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RESEARCH ARTICLE

Protection Against Late-Onset Doxorubicin Myotoxicity Using Creatine and Resistance Exercise

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ABSTRACT

This study examined the effects of creatine supplementation (Cr) and resistance training (RT) on the myotoxicity that accompanies treatment with the chemotherapy drug doxorubicin (DOX). Male rats were randomly assigned to control (CON), DOX, RT+DOX, Cr+DOX, and Cr+RT+DOX groups. DOX groups received 1 mg/kg daily DOX injections for 12 days, Cr groups were fed a diet supplemented with 3% Cr, and RT groups were housed in resistance training model cages. Forelimb grip strength was assessed at baseline and at day 40 at which time the soleus and extensor digitorum longus were excised and analyzed for positive and negative myogenic regulator factor expression. Grip strength increased from baseline to day 40 only in CON and Cr+RT+DOX groups but not in DOX, Cr+DOX or RT+DOX suggesting that combined Cr and RT helps maintain grip strength with DOX treatment. Myostatin expression was lower in solei from RT+DOX, Cr+DOX, and Cr+RT+DOX when compared to CON but not in DOX, and a trend toward higher muscle ring finger (MuRF) expression in the DOX only group was observed. These data suggest that Cr supplementation with RT may be an effective non-pharmacological therapeutic strategy to battle DOX myotoxicity through modulation of negative myogenic regulatory factors.

Keywords: Anthracycline, Grip Strength, Glycolytic Muscle, Myogenic Regulatory Factors, Oxidative Muscle

INTRODUCTION

Doxorubicin (DOX, trade name Adriamycin®) is a highly effective chemotherapeutic treatment for solid tumors and hematological malignancies.[1]Doxorubicin has been a conventional chemotherapy treatment for over 30 years, and patients often experience toxicities which causes severe side-effects and future health problems. For example, cardiac damage induced by Doxorubicin leads to the development of cardiomyopathy and congestive heart failure, making it the primary focus of Doxorubicin research;[2,3] however, patients undergoing Doxorubicin treatment commonly report muscle weakness and fatigue as a side-effect,[4] which may be the result of Doxorubicin -induced myotoxicity. Doxorubicin -induced myotoxicity results in the loss of muscle mass, decreased cross-sectional area, and skeletal muscle contractile impairment.[5-7] Overall, this compromises the patient's skeletal muscle function and quality of life while reducing the chances of prolonged survival after treatment.[8] This demonstrates the need to discover the mechanisms responsible for Doxorubicin-induced myotoxicity and to develop interventions that counter Doxorubicin-induced myotoxicity to maintain muscle function.

Doxorubicin treatment increases oxidative stress causing DNA damage,[9] altered molecular signaling,[10] and induction of apoptosis.[11] Creatine monohydrate (Cr) is a popular ergogenic aid that has antioxidant activity,[12] enhances phosphocreatine/inorganic phosphate ratio, and increases muscle force production, cross-sectional area, and satellite cell differentiation.[13,14] Clinical investigations of different diseases shown improvements in fatigue resistance and muscle function with the use of Cr supplementation.[15] Furthermore, type II skeletal muscle incubated for 30 minutes *ex vivo* prior to Doxorubicin exposure mitigated the reduction in force and maximal rate of force production induced by Doxorubicin.[16]

Exercise interventions also lessen adverse side effects of cancer treatments.[17] For example, resistance training (RT) improves muscular strength and antioxidant enzymes, which reduces oxidative stress,[18] while decreasing muscle ring finger protein 1 (MuRF-1) expression.[19] Furthermore, the combination of RT and Cr supplementation has been observed to have a more considerable improvement in muscular strength and reduced oxidative stress than RT and Cr supplementation alone when compared to a sedentary control group.[20] Thus, a combination of RT and Cr supplementation may combat Doxorubicin-induced myotoxicity to a greater extent than either intervention alone.

Skeletal muscle is one of the few tissues that can efficiently regenerate after injury due to satellite cells; however, maintaining a balance in expression of negative and positive muscle regulatory proteins is necessary for healthy muscle regeneration and muscle function. Muscle satellite cell activation generates myogenic precursor cells that differentiate to repair and replace damaged fibers. Specification of satellite cell differentiation depends on the basic helix-loop-helix transcription factors known as myogenic regulatory factors (MRF): myogenic regulatory factor 5 (Myf5), myogenic differentiation 1 (MyoD), myogenin, and muscle-specific regulatory factor 4 (MRF4).[21] DOX has been reported to inhibit myogenic differentiation through the downregulation of the gene transcripts of MyoD and myogenin.[22,23] Myostatin, a transforming growth factor-beta superfamily member, negatively regulates skeletal muscle by inhibiting myoblast differentiation and downregulates MyoD and myogenin gene expression.[21,24] Furthermore, myostatin promotes protein degradation by increasing ubiquitin ligase MuRF-1 protein.[24] Doxorubicin has been previously reported to increase skeletal muscle myostatin mRNA[1] and expression of MuRF-1 protein and mRNA[1,25] indicating that maintaining MRF and inhibiting the increase of myostatin and MuRF-1 protein expression may be critical for patients receiving Doxorubicin to maintain muscle function.

The combination of Cr supplementation and RT increases satellite cell differentiation proteins and reduces serum myostatin expression,[26,27] which may protect against Doxorubicin-induced myotoxicity. To our knowledge, there have been no studies examining the effects of *in vivo* Cr supplementation combined with RT as an intervention to minimize DOX-induced skeletal muscle dysfunction and alterations in molecular signaling. The purpose of the study was to determine the *in vivo* effects of Cr supplementation, RT, and a combination of Cr supplementation and RT on muscle function two weeks post-DOX treatment. Furthermore, this study explored if MRF, myostatin, and MuRF-1 proteins are part of the mechanisms responsible for Doxorubicin-induced muscle dysfunction. It was hypothesized that *in vivo* Cr supplementation combined with RT would mitigate the Doxorubicin-induced muscle dysfunction and regulate the protein expression of MRF, myostatin, and MuRF-1.

METHODS

All procedures were approved by the University of Northern Colorado Institutional Animal Care and Use Committee and were in compliance with the Animal Welfare Act guidelines. A total of 30, 10-week-old male Sprague-Dawley rats (~300g) were obtained from Envigo (Indianapolis, IN, USA) and were singly housed in an environmentally controlled facility on a 12:12-hour light:dark cycle and provided chow and water *ad libitum*. Rats were randomly assigned to one of five groups: control (CON, *n*=6), DOX (DOX, *n*=6), resistance exercise intervention and DOX (RT+DOX, *n*=6), 3% Cr supplementation and DOX (Cr+DOX, *n*=6), and combination of 3% Cr supplementation, resistance exercise intervention and DOX (RT+Cr+DOX, *n*=6).

In vivo forelimb grip strength and body mass were measured before dietary intervention (baseline) and before sacrifice. A commercially available mesh pull-bar attached horizontally to a force meter designed for rats was used to measure *in vivo* forelimb grip strength (Columbus Instruments, Columbus OH). By holding on to the tail, the animal was gently lifted over and lowered toward the mesh pull-bar. Once the animal had a secure grip with the front two paws, the animal was pulled away from the force meter slowly and steadily until the rat released the mesh pull-bar. The force meter measured the force applied to the mesh pull-bar and recorded the highest value achieved. The animal was returned to its cage and allowed five minutes of recovery before the next trial. A total of five trials were performed for each of the two sessions using the same protocol. The mean of all five trials was used as the animal's grip strength for that session. All grip strength procedure measurements were done by the same technician for all animals to eliminate inter-tester error.

Cr supplementation began two weeks before DOX injection and continued throughout the study. Food consumption was measured daily during the study to determine if there was a group difference in chow consumption. After 2-weeks, animals received intraperitoneal injections of 1 mg/kg of DOX or saline (0.9% NaCl) daily for 12 consecutive days. Immediately following the first injection, resistance exercise groups were housed in specialized cages where food and water were progressively raised requiring them to rise to an erect bipedal stance to eat and drink. Resistance training was done progressively with the cage height starting at 28 cm and raised 2.5 cm every third day until a height of 33 cm was reached, and this height was maintained for the remainder of the protocol.[28] After 14 days of resistance exercise, animals were housed in standard cages for 24 hours before being euthanatized. Animals were anesthetized with sodium pentobarbital (50 mg/kg), and a when a tail pinch reflex was absent, the heart was removed followed by the removal of the left soleus (SOL) and extensor digitorum longus (EDL) which were trimmed free of connective tissue, weighed, flash frozen in liquid nitrogen, and stored at -80°C for later biochemical analysis.

The SOL and EDL from the left hind limb were manually homogenized in radioimmunoprecipitation assay (RIPA) buffer (1:10 weight: volume) with 10µL of protease enzyme inhibitor (Sigma-Aldrich: St. Louis, MO). After homogenization, samples were centrifuged for 10 minutes at 10,000*g* at room temperature. The supernatant was collected, and total protein was quantified using the Bradford method (Sigma-Aldrich: St. Louis, MO) with a Genesys 20 spectrophotometer (ThermoSpectronic: Rochester, NY) at 595 nm.[29] Based on each sample's protein concentration, RIPA buffer was added to standardize protein concentrations and an equal volume of Lammeli sample buffer was then added thus preparing the proteins for electrophoresis.

Western blot analysis was conducted to quantify the expression of myostatin, MuRF-1, and the MRFs MyoD, Myf5, myogenin, and Mrf4 in the SOL and EDL Briefly, samples were heated in boiling water for 2 minutes and chilled on ice for 5 minutes. Fifteen µL of each sample was loaded onto 4-20% gradient Tris-Glycine precast gels (LifeTechnologies, Carlsbad, CA). Gels were run at 125 V (constant voltage) and 60 mA current for 2 hours in an Xcell II blot module (Invitrogen, LifeTechnologies). Proteins were transferred to 0.45 µm pore size polyvinylidene fluoride (PVDF) membrane (Novex, LifeTechnologies) over 90 minutes at 25 V and 100 mA. Protein transfer to the PVDF membrane was ensured by the presence of SeeBlue® Plus2 protein ladder (Novex, LifeTechnologies). PVDF membrane was then blocked for one hour in 15 mL of TBST (50 nM Tris [pH 7.4], 100 mM NaCl, 0.1% Tween-20) + 5% nonfat dry milk and incubated with gentle agitation overnight in 10 mL of primary antibody solution.

Detection was done by enhanced chemiluminescence (ECL) (C-Digit, Li-Cor: Lincoln, NE), and ImageJ software (NIH: Bethesda, MD) was used to quantify protein expression. Five minutes before ECL imagining, 1.5 mL of luminol and enhancer (SuperSignal West Femto Maximum Sensitivity Chemiluminescent Substrate,

ThermoScientific: Waltham, MA) was added to the membrane. The primary antibodies of interest included mouse monoclonal GDF-8 (myostatin) and MuRF-1 (Santa Cruz Biotechnology, Dallas, TX), rabbit monoclonal MyoD (Santa Cruz Biotechnology, Dallas, TX), and rabbit polyclonal myogenin (Santa Cruz Biotechnology, Dallas, TX), Myf5 and MRF4 (Abcam: Cambridge, MA). The rabbit monoclonal antibody GAPDH (Santa Cruz Biotechnology, Dallas, TX) was used as a loading control for each PVDF membrane. Molecular weights of protein bands were ensured in reference to a MagicMark™ XP standard ladder (Novex, LifeTechnologies). Secondary antibodies (Santa Cruz Biotechnology) corresponded to associated species (mouse, rabbit) and included horseradish peroxidase (HRP) for adequate reactivity.

Statistical Analysis

All data are reported as mean ± SEM. Body mass and *in vivo* forelimb grip strength were analyzed by a paired T-test for each group to determine if there were changes within the group during the study. Between-group differences of body mass, *in vivo* forelimb grip strength, and protein expression were analyzed by a one-way analysis of variance (ANOVA). If a significant F-value was observed, Tukey's *post hoc* testing was performed to identify where differences existed. Significance set at $\alpha=0.05$.

RESULTS

Throughout the study, there were no changes in food consumption (data not shown). As seen in Table 1, body mass increased in all groups, but there were no between-group differences throughout the study ($P > 0.05$). Furthermore, no between-group differences were observed for SOL and EDL muscle masses ($P > 0.05$).

Table 1. Animal Characteristics

	CON	DOX	RT+DOX	Cr+DOX	RT+Cr+DOX
Baseline Body Mass (g)	338.5 ± 7.7	339.7 ± 17.5	338.3 ± 7.8	340.3 ± 5.3	348.2 ± 8.7
Pre-Injection Body Mass (g)	370.0 ± 8.0	373.7 ± 17.8	371.3 ± 8.7	372.7 ± 6.7	387.6 ± 10.6
Post-Injection Body Mass (g)	385.0 ± 10.4	363.8 ± 21.9	364.5 ± 10.0	360.8 ± 8.8	379.8 ± 14.4
Sacrifice Body Mass (g)	407.7 ± 9.9	371.5 ± 23.8	363.7 ± 18.3	357.7 ± 7.7	405.6 ± 14.2
Soleus Mass (mg)	143.6 ± 8.7	135.8 ± 9.0	149.0 ± 11.3	136.4 ± 11.9	146.5 ± 11.9
EDL Mass (mg)	155.5 ± 6.9	125.0 ± 5.4	140.0 ± 10.3	125.4 ± 11.4	130.3 ± 6.8

CON, control group (no intervention); DOX, doxorubicin group (only doxorubicin); RT+DOX, resistance training + doxorubicin; Cr+DOX, 3% creatine monohydrate supplementation + doxorubicin; RT+Cr+DOX, resistance training + 3% creatine monohydrate supplementation + doxorubicin; EDL, extensor digitorum longus.

CON, control group (no intervention); DOX, doxorubicin group (only doxorubicin); RT+DOX, resistance training + doxorubicin; Cr+DOX, 3% creatine monohydrate supplementation + doxorubicin; RT+Cr+DOX, resistance training + 3% creatine monohydrate supplementation + doxorubicin; EDL, extensor digitorum longus.

In vivo muscle function was analyzed by handgrip force production. There were no between group differences at each time point in which *in vivo* handgrip was recorded ($P > 0.05$); however, RT+Cr+DOX and CON groups had significant increases in *in vivo* force production from baseline to day 40 ($P < 0.05$) which was not observed in the DOX, RT+DOX and Cr+DOX groups (Fig. 1A, $P > 0.05$). Additionally, there was a significant increase in percent difference from baseline to the time sacrifice in RT+Cr+DOX group when compared to Cr+DOX group ($P<0.05$) with a trend toward increased percent change when compared to the DOX group (Fig. 1B., $P = 0.064$).

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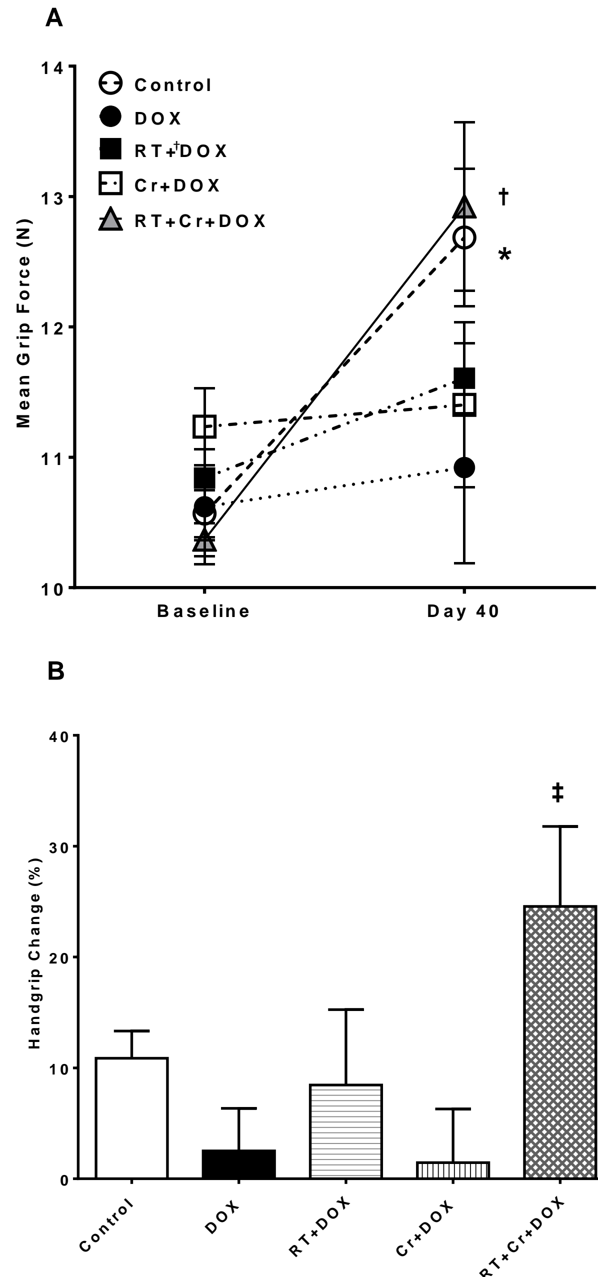

Figure 1. *In vivo* handgrip at baseline and at day 40. B: *In vivo* handgrip percent change from baseline to day 40. CON, control group (no intervention); DOX, doxorubicin; RT+DOX, resistance training + doxorubicin; Cr+DOX, 3% creatine monohydrate supplementation + doxorubicin; RT+Cr+DOX, resistance training + 3% creatine monohydrate supplementation + doxorubicin. Baseline measure at day 0, sacrifice measure on day 40. *CON *in vivo* handgrip significantly higher at day 40 compared to baseline ($P < 0.05$). †Cr+DOX *in vivo* handgrip significantly higher at day 40 compared to baseline ($P < 0.05$). ‡ Significantly higher than Cr+DOX ($P < 0.05$).

In the SOL, there were no differences in the expression of the positive regulatory factors Myf5, MyoD, myogenin, and MRF4 (Fig. 2), but there were tends toward RT+Cr+DOX expressing higher levels of Myf5 and Myogenin and all DOX treated groups expressing lower levels of MyoD. Likewise, in the EDL, there were no differences in Myf5, MyoD, myogenin, and MRF4 (Fig. 3, $P > 0.05$) with an observed trend of lower levels of MyoD in all DOX treated groups.

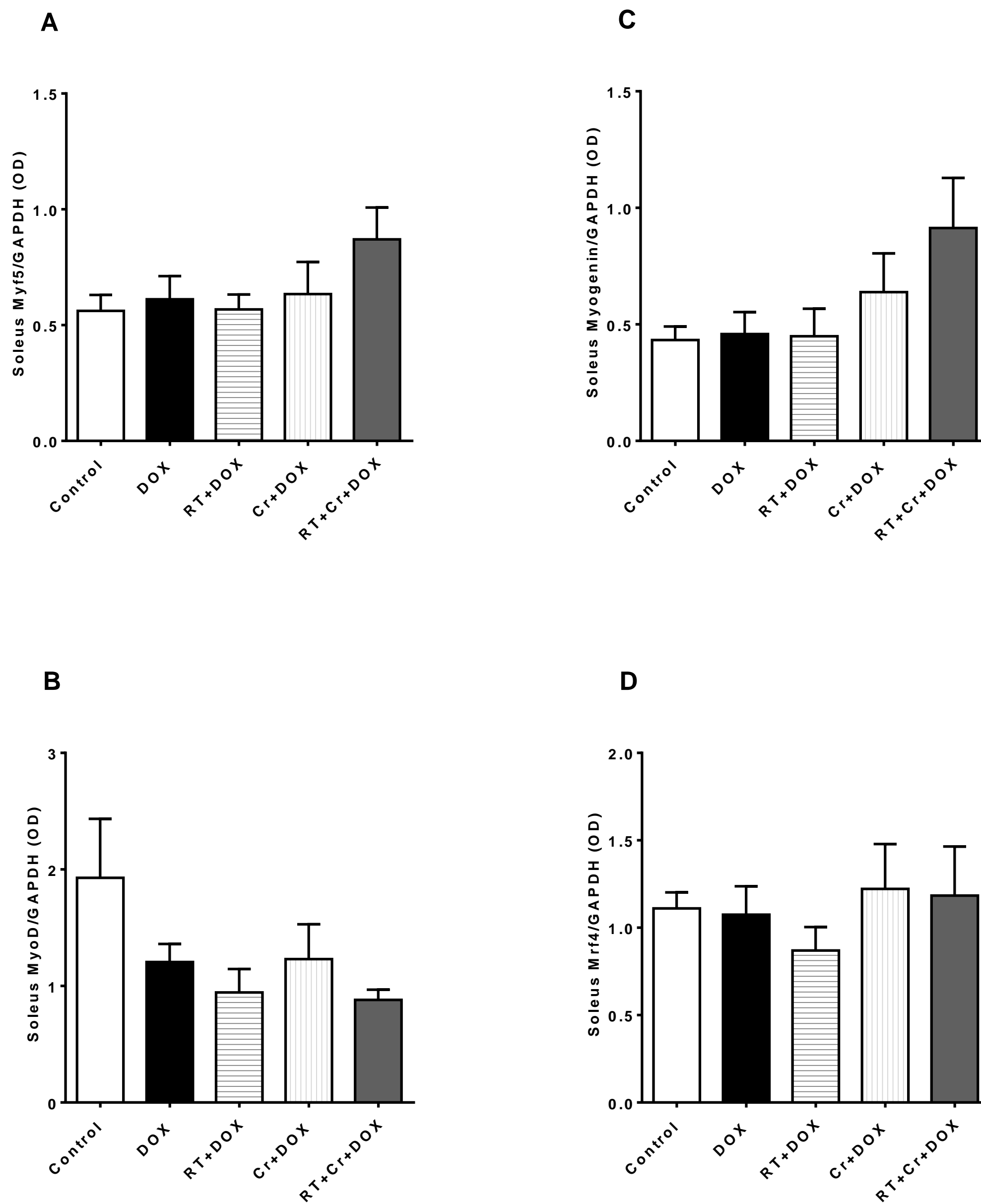

Figure 2. A: Soleus myogenic regulatory expression. A, Myf5 expression; B, MyoD expression; C, myogenin expression; D, MRF4 expression. Control, no intervention; DOX, doxorubicin; RT+DOX, resistance training + doxorubicin; Cr+DOX, 3% creatine monohydrate supplementation + doxorubicin; RT+Cr+DOX, resistance training + 3% creatine monohydrate supplementation + doxorubicin.

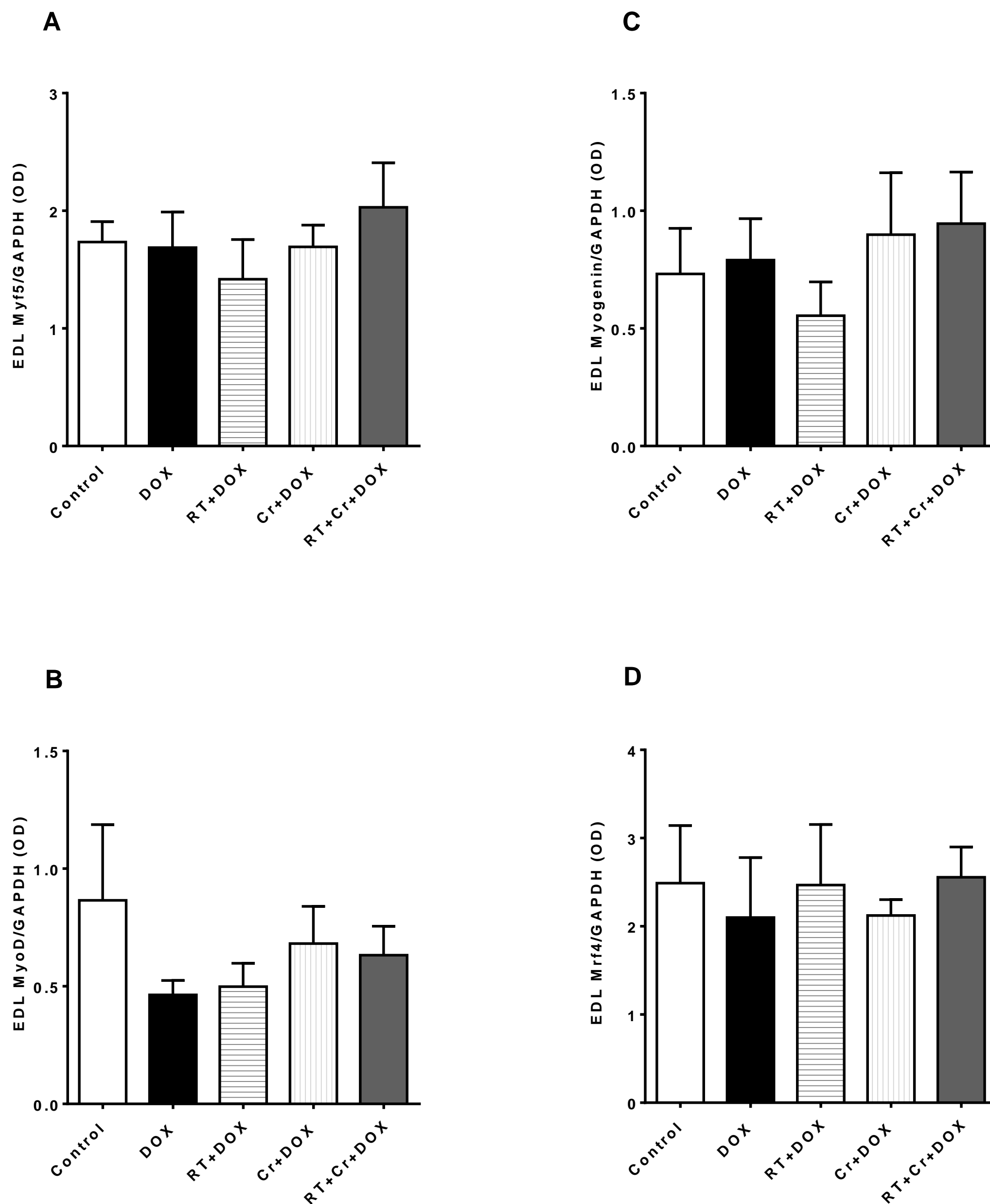

Figure 3. Extensor digitorum longus (EDL) myogenic regulatory factor expression. A, Myf5 expression; B, MyoD expression; C, myogenin expression; D, MRF4 expression. Control, no intervention; DOX, doxorubicin; RT+DOX, resistance training + doxorubicin; Cr+DOX, 3% creatine monohydrate supplementation + doxorubicin; RT+Cr+DOX, resistance training + 3% creatine monohydrate supplementation + doxorubicin.

With the negative myogenic regulators in the SOL, myotstatin was significantly lower in RT+DOX, Cr+DOX, and RT+Cr+DOX when compared to Control (Fig. 4A, $P < 0.05$), and although there were no between group Murf1 expression differences in the SOL (Fig. 4B, $P > 0.05$), a trend toward DOX expressing higher levels of Murf-1 than all other groups was observed. In the EDL, there were no between-group difference in myostatin (Fig. 5A) and Murf-1 (Fig. 5B, $P > 0.05$), but there was a trend toward RT+Cr+DOX expressing higher levels of myostatin and lower levels of Murf-1 than all other groups.

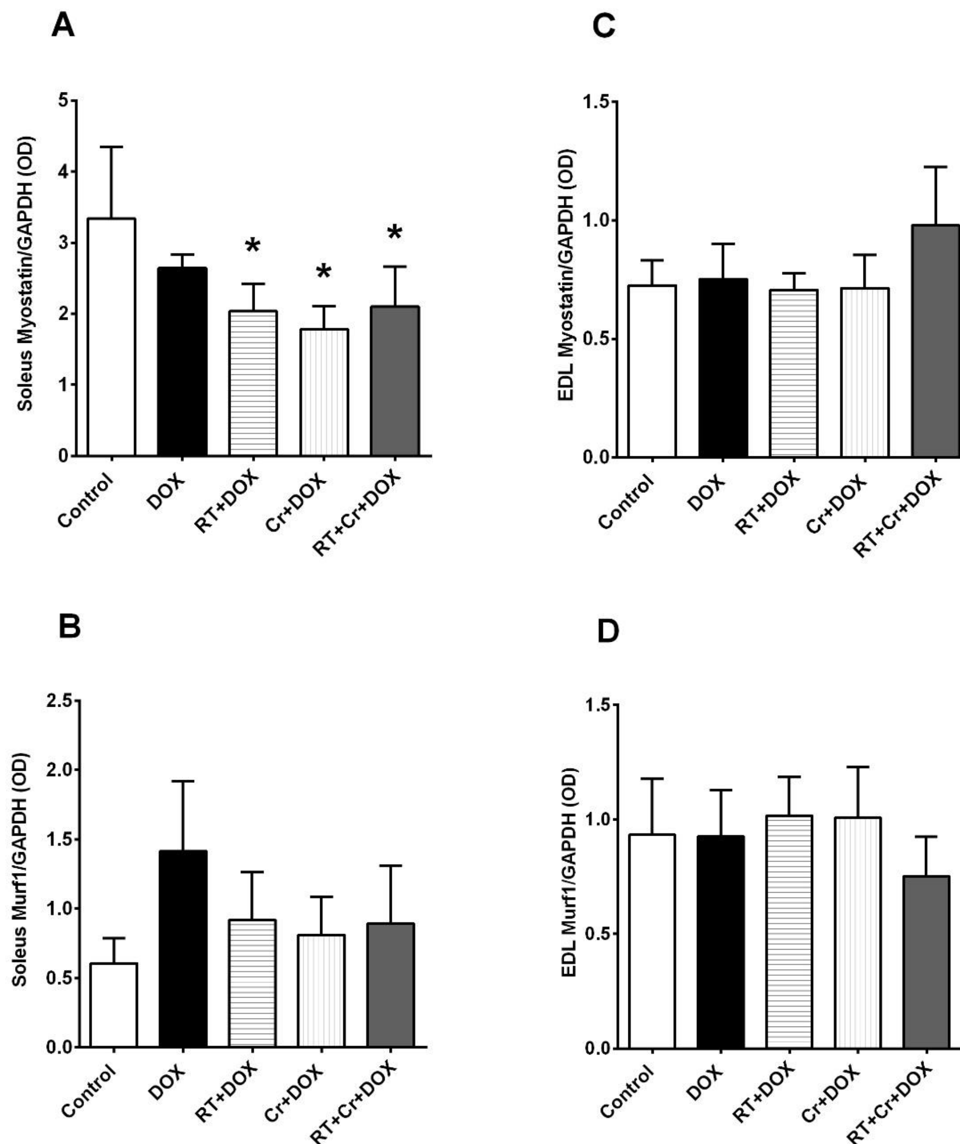

Figure 4. Negative regulatory factor expression in the soleus and Extensor digitorum longus (EDL). A, soleus myostatin expression; B, soleus muscle ring finger-1 (Murf-1) expression; C, EDL myostatin expression; D, EDL Murf-1 expression; Control, no intervention; DOX, doxorubicin; RT+DOX, resistance training + doxorubicin; Cr+DOX, 3% creatine monohydrate supplementation + doxorubicin; RT+Cr+DOX, resistance training + 3% creatine monohydrate supplementation + doxorubicin. *Significantly lower than CON ($P < 0.05$).

DISCUSSION

As older populations keep growing, the number of cancer survivors continues to grow with it, and by 2026, 20.3 million individuals in the United States will be alive with a history of cancer.[30] The growing epidemic of cancer will increase the usage of DOX as a chemotherapy treatment to create a cellular environment not suitable for cancer growth. This demonstrates the need for the development of interventions pre- and post- DOX treatment to minimize side-effects and provide a higher quality of life post-DOX treatment. This study aimed to investigate the effects of RT, Cr supplementation, and the combination of RT and Cr supplementation on *in vivo* muscle function in rats 2-weeks after a 12-day DOX treatment. Furthermore, this study determined if MyoD, Myf5, myogenin, Mrf4, myostatin, and MuRF-1 proteins in oxidative, type I (SOL) and glycolytic, type II (EDL) skeletal muscles are linked to DOX-induced muscle dysfunction and

protection afforded by RT and/or Cr. Our primary results show that the combination of RT and Cr supplementation improved *in vivo* muscle function from baseline to time of sacrifice, which was not seen in DOX, RT+DOX, and Cr+DOX groups. Resistance training, Cr supplementation, and RT with Cr supplementation downregulated myostatin protein expression in oxidative skeletal muscle following DOX treatment. No changes in MRF, myostatin, and MuRF-1 protein expression following DOX treatment, however, were observed in fast, glycolytic EDL muscle, and interventions did not promote alterations in the proteins although a trend toward higher myostatin expression in RT+Cr+DOX was observed.

Multiple studies report that DOX exposure causes skeletal muscle weakness by inducing contractile impairments[5,31,32] and reduction in the diameter of type I and type II muscle fibers.[33] The mechanisms of DOX-induced skeletal muscle dysfunction are multifaceted and not completely understood. It is well recognized that DOX is cardiotoxic, and treatment may result in cardiac dysfunction[34] which can eventuate to heart failure.[35,36] The dysfunctional heart results in reduced peripheral blood flow which can promote skeletal muscle wasting, weakness, and dysfunction.[37-39]Although these “indirect” effects of DOX treatment on skeletal muscle are concerning, evidence exists of DOX having more “direct” effects on skeletal muscle. The direct, negative effects of DOX on skeletal muscle have been demonstrated using models of cell culture[40,41] and *ex vivo* organ baths.[16,42] Additionally, the impact of *in vivo* DOX treatment on skeletal muscle has been demonstrated with mechanisms of oxidative stress,[43] impaired Ca^{2+} handling,[44] apoptosis, and authophagy[45] being attributed to myotoxicity and impaired muscle performance.

It was once thought that DOX side effects were specific to the myocardium due to cardiomyocytes’ high mitochondrial volume, but DOX also accumulates in skeletal muscle and smooth muscle although not to the same extent as cardiac muscle.[46] Since the effects of DOX on skeletal muscle have been receiving increased attention, a focus on skeletal muscle-specific mechanisms that DOX may target has emerged. One such mechanism specific to skeletal muscle is its regenerative capacity which is signaled by a host of MRFs. Early reports suggested that DOX administration decreases myogenic regulatory factor expression and signaling[22,47]; however, more recently, it was reported that DOX treatment does not necessarily promote decreased MRF protein expression but rather may upregulate certain MRFs to potentially assist in repairing damage caused by a large bolus DOX dose.[48] It should be noted that the current study did not employ a large bolus dose but rather administered a more clinically-relevant DOX dosing scheme where the cumulative dose was spread out over the course of 12 days. With that being said, the dosing scheme of DOX is an important factor to consider when interpreting the MRF response. Although no significant changes in the positive MRFs were observed with DOX treatment, a trend toward DOX decreasing MyoD expression in both the SOL and EDL were observed suggesting that DOX may target MyoD signaling to a greater extent in skeletal muscle than Myf5, myogenin, and Mrf4. More work, however, is needed to explore this effect.

It should be noted that the current study not only analyzed the positive MRFs MyoD, Myf5, myogenin, and Mrf4, but it also analyzed the expression of the negative regulatory factors myostatin and MuRF1 in response to DOX. Although DOX treatment alone did not significantly impact myoststin and MuRF-1 expression, a trend toward DOX promoting an increase Murf1 in the SOL was observed suggesting a possible differential effect of DOX on muscle fiber type. Myostatin and MuRF-1 proteins serve as negative regulators of skeletal muscle growth by decreasing protein synthesis and increasing protein degradation. An acute bolus dose of DOX has been shown to increase myostatin and MuRF-1 gene expression in oxidative skeletal muscle;[1] however, the current DOX dosing did not increase myostatin or MuRF-1 protein expressions in either oxidative or glycolytic skeletal muscle. Furthermore, RT, Cr supplementation, and the combination of both Cr supplementation and RT decreased myostatin protein expression in oxidative muscle fibers when compared to CON in the current study. Lower MuRF-1 protein expression could contribute to maintaining oxidative muscle function, and future work is required to better understand this mechanism.

Interventions aimed at battling cancer treatment-related side effects have received attention, and the use of exercise specifically to combat DOX-induced side effects specifically has shown great promise. Endurance exercise prior to[49-51] and during[52,53] DOX treatment have been effective at protecting against cardiotoxicity, and interestingly, resistance training has also shown promise in protecting against DOX-induced cardiac dysfunction.[54] Exercise has also been shown to

protect against DOX-induced muscle dysfunction as well.[1,45,55,56] More specifically, endurance training and resistance training prior to DOX treatment provided protection against the increased muscle fatigue and decreased force production that accompanies DOX treatment, but the effects were dependent upon muscle type.[57]

More recently, there has been a focus on the use of Cr in battling DOX-induced skeletal muscle dysfunction. Creatine monohydrate supplementation in general enhances muscular storage of free creatine and phosphorylated creatine which can elevate protein synthesis[58] and protects skeletal muscle from proteolysis[59] and gained popularity in athletes as it promotes increased skeletal muscle cross sectional area and force production.[14,60] Creatine supplementation, however, has also been shown to improve skeletal muscle quality in Duchenne muscular dystrophy,[61] chronic heart failure,[62] amyotrophic lateral sclerosis[63], and Huntington's disease.[64] Furthermore, Cr has an antioxidant effect[65] protecting against oxidant-induced lipid peroxidation and protein carbonyl formation[66] and oxidative DNA and RNA damage[67,68] which aligns its use as an intervention to protect against DOX myotoxicity.

The potential benefits of Cr in attenuating DOX myotoxicity was demonstrated in an *ex vivo* Cr incubation and DOX treatment study.[16] Although *ex vivo* DOX treatment promoted decreases in muscle force production and increases in fatigue, pre-incubation with Cr attenuated this dysfunction. Another study explored the effects of Cr and DOX *in vivo* where laboratory rats were fed chow supplemented with 3% for 2 weeks prior to a bolus 15 mg/kg DOX treatment, and Cr supplementation attenuated DOX-induced muscle dysfunction.[69] Although the use of Cr in managing DOX myotxicity has shown promise in the aforementioned animal models, an 8-week Cr supplementation failed to improve skeletal muscle function in colorectal cancer patients[70] indicating that oral consumption of creatine monohydrate alone may not protect skeletal muscle from DOX in a clinical setting.

It is possible that the full prophylactic effects of Cr may only be realized when supplementation is combined with resistance exercise as it has been demonstrated, for example, that the combination of RT and Cr supplementation in older individuals has a greater effect on skeletal muscle function than just RT or Cr supplementation administered alone.[20,71] Bredahl et al.[42] explored the combined effects of RT and Cr in the rat where rats were resistance trained using the raised cage model employed in the current study, and skeletal muscle was then excised, incubated in Cr and treated with DOX *ex vivo*. Skeletal muscle function was then analyzed, and combined RT and Cr protected against the increased muscle fatigue brought on by DOX treatment to a greater extent than RT or Cr alone. The current study observed a similar effect with combined RT and Cr providing a greater degree of protection against DOX-induced muscle dysfunction that Cr or RT alone as indicated by preserved *in vivo* grip force. This preservation of skeletal muscle force production following DOX treatment would be of great benefit to cancer patients, and it is recommended that additional work be done to better understand the role that Cr and RT may play in protecting against cancer treatment-related weakness and fatigue and improving quality of life.

CONCLUSIONS

Muscle function is a strong independent predictor of cancer mortality, morbidity, and quality of life.[72,73] Thus, interventions that can maintain muscle function following chemotherapy will be of excellent benefit for cancer survivors. The current study reports that Cr supplementation in combination with RT improved *in vivo* muscle function from baseline to sacrifice, which was hindered with DOX treatment alone. This provides merit that the combination of Cr supplementation and RT is an effective non-pharmacological approach to target DOX induced myotoxicity. Additionally, the current study explored how DOX, Cr, and RT affect signaling pathways for skeletal muscle repair and regeneration, and results provide insight into exploring the role that MyoD, myostatin, and MuRF-1 specifically play in the progression of DOX myotoxicity and how these transcription factors are involved in protection afforded by RT and Cr.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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