitochondrially produced ATP to the recycling of phosphocreatine (PCr) and ADP (7). Furthermore, DOX also lowers levels of creatine (Cr) and creatine kinase (CK) (8) and has

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been shown to reduce creatine transporter (CrT) expression in cultured cardiomyocytes (9), all of which impact the ability of the host to produce ATP. DOX also interferes with the respiratory chain and inhibits oxidative phosphorylation leading to substantial disruptions in mitochondrial health and a rise in reactive oxygen species (ROS) (10,11), resulting in muscle cell damage.

Most treatment programs designed to minimize the side effects of DOX include a combination of nutrition and drug therapy (12,13); however, there is emerging evidence for the role of exercise as an adjuvant therapy before, during, and after cancer treatment (2,14,15). The findings of two recent comprehensive reviews (14,15) that investigated the effect of exercise on health outcomes, primarily in breast cancer patients, concluded that aerobic and/or resistance training (RT) exercise improved cardiovascular fitness, muscle strength, cancer-related fatigue, cognitive function, and cancer site–specific QOL. Furthermore, exercise appears to be safe for cancer patients with a very low reported incidence of exercise-related adverse events (15). Although exercise helps reduce treatment-related side effects, more research is needed to develop optimal exercise protocols with regard to type and intensity, as well as the timing of an exercise intervention.

Studies using animal models have also consistently shown that exercise protects against DOX-induced cardiotoxicity and skeletal muscle dysfunction (11,16–20); however, the majority of these studies focused on the effects of aerobic exercise before DOX treatment. Less information is known regarding the effect of RT before and during DOX treatment on muscle dysfunction. To help bridge this gap in knowledge, we previously examined the effect of RT on DOX-induced muscle function using a rat model and demonstrated that 10 wk of low-intensity RT before DOX treatment preserved muscle function, minimized skeletal muscle fatigue, and preserved body mass (BM) (16). Although our initial findings were promising, questions remain regarding the ability of RT to minimize DOX-induced muscle dysfunction during the treatment process. Furthermore, the majority of studies fail to use realistic dosing strategies. Prior studies that examined the effect of DOX on skeletal muscle dysfunction either incubated cells with relatively large doses of DOX *in vitro* (10) or administered a bolus dose *in vivo* (11,21), which do not reflect the manner of DOX administration used for cancer patients. In normal standard of care, patients typically receive a series of smaller incremental infusions over time. We hypothesized that incremental dosing of DOX *in vivo* would provide a more accurate representation of the DOX-induced muscle dysfunction experienced by cancer patients and that RT may attenuate the adverse effects of DOX during treatment. Therefore, the purpose of this study was to investigate the capacity of RT before and during incremental DOX administration to offset skeletal muscle dysfunction using a rat model.

METHODS

Animals. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Creighton University. Ten-week-old male Sprague-Dawley rats (Envigo RMS, Indianapolis, IN) (N = 36) weighing approximately 300 g were used as subjects. Animals were housed two per cage in standard deep plastic rat cages (20.32 cm H × 26.67 cm W × 48.26 cm D) and maintained on a 12-h/12-h light/dark cycle in a temperature and humidity-controlled environment. Animals had access to standard food (Envigo 2018 Global 18% Rodent Diet) and distilled water *ad libitum* for the duration of the study.

Training protocol and drug administration. The study was conducted over a 14-wk period in two phases: a 10-wk training phase followed by a 4-wk treatment phase. Initially, animals were randomly assigned to either a RT group (n = 18) or a sedentary (SED) group (n = 18). Animals assigned to the RT group were placed in cages where food and water were progressively elevated every 2 wk over the entire 14 wk study to a maximum height of 35 cm above normal. This exercise model is considered to be less stressful than other models (22) and was successfully used in our previous work (16). Animals assigned to the SED group were housed in standard cages. At the end of the 10-wk training phase, animals in the SED and RT groups were divided into subgroups (n = 9) and either received weekly injections of DOX (3 $mg \cdot kg^{-1}$) for 4 wk (SED + DOX and RT + DOX; cumulative dose of 12 $mg \cdot kg^{-1}$) or 0.9% saline (SAL) as a control (SED + SAL and RT + SAL). The selected dose replicated the lowest reported dose capable of producing DOX-induced muscle dysfunction in rats (1) and, in clinical terms, represented a dose of 75 $mg \cdot m^{-2}$ (23), which matches to intensified DOX treatment for metastatic sarcomas (24). Animals assigned to the RT groups remained in their specialized cages for the duration of the DOX treatment period. Non-survival surgery was performed at the end of week 14.

***In vivo* muscle function.** Rodent grip strength has been used in toxicology (25) and disease (26) investigations to assess *in vivo* muscle function. Therefore, *in vivo* muscle function was assessed using a grip strength meter (Columbus Instruments, Columbus, OH) at baseline and at 10 wk, 12 wk, and 14 wk. Grip strength was measured by first having the animal grab the pull bar; then, the rodent was gently pulled horizontally until it released its grip, which resulted in a grip strength measurement. Each animal performed three consecutive trials, and the average value for each trial was recorded as the representative grip strength.

***Ex vivo* muscle function.** At the end of week 14, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 $mg \cdot kg^{-1}$; Vibrac, Fort Worth, TX). When the animal was anesthetized and a tail pinch response absent, the soleus (SOL) and extensor digitorum longus (EDL) muscles from the right hind limb were excised and allowed to stabilize for 5 min in aerated (95% O_2/5% CO_2) Krebs buffer (K, 120 mmol NaCl, 5.9 mmol KCl, 2.5 mmol $CaCl_2$, 1.2 mmol $MgCl_2$, 25 mmol $NaHCO_3$, 17 mmol glucose, pH 7.4). After stabilization, micro-spring clip sutures were attached to the distal and proximal tendons of each muscle. Muscle function was analyzed using an *ex vivo* muscle

TABLE 1. Animal masses (g).

	Starting Mass (g)	Mass at 10 wk (g)
SED + SAL	316 ± 11.8	432 ± 11.7
RT + SAL	315 ± 14.7	453 ± 16.3*,**
SED + DOX	326 ± 15.5	435.1 ± 13.3
RT + DOX	323 ± 10.8	451 ± 10.1*

Symbols indicate significant differences.
*Significantly different from SED + SAL.
**Significantly different from SED + DOX ($P < 0.05$).

function apparatus (Radnotti, Covina, CA). The proximal end of the muscle was affixed to an isometric force transducer (Radnotti, Covina, CA), and the distal end was affixed to a stationary glass hook. Muscles were stimulated with platinum coded field-stimulating electrodes, and maximal force determinations were made by adjusting both voltage and tension until maximal twitch force was achieved. The rate of force development, rate of force decline, and maximal force were recorded for each twitch. Maximal force was calculated using the maximum force value minus the minimum force value. After determination of contractile force characteristics, the buffers were refreshed, and the tissues allowed to recover for 5 min before fatigue testing. Fatigue was determined using the same voltage settings from maximal twitch determination with a frequency of 83 Hz and pulse duration of 500 ms^{-1} (square wave pulses) (16). Muscles were stimulated to contract every second for 100 s^{-1}, and percent change from baseline maximal force (0 s) was recorded every 10 s. Contractile forces were analyzed using Labchart Reader (AD Instruments, Colorado Springs, CO).

Protein expression. Soleus and EDL from the left hind limb were homogenized in RIPA buffer (1:10 w/v; Sigma-Aldrich, St. Louis, MO) and analyzed for CrT and CK expression by Western blotting as previously described (27). Proteins were separated using SDS-PAGE (4%–20% gradient Tris-Glycine) gels (Life Technologies, Carlsbad, CA) at 125 V (constant voltage) and 4 mA for 2 h in a Xcell II blot module (Invitrogen, Life Technologies). Proteins were transferred to 0.45 micron polyvinylidene fluoride (Invitrogen, Life Technologies) membranes over 90 min at 25 V and 100 mA. Protein transfer to polyvinylidene fluoride membranes was verified by the presence of a SeeBlue® Plus2 protein ladder (Life Technologies). Membranes were then blocked for 1 h in Superblock blocking buffer (Life Technologies) and subsequently incubated with gentle agitation overnight with 10 mL of the primary antibody (Thermo Scientific, Waltham, MA; 1:1000). The rabbit monoclonal anti-Glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Abcam, 1:1000) was used as a loading control. Membranes were washed in Tris 25 mmol, KCl 3 mmol, NaCl 140 mmol, and 0.05% Tween 20 three times for 5 min, followed by the incubation in the appropriate species-specific secondary antibody. A secondary anti-rabbit antibody (Santa Cruz Biotechnology, Dallas, TX, 1:1000) labeled with horseradish peroxidase was used for detection. After three more 5-min washes in Tris 25 mmol, KCl 3 mmol, NaCl 140 mmol, and 0.05% Tween 20, membranes were prepared for protein detection using enhanced chemiluminescence (C-Digit, Li-Cor: Lincoln, NE). ImageJ software (NIH: Bethesda, MD) was used to quantify protein expression. Molecular weights of protein bands were ensured in reference to a MagicMark™ XP standard ladder (Novex, Life Technologies).

Statistical analysis. All data are presented as mean ± SD. Data were analyzed using unpaired *t* tests (comparisons made before DOX treatment) and two-way (exercise–drug) ANOVA. If a significant main effect or interaction was observed, a follow up Tukey's *post hoc* analysis was used to determine significant differences between conditions. Fatigue was quantified as percent change from baseline and analyzed using a one-way repeated-measures ANOVA with Dunnett's *post hoc* testing to better understand how force changed over time (i.e., fatigue). A significance level of $\alpha = 0.05$ was used for all statistical analyses. Graph Pad Prism (San Diego, CA) was used for statistical analysis.

RESULTS

General observations. At the start of the study, BM was not significantly different between the animals (Table 1). However, at week 10, animals in the RT + SAL (453 ± 16 g) group were significantly ($P < 0.05$) heavier than the SED + SAL (432 ± 12 g) and SED + DOX (435 ± 13 g) groups. Animals allocated to RT + DOX (451 ± 10 g) group were significantly ($P < 0.05$) heavier than SED + SAL. Although not statistically significant, RT + DOX were notably heavier than SED + DOX ($P = 0.06$). After treatment at 14 wk, significant differences in BM and EDL muscle mass were observed between groups with a significant main effect for drug (Table 2). Follow-up analyses revealed that the RT + SAL group had a significantly higher BM (469 ± 32 g) compared with all other conditions ($P < 0.05$). In addition, the SED + DOX group had a significantly lower BM (412 ± 13 g) compared with SED + SAL (445 ± 23 g, $P < 0.05$). There were no significant differences in BM between SED + SAL (445 ± 23 g) and RT + DOX (428 ± 7 g)–treated animals. Although the animals in the RT + DOX group weighed 15 g more than the animals in the SED + DOX group (428 ± 7 g vs 412 ± 13 g, respectively), the difference was not statistically significant ($P = 0.18$).

Interestingly, SOL mass was not affected by either exercise or DOX treatment. However, EDL mass was significantly ($P < 0.05$) lower in the SED + DOX (0.19 ± 0.03 g) group compared with the SED + SAL (0.30 ± 0.03 g), RT + SAL (0.32 ± 0.03 g), and RT + DOX (0.23 ± 0.04 g)–treated animals. EDL mass in the RT + DOX group was significantly lower compared with the RT + SAL group (Table 2).

TABLE 2. Final animal and tissue masses (g).

	Animal Mass (g)	SOL Mass (g)	EDL Mass (g)
SED + SAL	445 ± 23*,**	0.26 ± 0.01	0.3 ± 0.03*
SED + DOX	412 ± 13**,***	0.22 ± 0.05	0.19 ± 0.03**,***
RT + SAL	469 ± 32*,**	0.26 ± 0.05	0.32 ± 0.03*
RT + DOX	428 ± 7**	0.22 ± 0.06	0.23 ± 0.04*,***

Symbols indicate significant differences.
*Significantly different from SED + DOX ($P < 0.05$).
**Significantly different from RT + SAL ($P < 0.05$).
***Significantly different from SED + SAL ($P < 0.05$).

Grip strength. At baseline, no significant differences were observed in grip strength between the groups (Fig. 1A). However, at 10 wk, the RT + SAL and RT + DOX animals had significantly ($P < 0.05$) greater grip strength compared with the SED animals (Fig. 1B). At week 12 (2 wk after starting treatment), no significant differences in grip strength were observed between any of the four subgroups (Fig. 1C); however, at week 14 (4 wk after starting treatment), there were significant main effects for drug and exercise ($P < 0.05$). Follow-up analysis revealed significant differences in grip strength between SED + DOX versus SED + SAL, RT + DOX, and RT + SAL ($P < 0.05$) (Fig. 1D).

***Ex vivo* muscle function.** Contractile measurements of the SOL and EDL based on exercise and drug are shown in Figure 2. For the SOL, no differences in maximal force (Fig. 2A) or maximal rate of force development (Fig. 2B) were observed. However, there was a significant main effect for drug on rate of SOL force decline (Fig. 2C; $P < 0.05$). In general, the DOX-treated animals had a slower rate of SOL force decline compared with SAL control animals (Fig. 2C). *Post hoc* analyses revealed a significant difference between RT + SAL versus SED + DOX–treated animals for SOL force decline ($P < 0.05$).

Significant main effects for exercise and drug were found for maximal force (Fig. 2D) and rate of force decline for the EDL (Fig. 2F, $P < 0.05$). In general, RT animals had greater EDL contractile forces and faster relaxation rates compared with SED animals, and DOX-treated animals demonstrated lower EDL maximal force and slower rates of relaxation. *Post hoc* analyses revealed a significant difference between RT + SAL versus SED + DOX-treated animals for EDL maximal force ($P < 0.05$).

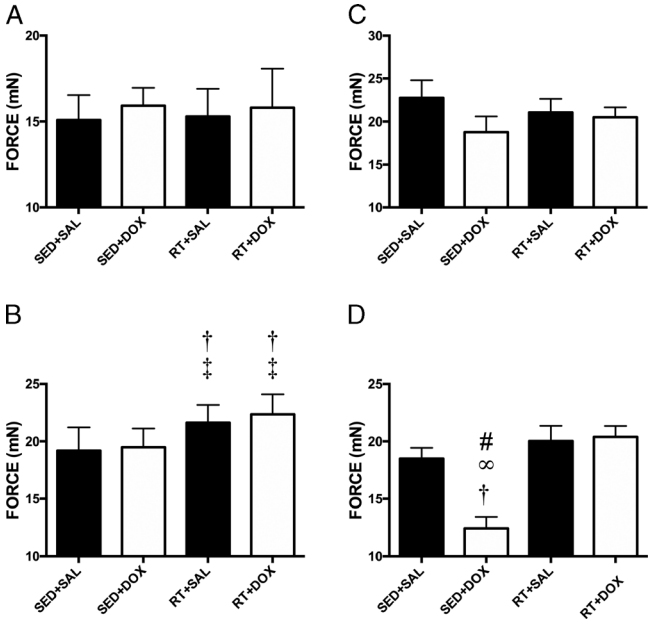

FIGURE 1—Grip strength (mN) at 0 wk (A), and 10 wk (B), 12 wk (C), and 14 wk (D). SED, sedentary; SAL, saline; DOX, doxorubicin. Symbols indicate significant differences. *Significantly different from SED; †Significantly different from SED + SAL ($P < 0.05$); ‡Significantly different from SED + DOX ($P < 0.05$); #Significantly different from RT + SAL ($P < 0.05$); ∞Significantly different from RT + DOX ($P < 0.05$).

Fatigue. Compared with baseline, significant ($P < 0.05$) reductions in SOL maximal force were observed at 20 s^{-1} for animals in the SED + DOX group, at 30 s^{-1} for the RT + SAL and RT + DOX groups, and at 40 s^{-1} for the SED + SAL group (Fig. 3A). Results for the EDL showed that significant ($P < 0.05$) reductions in maximal force occurred at 10 s^{-1} for the SED + DOX–treated animals and at 20 s^{-1} for the RT + DOX, SED + SAL, and RT + SAL groups (Fig. 3B).

Protein expression. In the SOL, a significant main effect for drug was observed with DOX-treated animals exhibiting a lower expression of CK compared with SAL-treated animals (Fig. 4B). However, CrT expression in the SOL was unaffected by exercise or DOX treatment. Protein analysis of excised EDL revealed a significant main effect for exercise on CrT expression ($P < 0.05$). In general, all RT animals demonstrated a higher expression of CrT expression compared with SED animals (Fig. 4C). There were no significant changes in EDL CK expression (Fig. 4D).

DISCUSSION

Doxorubicin is one of the most widely used chemotherapeutic agents and is a highly effective treatment for a variety of cancers (28); however, it causes a number of severe side effects that can greatly alter the patient's QOL and ability to perform ADLs (2). A number of investigations have shown that physical activity attenuates the adverse effects of DOX on cardiovascular and muscle function (16–20,29,30). The current study sought to build on these investigations by using a clinically relevant DOX dosing schedule in conjunction with a low intensity RT model in rats that was designed to represent an active individual who continues his or her training via a low intensity RT program, such as plyometric or body weight training, during chemotherapy.

As expected, RT resulted in an increase in BM and grip strength at the end of the 10-wk training period compared with the SED condition. At the end of the 4-wk DOX treatment period (week 14 of the study), there were no significant differences in grip strength between the SED + SAL, RT + SAL, and RT + DOX groups, indicating that RT during DOX treatment helped maintain muscle strength. BM was 15 g higher in the RT + DOX group compared with SED + DOX, which may have some practical significance, however, the difference was not statistically significant ($P = 0.18$). The EDL muscle mass was significantly higher in the RT + DOX versus SED + DOX condition, which also suggests a protective effect from RT. The results for EDL maximal force and force development were similar in that the decline in skeletal muscle function in the RT + DOX group was not statistically different compared with the SED + SAL or RT + SAL–treated animals. Furthermore, the results from the SOL and EDL fatigue testing showed an earlier time to fatigue among SED + DOX animals. However, when DOX was combined with RT, the onset of fatigue was attenuated for both the SOL and EDL. Together, these findings suggest that low-intensity RT could play a key rehabilitative or preventive role in mitigating the adverse

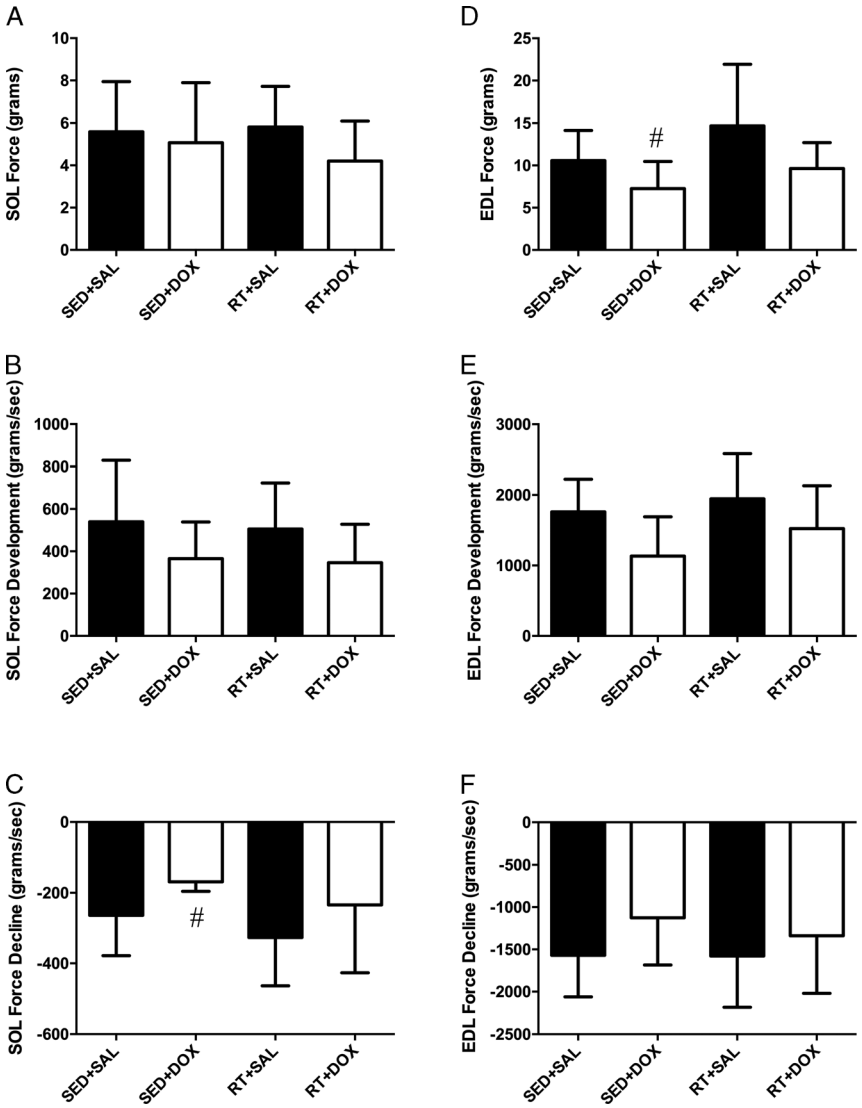

FIGURE 2—Muscle characteristics. SOL maximal force (A); SOL maximal rate of force development (B); SOL maximal rate of force decline (C); EDL maximal force (D); EDL maximal rate of force development (E); EDL maximal rate of force decline (F). SED, sedentary; Symbols indicate significant differences. †Significantly different from SED + SAL ($P < 0.05$); ‡Significantly different from SED + DOX ($P < 0.05$); #Significantly different from RT + SAL ($P < 0.05$); ∞Significantly different from RT + DOX ($P < 0.05$).

effects of chemotherapy with DOX, which is in agreement with our previous work (16).

Interestingly, the results of the current study for the EDL and SOL *ex vivo* tests are different from our previous work (16). We previously reported that a single-bolus dose of 15 $mg \cdot kg^{-1}$ DOX, resulted in significant declines in contractile mechanics for both the SOL and EDL 5 d posttreatment (16). Furthermore, our prior study also demonstrated that 10 wk of RT before DOX treatment resulted in the protection of both SOL and EDL from DOX-induced muscle dysfunction (16). In contrast, the current study showed that incremental DOX dosing had the greatest effect on EDL versus SOL muscle as evidenced by the reduced EDL maximal force and loss of EDL muscle mass in comparison to the SOL. The discrepancy in the findings between our two studies may be explained, in part, by differences in the dosing protocol (bolus vs incremental) and the duration of DOX treatment (5 d vs 4 wk).

Muscle loss is a common occurrence with DOX treatment, and the degree of atrophy and affected muscles varies between investigations (1,21,28). As suggested above, differences in the findings between studies are likely attributable to differences in the dose protocol and treatment duration, as well as the accumulation of DOX metabolites (i.e., doxorubicinol). In addition, the amount of naturally occurring antioxidants in the host may affect the susceptibility of the tissue to oxidative stress. Although not examined in the current study, it is generally accepted that long duration, low-dose DOX treatment leads to a sustained increase in ROS throughout the treatment period (31). In such a case, more aerobic tissues, such as the SOL, would have a greater resistance to oxidative stress compared with more anaerobic tissues (i.e., the EDL) due to the differences in basal levels of antioxidants in each tissue (18). Therefore, as DOX treatment progresses, the degree of cellular damage and cell death would eventually manifest in the loss of

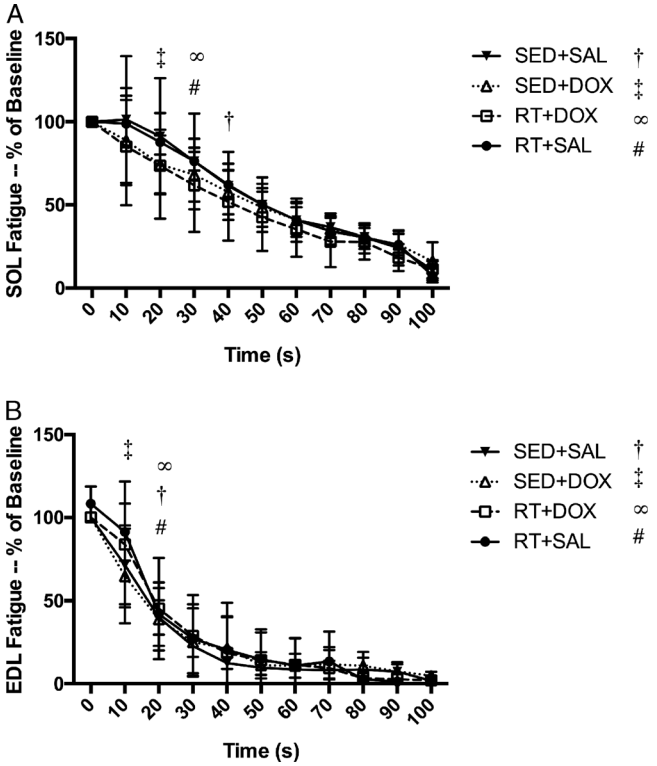

FIGURE 3—Fatigue for a 100-s fatigue protocol for the soleus (SOL, A) and EDL (B). Symbols indicate significant difference from base line. †SED + SAL significantly different from base line ($P < 0.05$); ‡SED + DOX significantly different from baseline ($P < 0.05$); #RT + SAL significantly different from baseline ($P < 0.05$); ∞RT + DOX significantly different from base line ($P < 0.05$).

more anaerobic tissue and could explain the loss of EDL tissue and reduced muscle function observed in the current study, with fewer adverse effects on SOL tissue. Accordingly, it may also be reasonable to suggest that DOX dosing strategies and treatment duration could help predict the muscles types that may be most affected and the degree of muscle dysfunction in those muscles. For example, our current findings suggest that type 2 muscle (i.e., high power, anaerobic fibers), such as the EDL, are most affected during incremental DOX treatment; however, the severity of muscle loss, dysfunction, and fatigue were all attenuated with RT.

A hallmark of chemotherapy treatment with DOX is a decline in mitochondrial function (10). Therefore, the DOX-induced muscle dysfunction and fatigue observed in the current study may also be explained by disruptions in mitochondrial function and increased rates of oxidative stress via DOX-induced generation of ROS (31). As previously mentioned, as DOX treatment progresses, there is a continual rise in oxidative stress ultimately leading to organelle dysfunction, cell death, and eventual tissue loss (32,33). These effects become more apparent during analysis of fatigue. At the cellular level, contractile fatigue is initially due to decreased cross-bridge force development followed by a decrease in the number of cross-bridges (34). Therefore, the earlier time to fatigue observed with DOX is likely attributed to declines in ATP availability and a reduced amount of contractile proteins (5,7), which limits the force generating capacity and number of cross-bridges. To further illustrate the detrimental effects of DOX treatment on energy availability, previous investigations reported significant decreases in the ratio of PCr to ATP (35) and a decreased capacity of the PCr shuttle to maintain adequate energy stores (7), which also greatly diminishes the functional capacity of muscle tissue and leads to fatigue. Previous work (12) also demonstrated that cardiomyocytes exposed to DOX lose their ability to properly couple mitochondrially produced ATP to the recycling of PCr and ADP, commensurable with a reduction in Cr and CK (8).

In addition to the disruptions in cellular function discussed above, Santacruz et al. (9) reported that DOX reduces Cr transport, decreases V_{max}, lowers $K_{m,}$ and reduces CrT levels present on the cell surface in cultured cardiomyocytes (9). In agreement, we observed a decline in SOL CK expression with DOX treatment; however, in contrast to Santacruz et al. (9), we did not observe a decline in CrT expression. The lack of comparable findings for CrT expression between studies may be due to the differences in DOX administration (i.e., bolus dose vs incremental dosing). Our study is novel in that DOX was administered over 4 wk in an effort to create a scenario similar to that experienced by a patient receiving DOX treatment. Therefore, it may be hypothesized that the decrease in CrT expression is rectified with time or that the decline in CrT expression is dose-dependent.

The findings of the current study showed that when RT was performed during DOX treatment, the level of DOX-induced muscle dysfunction was notably lower as evidenced by the attenuated decline in grip strength and EDL mass. Interestingly, all RT animals had a significantly greater expression of EDL CrT compared with SED animals (Fig. 4C). Resistance training is reliant on the phosphagen system, and chronic RT is associated with improved PCr function (36). This adaptation is best observed in type 2 muscle fibers and supports the findings of this investigation in that an increase in intracellular Cr and improved PCr function may have diminished oxidative damage in the EDL. Although Cr has been shown to reduce oxidative damage in a variety of human, animal, and cellular models (37–39), more studies are needed to verify this hypothesis. Additionally, EDL muscle mass was significantly higher in RT + DOX versus SED + DOX at the end of the 4-wk treatment phase, which provides further evidence for the protective effects of RT. However, because BM in the RT animals was significantly higher compared with the SED control animals at week 10 before DOX treatment (Table 1), the observed difference in EDL mass posttreatment could be attributed to greater muscle size before DOX treatment began.

Although the results of our study suggest that RT may reduce muscle dysfunction during DOX treatment, there are some limitations that deserve mention. First and foremost, animals in this study did not have cancer. Although the results of this investigation are encouraging, future studies are warranted using a tumor bearing animal model to ensure that exercise does not interfere with treatment or alter tumor progression. Because this study had a homogeneous population of male

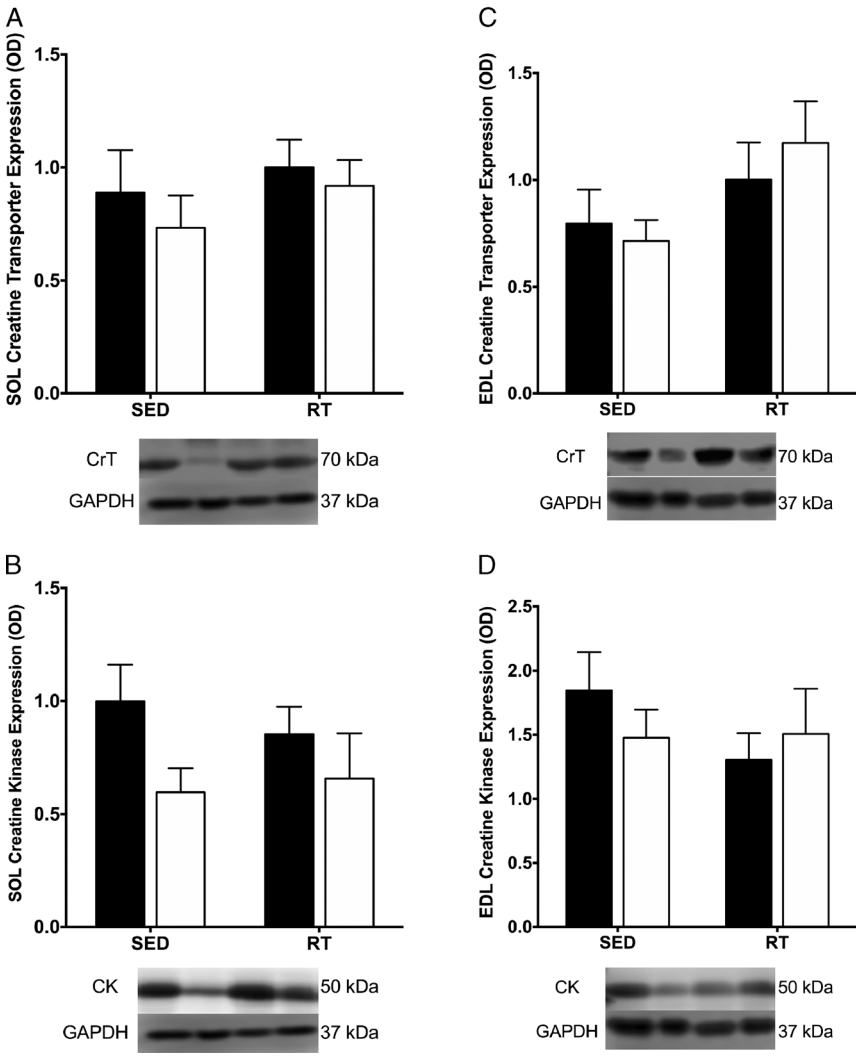

FIGURE 4—Protein expression. SOL CrT (A), SOL creatine kinase (CK, B), EDL CrT (C), and EDL CK (D).

rats, no female-specific adaptions were investigated. Therefore, future investigations should include a heterogeneous population to better identify sex-specific adaptations. We also did not assess forelimb muscle mass, which could have affected our grip strength measurements, nor did we assess intracellular levels of ATP, Cr, oxidative damage, antioxidant concentrations, or PCr content. Future studies should evaluate these variables to provide a better understanding of how RT affects DOX-induced muscle dysfunction. In addition, because DOX treatment is known to cause severe cardiac toxicities (20), future exercise intervention studies should explore how RT during DOX treatment affects ventricular thickness and volume, as well as overall cardiac function. Future research should also consider the effect of RT on health outcomes during longer durations of cancer treatment, as well as its effect on the recovery period during the course of several treatment cycles.

CONCLUSIONS

The present study used a rat model to examine the effects of RT before and throughout DOX treatment in an attempt to represent an active individual who continues to engage in RT after a cancer diagnosis. The exercise model used in the current study would be akin to a low intensity RT program, such as plyometric or body weight training. The results suggest that RT increased BM and muscle strength before treatment and minimized the degree of DOX-induced muscle dysfunction and fatigue compared to sedentary control animals. Specifically, RT during DOX treatment preserved maximal force, rate of force decline, rate of force development, grip strength, and CK expression compared with DOX treatment alone. Although more research is necessary to replicate these findings using heterogenous samples and different treatment models, the results suggest that cancer patients who regularly engage in RT before diagnosis should continue to engage in low intensity RT-type activities during chemotherapy because it is likely to preserve strength and attenuate cancer-related fatigue, affording them a greater capacity to perform ADLs and improve their QOL.

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