Global Journals LaTeX JournalKaleidoscope™
Artificial Intelligence formulated this projection for compatibility purposes from the original article published at Global Journals. However, this technology is currently in beta. *Therefore, kindly ignore odd layouts, missed formulae, text, tables, or figures.*

Effects of Exercise on Doxorubicin Accumulation and Multidrug Resistance Protein Expression in Striated Muscle

David Hydock[1], Colin J Quinn[2] and Noah M. Gibson[3]

[1] University of Northern Colorado

Received: 12 December 2015 Accepted: 4 January 2016 Published: 15 January 2016

Abstract

The chemotherapy drug doxorubicin (DOX) is well known to induce cardiac and skeletal muscle dysfunction. Previous studies demonstrate that exercise can mitigate dysfunction, reduce myocardial DOX accumulation, and depress markers of oxidative stress, but a putative mechanism is unknown. The aim of this study was to determine whether multidrug resistance protein (MRP) expression contributes to the protective effects of exercise against DOX-induced muscular dysfunction. Lower left ventricle (LV) and soleus DOX concentrations were observed in exercised animals, and MRP- 1, MRP-2, and MRP-7 expression was significantly increased in the LV with exercise. No MRP variations were apparent in skeletal muscles following the exercise protocol. As a marker of oxidative stress, malondialdehyde+4 hydroxyalkenal levels were analyzed, and exercise reduced both cardiac and skeletal muscle levels from exercised trained animals treated with DOX had significantly lower levels than SED-DOX. This study suggests increased MRP expression with exercise may contribute to exercise-induced protection in cardiac muscle but not skeletal muscle.

Index terms— ABC transporters, adriamycin, anthracyclines, cardiotoxicity, chemotherapy, oxidative stress, myotoxicity.

1 I. Introduction

nthracycline antibiotics are commonly used chemotherapeutic agents that have a wide range of applications to treat many cancer types including solid tumors. Doxorubicin (DOX, trade name: Adriamycin) is one of the most effective anthracyclines; however, it is associated with cardiotoxicity. This cardiotoxicity is dose-dependent and can eventually lead to congestive heart failure. Mechanisms of the cardiotoxic milieu associated with anthracyclines are mainly attributed to oxidative stress. DOX molecules undergo redox cycling at complex I of the electron transport chain 1 . Additionally, DOX impairs mitochondrial respiration and elevates the formation of reactive oxygen species (ROS). An overproduction of ROS can damage cellular components and induce signaling pathways that lead to apoptosis 2,3 , necrosis 4 , and autophagy 5,6 .

In addition to the cardiotoxicity of anthracyclines, myotoxic effects are also evident and can manifest as increased fatigue and reduced force production **??**6, **??**7 . This has been reported as being due to oxidative stress as well, which can affect calcium handling and actin-myosin cross bridge cycling **??**8, **??**9 . While there is relatively little research examining these mechanisms, it has been reported that exercise plays a beneficial role by mitigating skeletal muscle dysfunction **??**0 , autophagic signaling 5, **??**1 , and proteolysis 22 .

The multidrug resistance protein (MRP) family, which is part of the super family of ATP binding cassette (ABC) transporters, includes a group of drug efflux pumps and transporters known to extrude cellular anthracyclines **??**23] **??**24][25][26] . Gene transfected upregulation of MRPs has been shown to confer multidrug resistance on cultured cells by decreasing intracellular drug concentrations 27 .

Additionally, MRP-1 knockout cardiomyocytes show increased 4-hydroxynonenal production, a marker of oxidative stress, when exposed to DOX 28 . Parry and Hayward 29 indicated cardiomyocyte expression of MRP-1 and MRP-2 was increased in previously-exercised rats receiving DOX versus paired sedentary animals.

Our laboratory has previously reported that exercise preconditioning is associated with a decrease in cardiac DOX accumulation 9 and reduced lipid peroxidation 30 . Additionally, Marques-Aleixo et al. 31 Endurance exercise interventions have been used as an effective strategy to mitigate the severity of anthracycline cardiotoxicity 7,8 . With both voluntary wheel running and treadmill training, our laboratory has observed attenuated DOX-induced cardiac dysfunction analyzed both in vivo and ex vivo [9][10][11] . With echocardiographic functional analyses, exercise preconditioned animals receiving DOX show increased fractional shortening, aortic and mitral valve maximal blood flow velocity, and mean blood flow velocity when compared to sedentary animals receiving DOX **??**2, **??**3 . Furthermore, isolated working heart experiments have shown preservation in left ventricular developed pressures, maximal rate of pressure development, and maximal rate of pressure decline with exercise 11, **??**4 . demonstrated treadmill and free-wheel running in rats preserved oxidative phosphorylation proteins, mitochondrial complexes' I and V content and activity, and mitochondrial biogenesis while decreasing oxidative stress associated with DOX treatment. Taken together, prior exercise may influence MRP content and consequent uncontrolled ROS generation. The purpose of this study was to determine if MRP expression in striated muscle is up regulated with exercise and if its expression is linked with decreases in DOX accumulation and oxidative stress.

2 II. Methods

3 a) Animal Care and use

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. Ten week old male Sprague-Dawley rats (n=32) were purchased from Harlan Laboratories (Indianapolis, IN) and housed in an environmentally controlled facility on a 12:12 hour light: dark cycle. Rat chow (Teklad 2016: Harlan) and distilled water were provided ad libitum. Rats were randomly assigned to sedentary (SED, n=16) or wheel run (WR, n=16) groups. WR rats were housed in cages equipped with voluntary running wheels and allowed 24-hour access to wheels for 10 weeks. SED animals were restricted to normal cage activity for the duration of the study. Following completion of the ten weeks of exercise, WR animals were removed from wheel cages.

Twenty-four hours following removal from wheel access, both groups (SED & WR) were randomly assigned to receive a single bolus injection of DOX or saline as a placebo (SED-DOX, n=8; SED-SAL, n=8; WR-DOX, n=8; WR-SAL, n=8). DOX animals received 15 mg DOX/kg body mass i.p. and SAL animals received an equivalent volume of 0.9% SAL i.p. injection. All animals were euthanized 24 hours following DOX or SAL injection with an i.p injection of heparini zed (500 U) sodium pentobarbital (50 mg/kg). Immediately following the absence of a tail-pinch reflex, the heart was rapidly excised, and the aorta cannulated and flushed of blood with Krebs-Hansleit buffer (in mM, 120 NaCl, 5.9 KCl, 2.5 CaCl2, 1.2 MgCl2, 25 NaHCO3, 17 glucose, and 0.5 EDTA, pH 7.4) through retrograde perfusion. Upon clearance of blood, the left ventricle (LV) was isolated and flash frozen in liquid nitrogen for subsequent biochemical analyses. Skeletal muscles were isolated quickly following heart excision. The soleus (SOL) and extensor digit orum longus (EDL) were obtained from the right hind limb to represent type I and type II dominant muscle, respectively and flash frozen in liquid nitrogen.

4 b) Quantification of doxorubicin

High performance liquid chromatography (HPLC) was used to quantify DOX accumulation as previously described **??**7 . Approximately 50 mg of LV or skeletal muscle tissue was diluted with a 0.067 M phosphate buffer (pH 7.4) and homogenized using a Virtishear homogenizer (Virtis: Gardner, NJ). Homogenates were then subjected to protein precipitation by adding 200 ?L of a 50:50 (v/v) mixture of HPLC-grade methanol and 40% ZnSO4 to 150 ?L of homogenized tissue. Fifty ?L of daunorubicin (DAUN) (Sigma: St. Louis, MO) at an initial concentration of 500 ng/mL was added to the sample as an internal standard. The sample was vortexed for 1 min before centrifugation at 1,500 g for 10 minutes. The supernatant was filtered through a 0.2 ?M syringe filter and injected directly onto the column to initiate the analytical method. Quantification of DOX and DAUN was performed using a Shimadzu HPLC system (Shimadzu: Kyoto, Japan) equipped with the following components: DGU-14A degasser, dual pump LC-10A LC chromatograph, SPD-M10A diode array detector, SLC-10A system controller, and RF-10Axl fluorescence detector. The stationary phase consisted of a reverse phase Zorbax C8 column (Agilent Technologies: Santa Clara, CA). The fluorescence detector used to quantify DOX and DAUN was operated at an excitation of 470 nm and emission of 550 nm. A volume of 20 ?L of sample was injected directly onto the column.

5 c) MRP expression d) Lipid peroxidation

A commercially available assay kit (Bioxytech LPO-586, Oxis Research: Foster City, CA) was used to measure malondialdehyde + 4-hydroxyalkenals (MDA + 4-HAE) as an indicator of cellular lipid peroxidation. Tissue homogenates in RIPA were measured for the assay. A 200 µL aliquot of each sample was added to a micro

centrifuge tube followed by 650 µL of N-methyl-2-LV and skeletal muscle homogenates were analyzed for MRP-1, MRP-2, and MRP-7 by Western blotting. Approximately 100 mg of tissue was homogenized in a dilution of 1:10 w:v in RIPA buffer (Sigma-Aldrich) using a Virtishear homogenizer. Homogenates were centrifuged at 10,000 g for 10 minutes, and supernatant was removed for analysis. Total protein was determined by a Bradford assay 31 . Protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% polyacrylamide gel and transferred to a nitrocellulose membrane in a Novex Sure Lock blotting unit (Invitrogen: Carlsbad, CA). Membranes were incubated overnight with primary antibodies (Santa Cruz Biotechnology: Dallas, TX) for MRP-1 (anti-goat), MRP-2 and MRP-7 (anti-rabbit) in a 1:500 dilution and developed using Novex Western Breeze Immunodetection kits (Invitrogen). Developed membranes were scanned and proteins bands were analyzed using densitometry (ImageJ: NIH, Bethesda, MD). phenylindole in acetonitrile and briefly vortexed. Next, 150 µL of methanesulfonic acid was added, vortexed, and incubated at 45°C for 60 minutes. Samples were then centrifuged at 10,000 g for 10 minutes. The resulting supernatant was transferred to a cuvette, and absorbency was measured using a spectro photometer at 586 nm. MDA + 4-HAE was estimated from a standard curve. All samples were assayed in duplicate, and any samples varying more than 5% were reassayed.

6 e) Statistical analysis

All data are expressed as mean ± standard deviation (mean±SD). A Student's t-test was performed to compare tissue DOX accumulation in DOX-treated groups (SED-DOX vs. WR-DOX). A two-way analysis of variance (ANOVA, drug x exercise) was performed to determine significant main effects and interactions for MRP expression and MDA + 4-HAE in LV, SOL, and EDL. If a significant difference was observed, a Tukey's post hoc test was performed to identify where differences existed. For all procedures, significance was set at the ?= 0.05 level.

7 III. Results

8 a) Animal characteristics

Heart and skeletal muscle masses are presented in Table **??**. No main effects or interactions ($p>0.05$) were observed between groups in skeletal muscle mass. A drug effect was observed for heart mass with animals receiving DOX having a lower heart mass ($p=0.025$), post hoc testing revealed that WR-DOX had significantly lower heart mass than SED-SAL ($p<0.05$).

9 b) Doxorubicin accumulation

Figure **??** illustrates DOX accumulation in cardiac and skeletal muscle. DOX accumulation varied between tissue types, which is consistent with previous reports from our laboratory 17 . As expected, the LV accumulated the greatest quantity of DOX with a significantly lower level observed in WR animals when compared to SED ($p<0.05$, WR: 1055±188 ng/g versus SED: 1284±150 ng/g, Figure **??**A). Similarly, exercise groups had a lower level of DOX in the SOL ($p<0.05$, WR: 505±99 ng/g versus SED: 626±86 ng/g, Figure **??**B). In contrast to the oxidative muscle fibers found in the heart and SOL, no DOX level differences were observed in the EDL between exercised and sedentary animals ($p>0.05$, WR: 219±89 ng/g versus SED: 287±85 ng/g, Figure **??**C).

10 c) MRP expression

Analysis of MRP expression in the LV by Western blotting revealed a significant DOX effect with an up regulation of MRP-1, MRP-2, and MRP-7 (+79%, +107%, and +193%, respectively; $p<0.05$) as well as a significant exercise effect (+56%, +99%, and +179%, respectively; $p<0.05$, Figure 2). In addition, MRP-1 and MRP-7 appear to have significant ($p<0.05$) additive increases in WR-DOX when compared to WR-SAL (MRP-1: +80%; MRP-7: +150%). In contrast to LV MRP expression, no significant main effects were observed in SOL MRP-1, MRP-2, or MRP-7 or EDL MRP-2 or MRP-7 expression (Figures 3 & 4). An interaction was observed in SOL MRP-7 ($p=0.0240$). No main effects or interactions were observed for MRP-2 or MRP-7 in the EDL ($p>0.05$). It should be noted that MRP-1 expression was not detected in the EDL.

11 d) Lipid peroxidation

MDA + 4-HAE was quantified as a marker of oxidative stress-induced cell damage (Fig. 5). With all tissues analyzed, WR reduced lipid peroxidation in DOXtreated tissues (LV, -23%; SOL, -44%; EDL, -34%), suggesting exercise protects against DOX induced lipid peroxidation. Significant drug and activity effects were observed in the LV ($p<0.05$) while significant drug effects and interactions were observed in SOL and EDL ($p<0.05$). SED-DOX MDA + 4-HAE was significantly greater than all other groups across in the three tissues examined ($p<0.05$).

12 IV. Discussion

Exposure to DOX provokes damage to the structure and function of both cardiac and skeletal muscles ??6, ??7,33,34 . DOX-induced structural alteration was evident by decreases in heart mass. Exercise as a preventative and complementary treatment, however, has demonstrated increased tolerance to DOX in cardiac and skeletal muscle tissues 7, ??2,35,36 . Specifically, reduction of tissue DOX accumulation with exercise preconditioning has previously been linked to the maintenance of cardiac function following DOX treatment 9 . The current experiments provide further evidence in support of a mechanism that may contribute to exercise-induced cardio protection in this setting. It is suggested that an up regulation of MRP expression in the myocardium is associated with a decrease in intracellular DOX accumulation. In addition, these data also suggest that MRP expression contributes to the reduced LV oxidative stress observed in exercised groups. This study observed drug and activity main effects with significantly elevated of MRP-1, -2 and -7 expression in the LV with DOX and prior treadmill training, respectively. Additionally, an interaction was observed in LV expression of MRP-2. No significant changes in skeletal muscle MRP expression with exercise or drug treatments; however, WR groups exhibited less DOX accumulation in the SOL and lower levels of oxidative stress in both the SOL and EDL.

Myocardial alterations of MRP expression and oxidative stress with exercise predicate the examination for a relationship with cell health. Glutathione (GSH) is an antioxidant present in a variety of cells, and DOX has been shown to deplete populations, specifically in the heart 37 . Kwok and Richardson 38 showed DOX-treated myocardial cells to increase levels of oxidative stress. Lipid peroxidation products (alkenals) react with GSH, reducing levels and have been correlated with apoptosis 39 . Highly oxygen-dependent tissues may be particularly susceptible to reactive oxygen species damaging proteins and lipids, leading to cell dysfunction and death. MRP-1, MRP-2 and MRP-7 are known to extrude anthracyclines, such as DOX, as well as GSH conjugates 40,41 . Krause et al. 42 reported that 60 minutes of exercise per day for only 1 week induced an up regulation of MRP-1 in rat myocardium (2.4-fold). This resulted in an increase in MRP-1 function and reduced redox imbalance associated with DOX exposure. In the current study, DOX treatment induced elevated levels of MRP-1, -2, and -7 in the LV. Levels of MRP-1 and -7 were highest in WR-DOX groups suggesting an additive effect. Accordingly, levels of DOX accumulation in LV were significantly higher in the DOX-treated SED than WR groups.

The aforementioned study by Krause et al. 42 reported that MRP-1 expression in whole gastrocnemius was absent. This is consistent with our results in the EDL, a muscle of similar fiber type; however, we did detect MRP-1 expression in the SOL, a muscle with greater oxidative capacity. It may be the case that the greater the oxidative muscle fiber content, the more apt the cells are to handling oxidative stress with the expression of MRP-1. Another possible explanation for MRP-1 expression variability is the localization of the protein in the mitochondrial membrane following DOX exposure. It was reported that following DOX treatment, a 2-fold MRP-1 expression aggregated at the mitochondrial membrane of cardiomyocytes versus observations in whole heart homogenate samples 28 . This evidence suggests that MRP-1 may be expressed specifically where greatest redox cycling occurs. Additionally, DOX preferentially accumulating at the mitochondria, due to cardiolipin binding, may drive higher localized MRP expression 17 . Furthermore, the mitochondrial content between muscle fiber types varies greatly (20-30% in cardiomyocytes, 6% in oxidative skeletal muscle, and 2-3% in glycolytic skeletal muscle) which may contribute to the differential expression between muscle types. However, no studies have examined the effect of exercise on MRP-1 expression in the mitochondrial membrane, and thus, this warrants future investigation.

Our lab has observed a significant decline in skeletal muscle function 24 hours following DOX treatment with increasing loss of function 5 days following treatment 17 . While skeletal muscle did not show significant changes in MRP expression, there was a significant decrease in SOL DOX accumulation and a trend toward a decrease in EDL DOX accumulation. One possible explanation for this may be the increase in mitochondrial function with chronic endurance training regardless of mitochondrial quantity. Exercise induces an improvement in skeletal muscle metabolic capacity, which enhances the function and efficiency of the mitochondria. This could be achieved through a process whereby increased mitochondrial biogenesis replaces mitochondria damaged by DOX exposure resulting in an overall healthier mitochondrial population within the cell without an increase in mitochondrial density per se. DOX has been shown to acutely (2 hours-post) reduce mitochondrial function by inhibiting complex-I and II and increasing H 2 O 2 production as well as disrupting membrane potential and compromising respiratory capacity 72 hours following exposure 43 . Prior exercise may contribute to reducing this dysfunction by improved mitochondrial function at the onset of treatment there by better maintaining function and possibly improving mitochondrial turnover following DOX-induced damage. Further studies are necessary to elucidate these mechanisms.

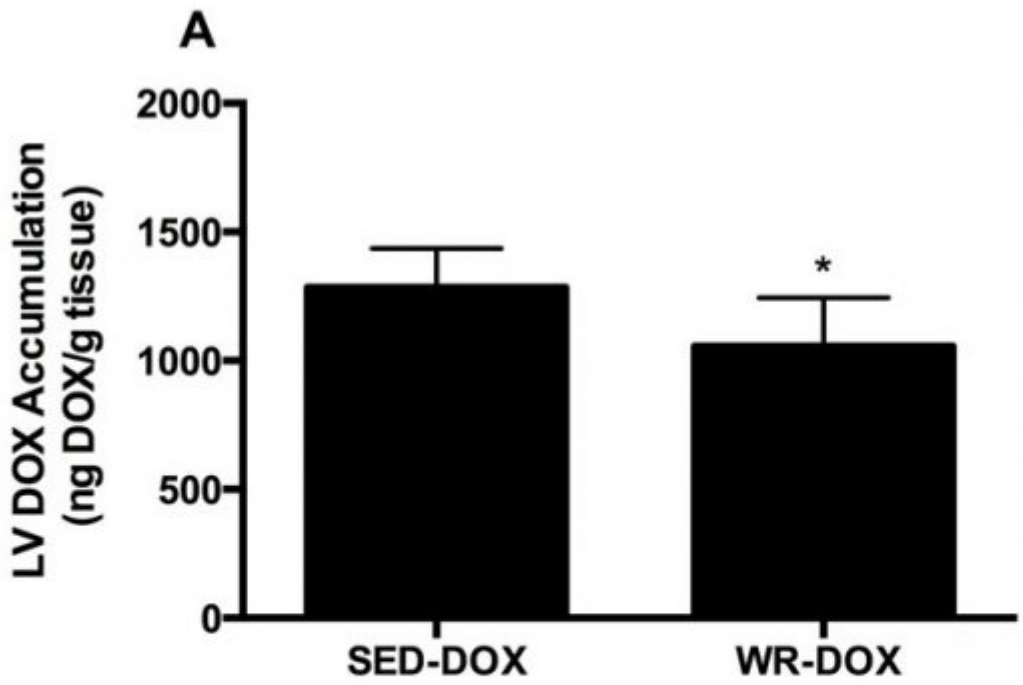
It should be noted that the xenobiotic function of MRP-1 in rodent species exhibits lesser DOX export capacity than that of human cells 44,45 ; however, MRP-1 maintains its ability to transport oxidized glutathione, which may contribute to the protection against DOXinduced oxidative stress with exercise preconditioning. Additionally, Hopper-Borge et al. 27 characterized MRP-7's DOX resistance as significantly lower activity levels than MRP-1 and -2. In spite of lesser transport activity, greater expression of MRP-1 and -7 seen in this study may have contributed to lesser DOX accumulation in the LV in WR animals. Of additional interest, elevated MRP expression has been linked to vincristine resistance and increased release of GSH disulfides thereby reducing oxidative stress 46, ??7 . To our knowledge, this is the first study examining striated muscle expression of MRP-2 and MRP-7 with exercise.

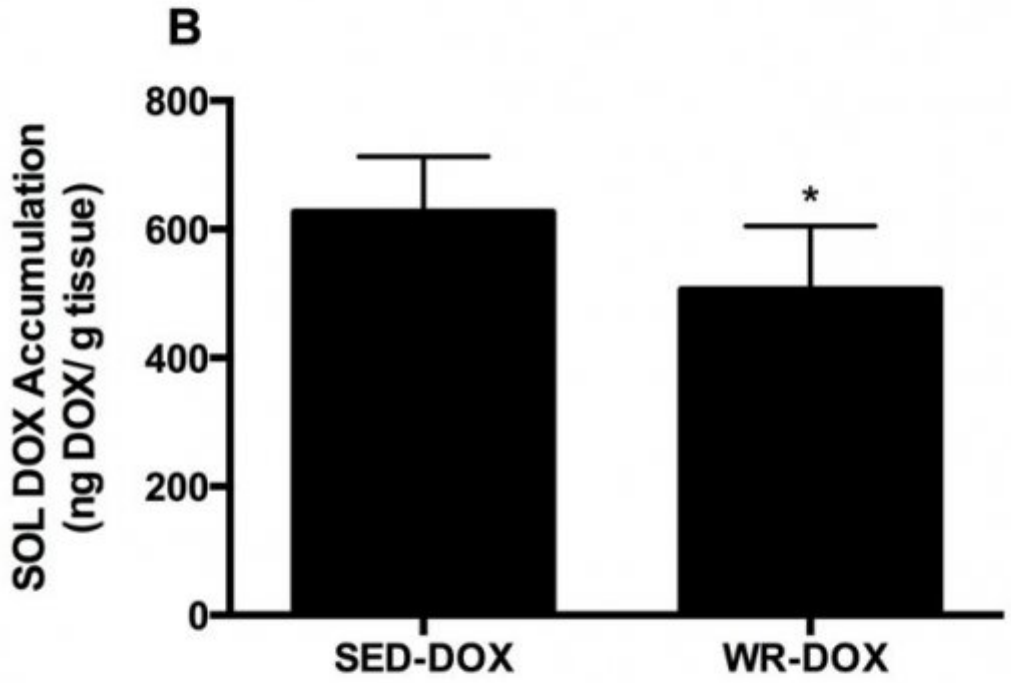
13 V. Conclusion

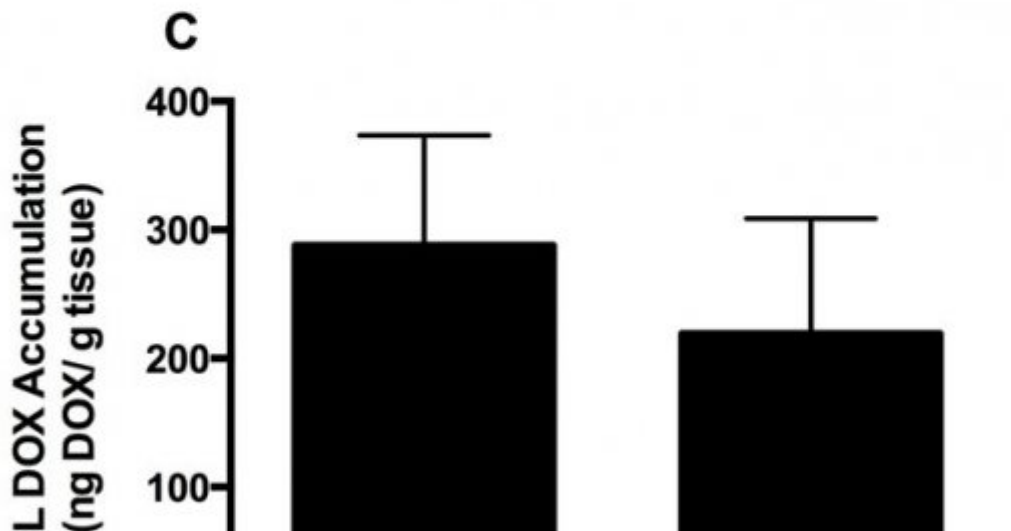
The current report examined the role of MRP expression as a possible mechanism of protection against DOX-induced striated muscle toxicities. Exercise promoted an up regulation of MRPs, decreased DOX accumulation and decreased lipid peroxidation in the LV. However, no modulation of MRP expression was observed in either the SOL or EDL, but a decrease in DOX accumulation was observed in the SOL. Additionally, a reduction in lipid peroxidation was observed with WR in both skeletal muscles analyzed. These findings suggest that cardioprotection may be induced by an up regulation of MRPs, which may reduce DOX accumulation and increase the ability of the heart to with stand oxidative stress, but MRP changes do not explain the observed exercise-induced reductions in lipid peroxidation in skeletal muscle with DOX treatment. These findings also further contribute to the notion that chronic exercise training plays an important and applicable role in reducing DOX-induced

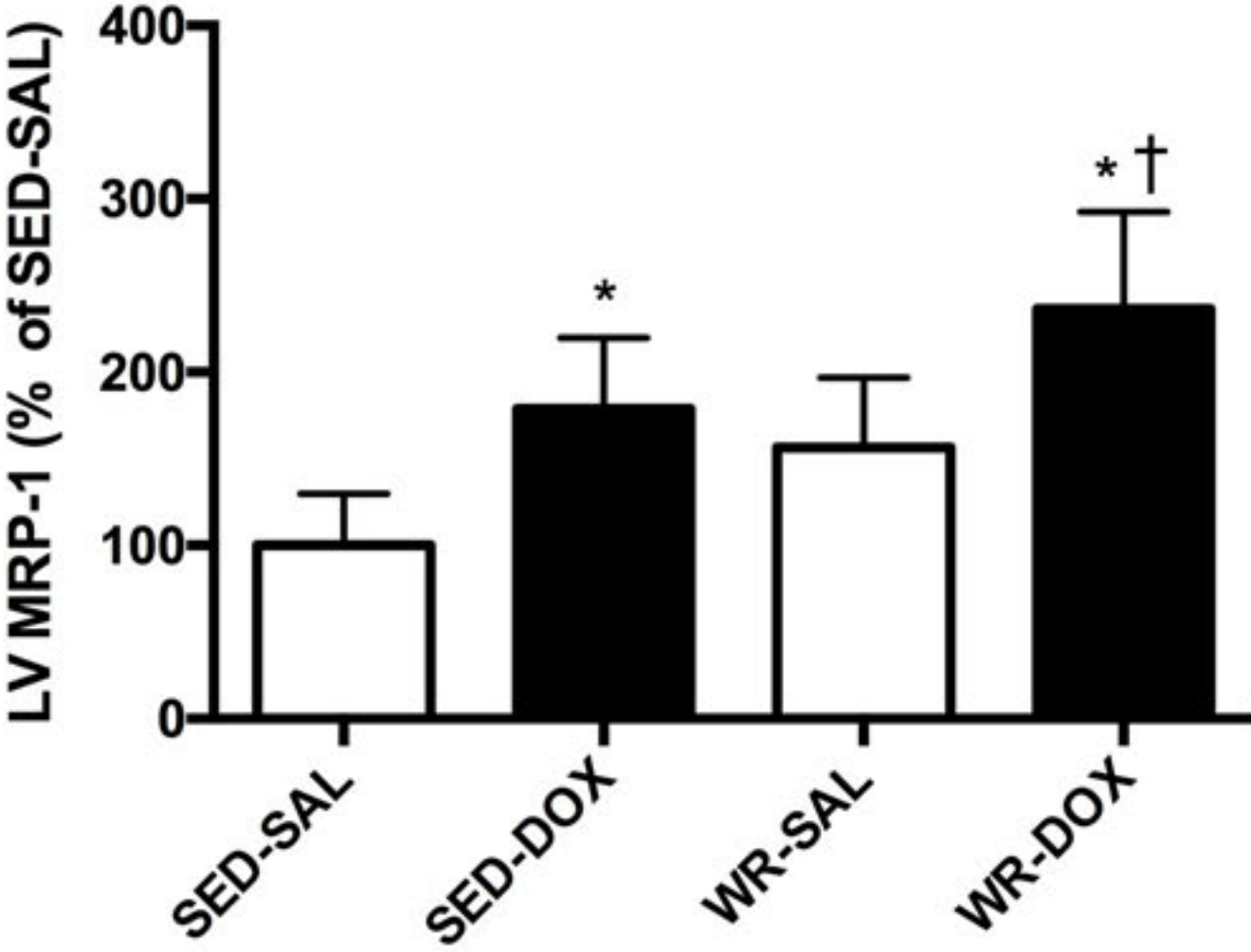
Figure Captions

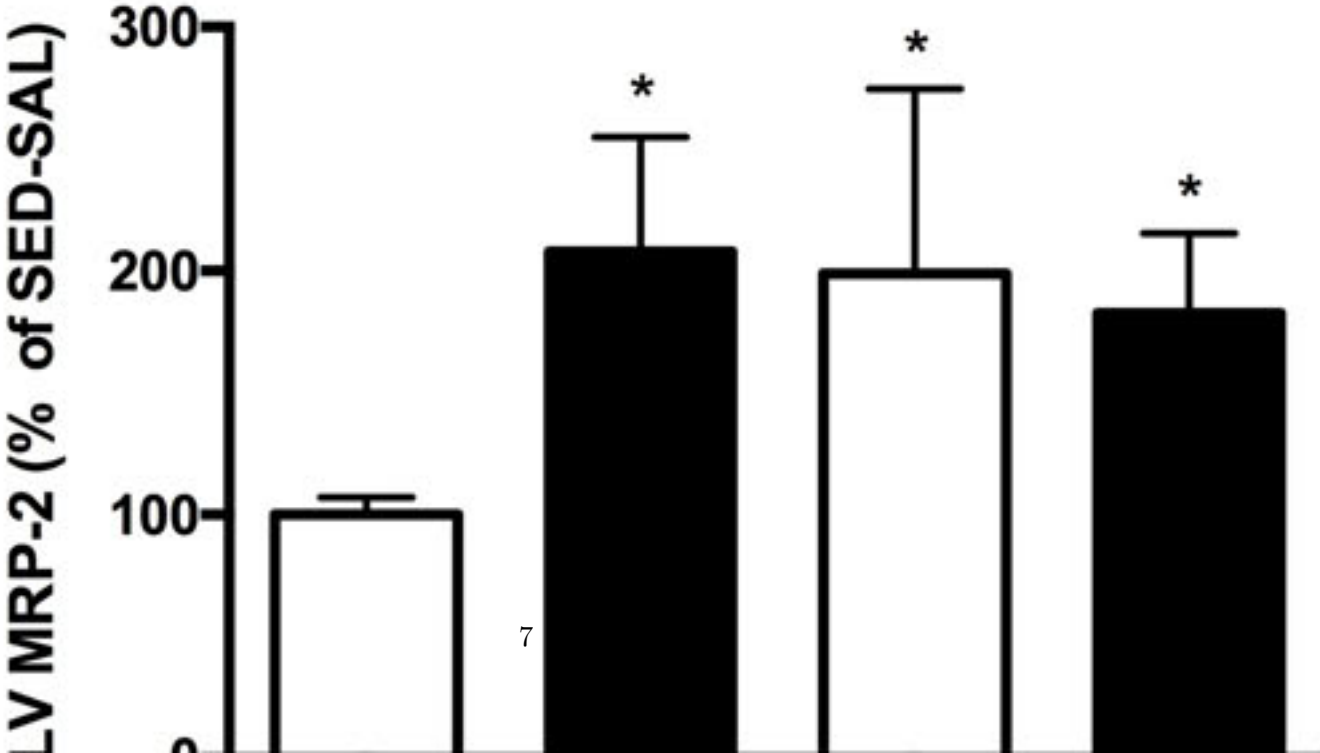
[1]

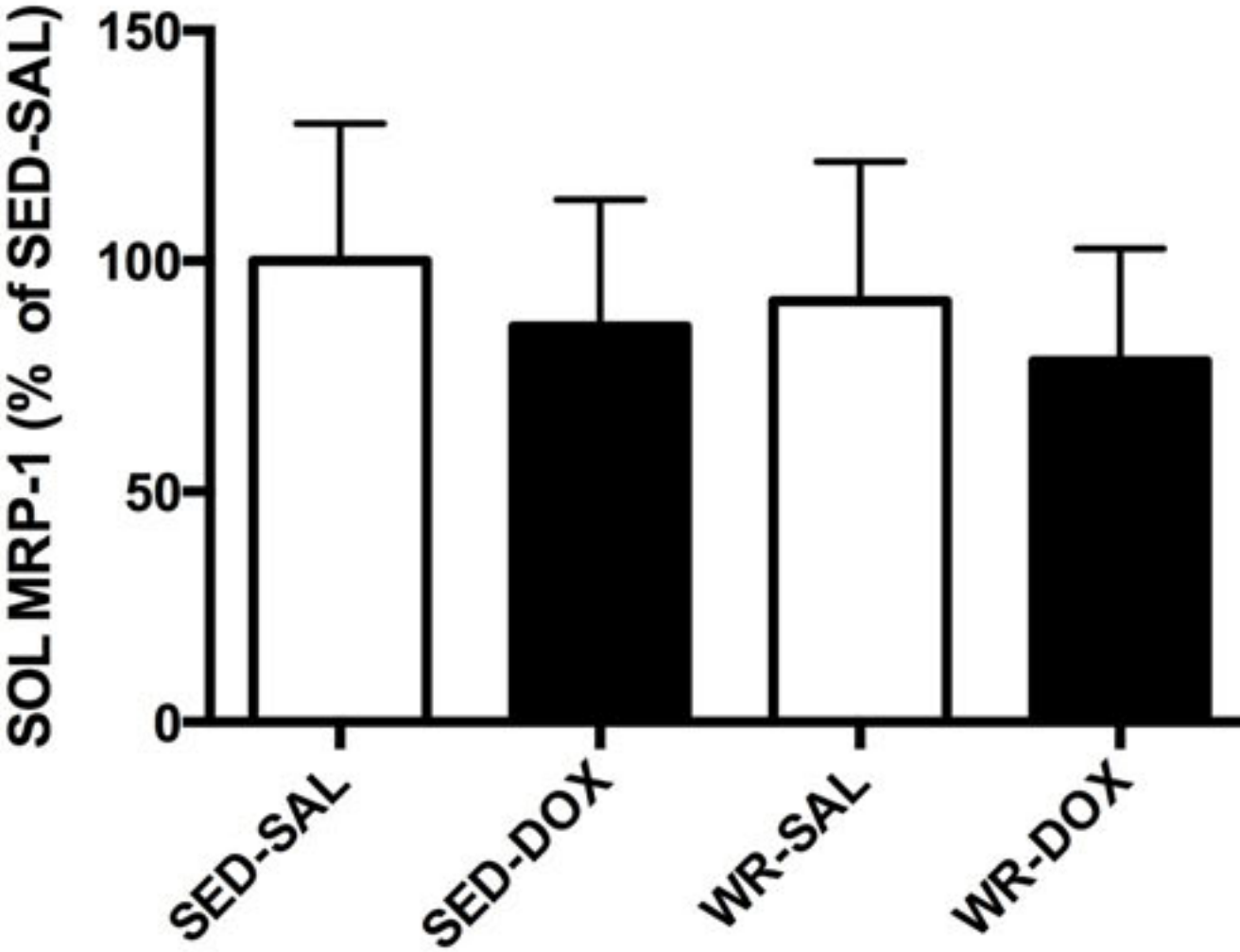

A
LV DOX Accumulation (ng DOX/g tissue)
2000
1500
1000
500
0
*
SED-DOX
WR-DOX

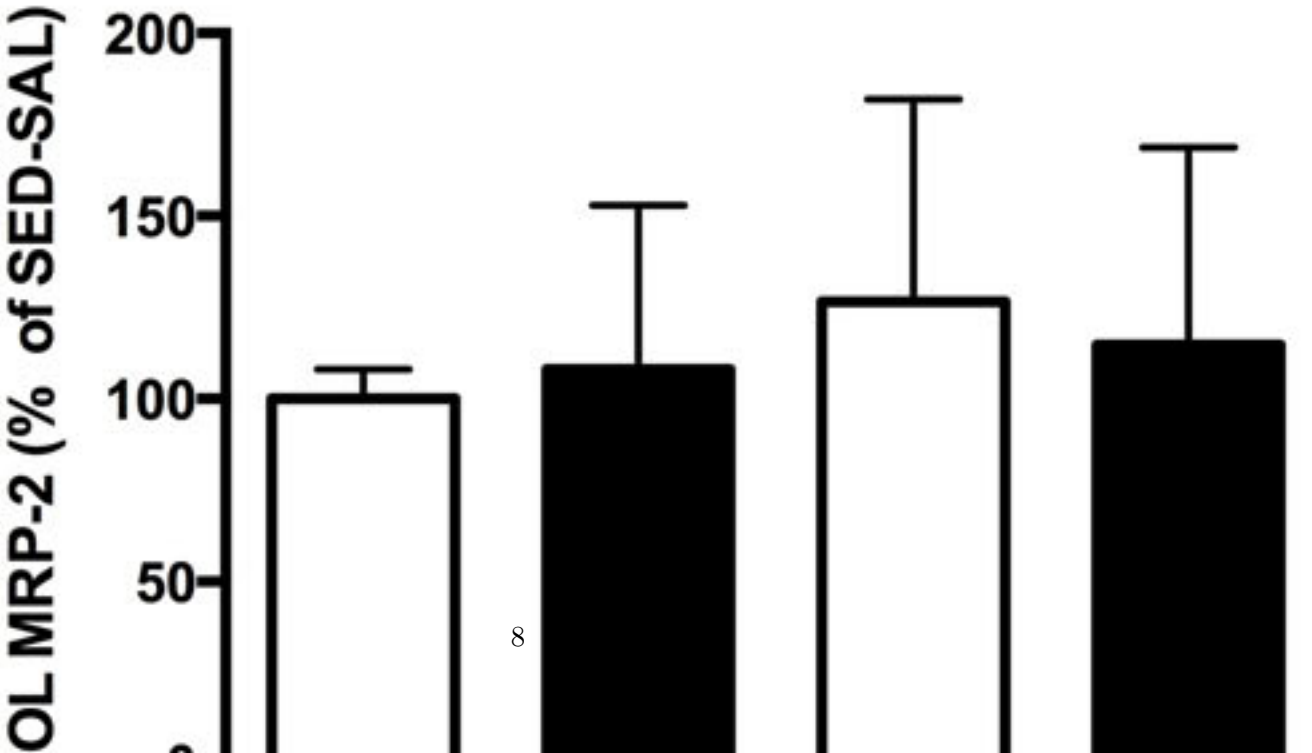

B
SOL DOX Accumulation (ng DOX/ g tissue)
800
600
400
200
0
*
SED-DOX
WR-DOX

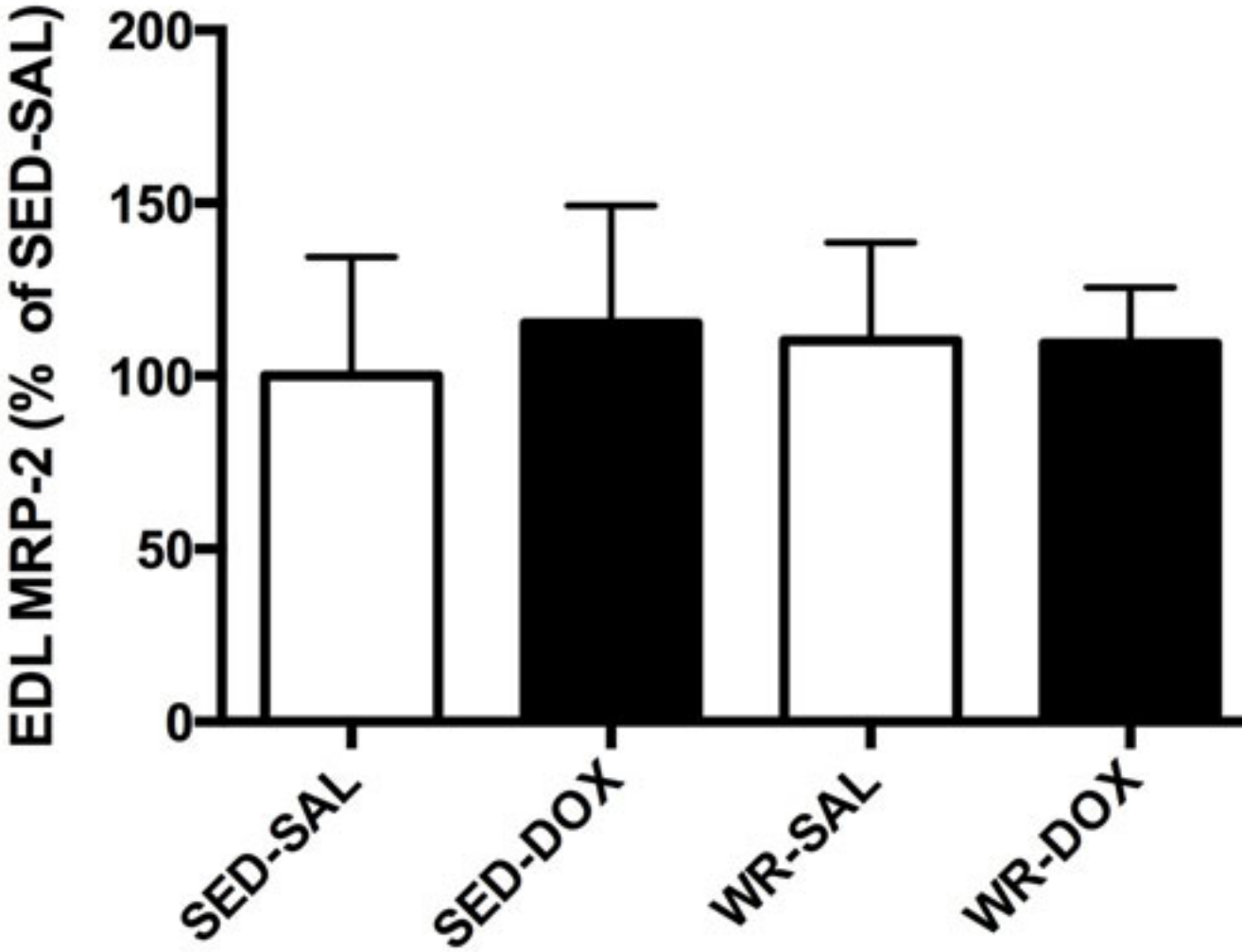

C
L DOX Accumulation (ng DOX/ g tissue)
400
300
200
100

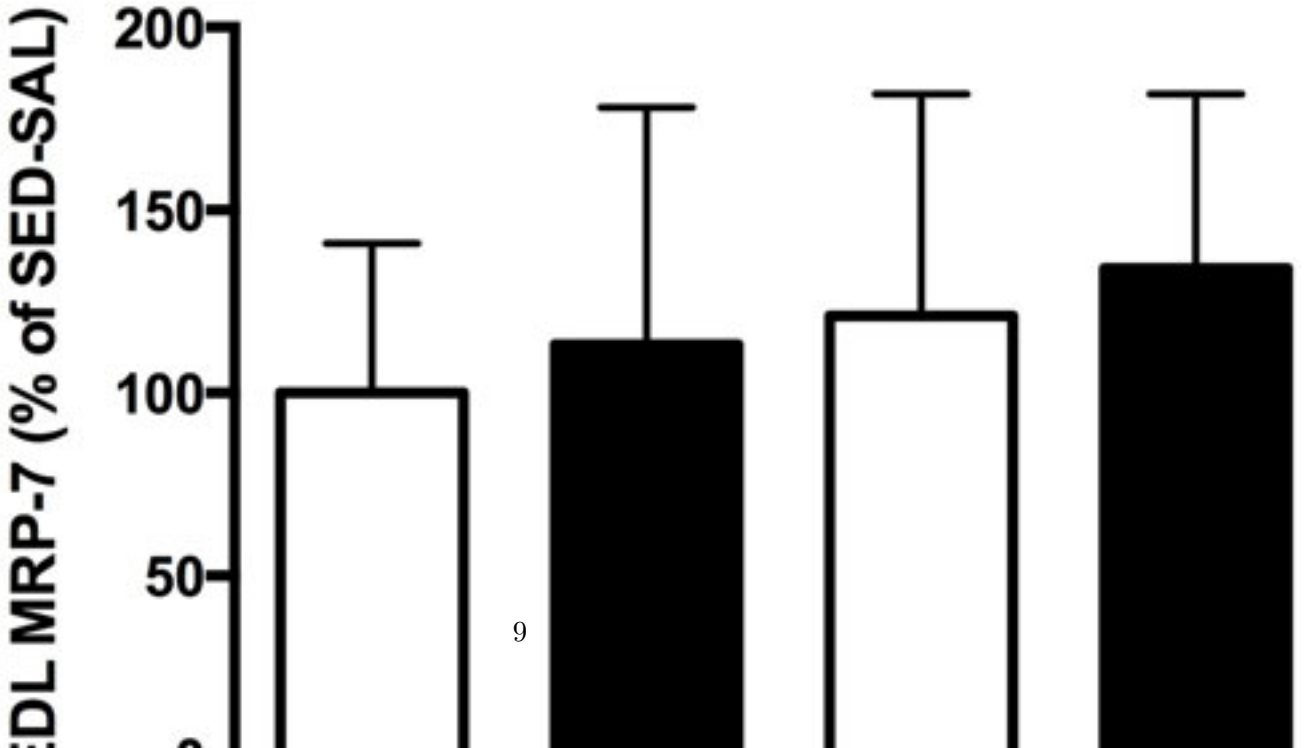

LV MRP-1 (% of SED-SAL)
400
300
200
100
0
*
* †
SED-SAL
SED-DOX
WR-SAL
WR-DOX

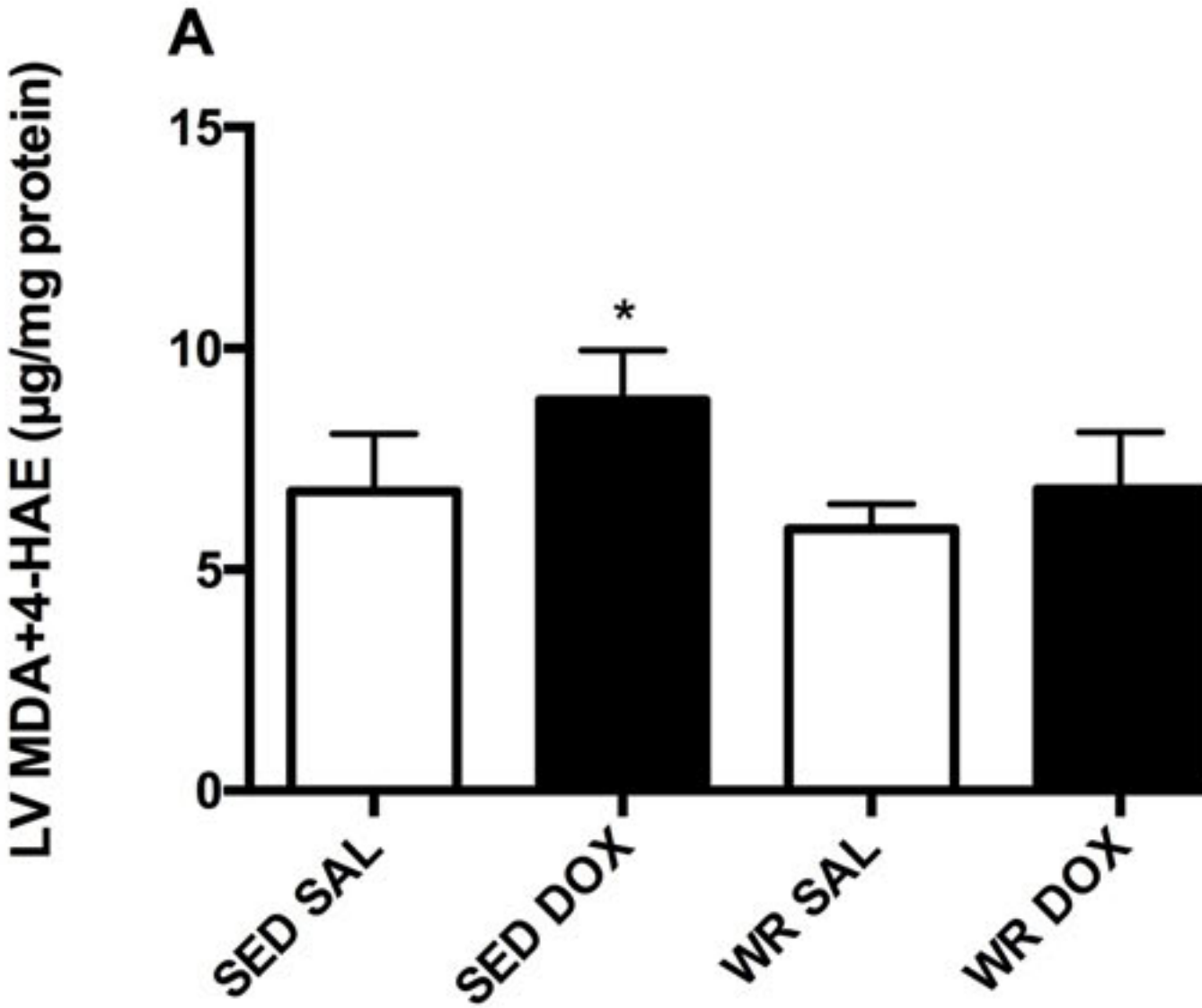

LV MRP-2 (% of SED-SAL)
300
200
100
*
*
*

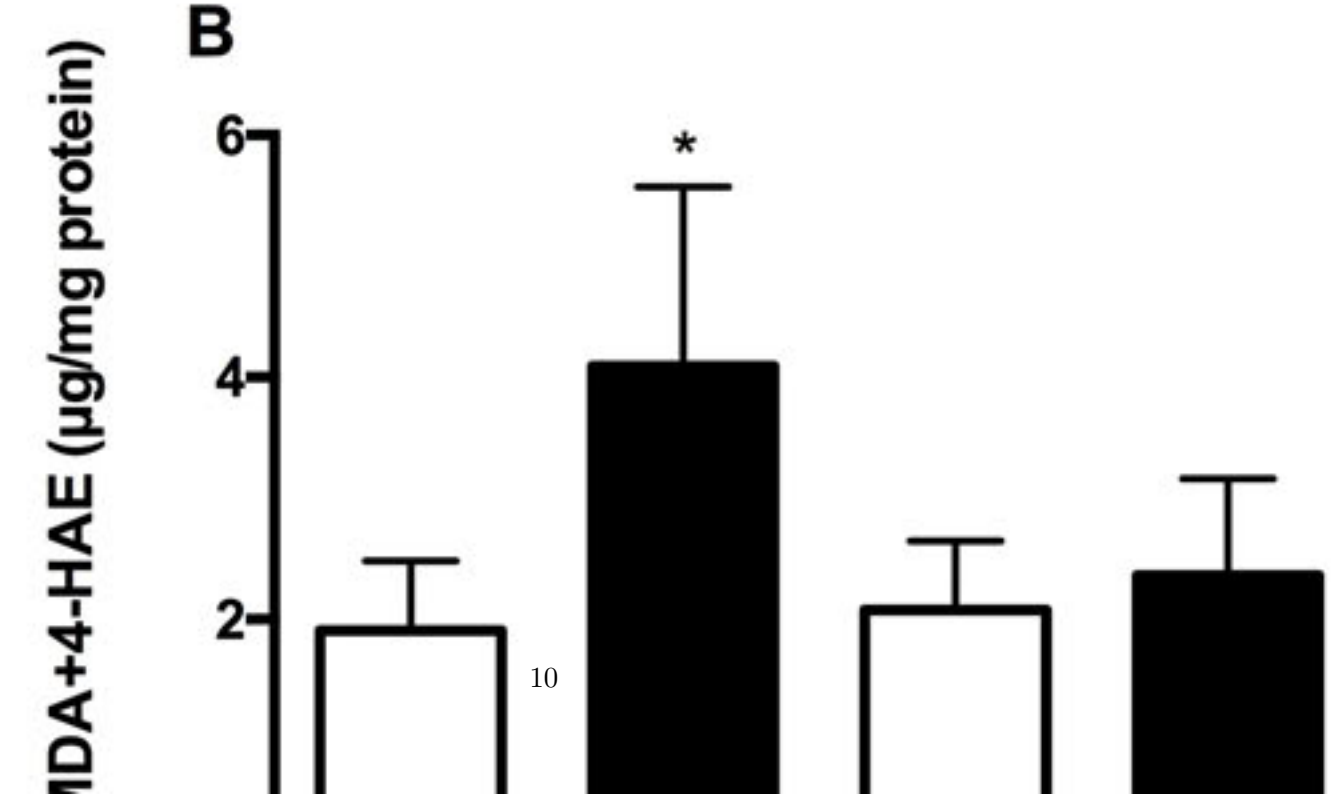

SOL MRP-1 (% of SED-SAL)
150
100
50
0
SED-SAL
SED-DOX
WR-SAL
WR-DOX


SOL MRP-2 (% of SED-SAL)
200
150
100
50


EDL MRP-2 (% of SED-SAL)
200
150
100
50
0
SED-SAL
SED-DOX
WR-SAL
WR-DOX


EDL MRP-7 (% of SED-SAL)
200
150
100
50


A
LV MDA+4-HAE (µg/mg protein)
15
10
5
0
*
SED SAL
SED DOX
WR SAL
WR DOX


B
MDA+4-HAE (µg/mg protein)
6
4
2
*

side-effects which may improve quality of life, but more work needs to be done exploring tissue-specific mechanisms.

12. Chicco AJ, Schneider CM, Hayward R. Exercise training attenuates acute doxorubicin-induced cardiac dysfunction. Journal of cardiovascular pharmacology. 2006; 47: 182-189.
13. Marchandise B, Schroeder E, Bosly A, et al. Early detection of doxorubicin cardiotoxicity: interest of Doppler echocardiographic analysis of left ventricular filling dynamics. American heart journal. 1989; 118: 92-98.
14. Hydock DS, Lien C-Y, Jensen BT, Schneider CM, Hayward R. Exercise preconditioning provides long-term protection against early chronic doxorubicin cardiotoxicity. Integr Cancer Ther. 2011; 10: 47-57. 15. Hydock DS, Lien C-Y, Jensen BT, Parry Experimental biology and medicine. 2012; 237: 1483-1492.
16. Hydock DS, Lien CY, Jensen BT, Schneider CM, Hayward R. Characterization of the effect o

D D D D) K D D D D) K Direct effects of doxorubicin on skeletal muscle

[Bradford ()] 'A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding'. M M Bradford . *Anal Biochem* 1976. 72 p. .

[Hopper-Borge et al. ()] 'Analysis of the drug resistance profile of multidrug resistance protein 7 (abcc10): Resistance to docetaxel'. E Hopper-Borge , Z S Chen , I Shchaveleva , M G Belinsky , G D Kruh . *Cancer research* 2004. 64 p. .

[Kwok and Richardson ()] 'Anthracyclines induce accumulation of iron in ferritin in myocardial and neoplastic cells: Inhibition of the ferritin iron mobilization pathway'. J Kwok , D Richardson . *Molecular pharmacology* 2003. 63 p. .

[Sishi et al. ()] 'Autophagy upregulation promotes survival and attenuates doxorubicin-induced cardiotoxicity'. B J Sishi , B Loos , J Van Rooyen , A M Engelbrecht . *Biochemical pharmacology* 2013. 85 p. .

[Yoshida et al. ()] 'Chronic doxorubicin cardiotoxicity is mediated by oxidative dna damage-atm-p53-apoptosis pathway and attenuated by pitavastatin through the inhibition of rac1 activity'. M Yoshida , I Shiojima , H Ikeda , I Komuro . *Journal of molecular and cellular cardiology* 2009. 47 p. .

[Lebrecht et al. ()] 'Dexrazoxane prevents doxorubicin-induced long-term cardiotoxicity and protects myocardial mitochondria from genetic and functional lesions in rats'. D Lebrecht , A Geist , U P Ketelsen , J Haberstroh , B Setzer , U A Walker . *British journal of pharmacology* 2007. 151 p. .

[Minotti et al. ()] 'Doxorubicin cardiotoxicity and the control of iron metabolism: Quinone-dependent and independent mechanisms'. G Minotti , S Recalcati , P Menna , E Salvatorelli , G Corna , G Cairo . *Methods Enzymol* 2004. 378 p. .

[Kalyanaraman et al. ()] 'Doxorubicin-induced apoptosis: Implications in cardiotoxicity'. B Kalyanaraman , J Joseph , S Kalivendi , S Wang , E Konorev , S Kotamraju . *Molecular and cellular biochemistry* 2002. p. .

[Carvalho et al. ()] 'Doxorubicin-induced cardiotoxicity: From bioenergetic failure and cell death to cardiomyopathy'. F S Carvalho , A Burgeiro , R Garcia , A J Moreno , R A Carvalho , P J Oliveira . *Med Res Rev* 2014. 34 p. .

[Smuder et al. ()] 'Doxorubicin-induced markers of myocardial autophagic signaling in sedentary and exercise trained animals'. A J Smuder , A N Kavazis , K Min , S K Powers . *J Appl Physiol* 1985. 2013. 115 p. .

[Kavazis et al. ()] 'Effects of short-term endurance exercise training on acute doxorubicin-induced foxo transcription in cardiac and skeletal muscle'. A N Kavazis , A J Smuder , S K Powers . *Journal of applied physiology* 2014. 117 p. .

[Wonders et al. ()] 'Endurance exercise training preserves cardiac function in rats receiving doxorubicin and the her-2 inhibitor gw2974'. K Y Wonders , D S Hydock , S Greufe , C M Schneider , R Hayward . *Cancer chemotherapy and pharmacology* 2009. 64 p. .

[Ascensão et al. ()] 'Exercise as a beneficial adjunct therapy during doxorubicin treatment-role of mitochondria in cardioprotection'. A Ascensão , P J Oliveira , J Magalhães . *International journal of cardiology* 2012. 156 p. .

[Jensen et al. ()] 'Exercise mitigates cardiac doxorubicin accumulation and preserves function in the rat'. B T Jensen , C Y Lien , D S Hydock , C M Schneider , R Hayward . *Journal of cardiovascular pharmacology* 2013. 62 p. .

[Hydock et al. ()] 'Exercise preconditioning protects against doxorubicin-induced cardiac dysfunction'. D S Hydock , C Y Lien , C M Schneider , R Hayward . *Med Sci Sports Exerc* 2008. 40 p. .

[Hydock et al. ()] 'Exercise preconditioning provides longadenocarcinoma cell lines'. D S Hydock , C Y Lien , B T Jensen , C M Schneider , R Hayward . *International journal of oncology* 2007. 30 p. .

[Chicco et al. ()] 'Exercise training attenuates acute doxorubicin-induced cardiac dysfunction'. A J Chicco , C M Schneider , R Hayward . *Journal of cardiovascular pharmacology* 2006. 47 p. .

[Parry and Hayward ()] 'Exercise training does not affect anthracycline antitumor efficacy while attenuating cardiac dysfunction'. T L Parry , R Hayward . *Am J Physiol Regul Integr Comp Physiol* 2015. 309 p. .

[Xie et al. ()] 'Glutathione transferase protects neuronal cultures against four hydroxynonenal toxicity'. C Xie , M A Lovell , W R Markesbery . *Free Radical Biology and Medicine* 1998. 25 p. .

[Slot et al. ()] 'Mammalian multidrug-resistance proteins (mrps)'. A J Slot , S V Molinski , S P Cole . *Essays Biochem* 2011. 50 p. .

[Nunoya et al. ()] 'Molecular cloning and pharmacological characterization of rat multidrug resistance protein 1 (mrp1)'. K Nunoya , C E Grant , D Zhang , S P Cole , R G Deeley . *Drug Metab Dispos* 2003. 31 p. .

[Jungsuwadee et al. ()] 'Mrp1 localization and function in cardiac mitochondria after doxorubicin'. P Jungsuwadee , R Nithipongvanitch , Y Chen , T D Oberley , D A Butterfield , St Clair , DK . *Molecular pharmacology* 2009. 75 p. .

[Krause et al. ()] 'Mrp1/gs-x pump atpase expression: Is this the explanation for the cytoprotection of the heart against oxidative stressinduced redox imbalance in comparison to skeletal muscle cells?'. M S Krause , L P Oliveira , Jr Silveira , E M Vianna , D R Rossato , J S Almeida , BS . *Cell Biochem Funct* 2007. 25 p. .

[Dallas et al. ()] 'Multidrug resistance-associated proteins: Expression and function in the central nervous system'. S Dallas , D S Miller , R Bendayan . *Pharmacol Rev* 2006. 58 p. .

[El-Gawad and El-Sawalhi ()] 'Nitric oxide and oxidative stress in brain and heart of normal rats treated with doxorubicin: Role of aminoguanidine'. Abd El-Gawad , H M El-Sawalhi , MM . *Journal of biochemical and molecular toxicology* 2004. 18 p. .

[Grant et al. ()] 'Overexpression of multidrug resistance-associated protein (mrp) increases resistance to natural product drugs'. C E Grant , G Valdimarsson , D R Hipfner , K C Almquist , S P Cole , R G Deeley . *Cancer research* 1994. 54 p. .

[Marques-Aleixo et al. ()] 'Physical exercise prior and during treatment reduces sub-chronic doxorubicin-induced mitochondrial toxicity and oxidative stress'. I Marques-Aleixo , E Santos-Alves , D Mariani , D Rizo-Roca , A I Padrão , S Rocha-Rodrigues . *Mitochondrion* 2014.

[Powers et al. ()] 'Rigorous exercise training increases superoxide dismutase activity in ventricular myocardium'. S K Powers , D Criswell , J Lawler , D Martin , F Lieu , L Ji . *American Journal of Physiology-Heart and Circulatory Physiology* 1993. 265 p. .

[Gilliam et al. ()] 'The anticancer agent doxorubicin disrupts mitochondrial energy metabolism and redox balance in skeletal muscle'. L A Gilliam , K H Fisher-Wellman , C T Lin , J M Maples , B L Cathey , P D Neufer . *Free radical biology & medicine* 2013. 65 p. .

[Borst et al. ()] 'The multidrug resistance protein family'. P Borst , R Evers , M Kool , J Wijnholds . *Biochimica et biophysica acta* 1999. 1461 p. .

[Hirrlinger et al. ()] 'The multidrug resistance protein mrp1 mediates the release of glutathione disulfide from rat astrocytes during oxidative stress'. J Hirrlinger , J Koènig , D Keppler , J Lindenau , J B Schulz , R Dringen . *Journal of Neurochemistry* 2001. 76 p. .

[Deeley et al. ()] 'Transmembrane transport of endo-and xenobiotics by mammalian atp-binding cassette multidrug resistance proteins'. R G Deeley , C Westlake , S P Cole . *Physiol Rev* 2006. 86 p. .

[Doroshow et al. ()] 'Ultrastructural features of adriamycin-induced skeletal and cardiac muscle toxicity'. J H Doroshow , C Tallent , J E Schechter . *Am J Pathol* 1985. 118 p. .