Accepted Article

ACTIVITY OF KREBS CYCLE ENZYMES IN *mdx* MICE

Clarissa M. Comim, PhD[1]; Andreza Hoepers, BSc[1]; Letícia Ventura, BSc[1]; Viviane Freiberger, BSc[1]; Diogo Dominguini, BSc[2]; Francielle Mina, MSc[2]; Bruna P. Mendonça[2], MSc; Giselli Scaini, PhD[3]; Mariz Vainzof, PhD[4]; Emílio L. Streck, PhD[3]; João Quevedo, PhD[2,5]

[1]*Laboratory of Experimental Neuroscience, Postgraduate Program in Health Sciences, University of South Santa Catarina, Palhoça, SC, Brazil;*

[2]*Laboratory of Neurosciences, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciúma, SC, Brazil.*

[3]*Laboratory of Bioenergetics, Postgraduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, 88806-000 Criciúma, SC, Brazil.*

[4]*Human Genome Research Center, Biosciences Institute, University of São Paulo, São Paulo, São Paulo, Brazil*

[5]*Center for Experimental Models in Psychiatry, Department of Psychiatry and Behavioral Sciences, The University of Texas Medical School at Houston, Houston, TX, USA.*

RUNNING TITLE: Krebs cycle in *mdx* mice

ABBREVIATIONS: DMD - Duchenne muscular dystrophy; CNS - Central Nervous System; ATP - Adenosine triphosphate; CS - Citrate synthase; MDH - Malate dehydrogenase; IDH - Isocitrate dehydrogenase; SDH - Succinate dehydrogenase.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an ‘Accepted Article’, doi: 10.1002/mus.24704

This article is protected by copyright. All rights reserved.

FINANCIAL DISCLOSURE

This research were supported by grants from CNPq (CMC, ELS and JQ) and University of South Santa Catarina (CMC). MV, ELS and JQ are CNPq Research Fellows.

CONFLICT OF INTEREST

All authors have read the journal's policy on disclosure of potential conflicts of interest and have none to declare.

Corresponding author:

Clarissa M. Comim, PhD; Laboratory of Experimental Neuroscience, University of South Santa Catarina, Palhoça, SC, Brazil; email: clarissamc@gmail.com; phone: +55 48 3621 - 3363

Accepted Article

This article is protected by copyright. All rights reserved.

Accepted Article

ABSTRACT

Introduction: Duchenne muscular dystrophy (DMD) is a degenerative disease of skeletal, respiratory, and cardiac muscles caused by defects in the dystrophin gene. More recently brain involvement has been verified. Mitochondrial dysfunction and oxidative stress may underlie the pathophysiology of DMD. This study aims to evaluate Krebs cycle enzymes activity in the cerebral cortex, diaphragm, and quadriceps muscles of *mdx* mice. **Methods:** Cortex, diaphragm, and quadriceps tissues from male dystrophic *mdx* and control mice were used. **Results:** We observed increased malate dehydrogenase activity in the cortex, increased malate dehydrogenase and succinate dehydrogenase activities in the diaphragm, and increased citrate synthase, isocitrate dehydrogenase, and malate dehydrogenase activities in the quadriceps of *mdx* mice. **Conclusion:** this study shows increased activity of Krebs cycle enzymes in cortex, quadriceps, and diaphragm in *mdx* mice.

Key-words: Duchenne Muscular Dystrophy, Krebs cycle enzymes, Cortex, Diaphragm, Quadriceps, *mdx* mice.

This article is protected by copyright. All rights reserved.

Accepted Article

INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked fatal muscle wasting disease affecting approximately 1 in every 3,500 male births. It results from mutations in the dystrophin gene. Dystrophin is a large cytoskeletal protein located on the inner cytoplasmic surface of skeletal and cardiac muscle cell membranes. The dystrophin-glycoprotein complex connects the actin cytoskeleton to the extracellular matrix of the cell and plays an important role in sarcolemmal membrane stability[1,2]. DMD Patients exhibit severe and progressive pathology in skeletal, respiratory, and cardiac muscles[2,3]. Furthermore, it has been shown that DMD patients may have progressive cognitive impairment[4]. In an animal model of DMD, the progressive pathology has been shown to be associated with oxidative stress and energy metabolism alterations in skeletal muscle[5] and brain[6,7].

Several studies also support the hypothesis that metabolic impairment is involved in the pathophysiology of DMD. In the *mdx* mouse, studies indicate dysfunction in mitochondria and alteration in mitochondrial protein composition in the quadriceps[5]. Similar findings were observed in a skeletal muscle biopsy of a DMD patient[3]. In the CNS, alteration in the complex of mitochondrial respiratory chain associated with a decrease of creatine kinase activity has been reported[7]. Mitochondria are well known intracellular organelles which play a crucial role in adenosine triphosphate (ATP) production[8]. The Krebs cycle occurs within the mitochondrial matrix and contributes to production of large amounts of ATP via mitochondrial oxidative phosphorylation, which supplies more than 95% of the total energy requirement in the cells[9]. Also called

This article is protected by copyright. All rights reserved.

Accepted Article

the citric acid cycle, the Krebs cycle is the central point of oxidative metabolism, providing carbon for biosynthesis and reducing agents for generation of ATP. Inactivation of any Krebs cycle step can alter mitochondrial ATP production[10]. Some of the enzymes that form part of the Krebs cycle are citrate synthase (CS), malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), and succinate dehydrogenase (SDH). MDH catalyzes the conversion of oxaloacetate and malate, utilizing the NAD/NADH coenzyme system. This reaction plays a key part in malate/aspartate transport across the mitochondrial membrane, and in the Krebs cycle within the mitochondrial matrix[11]. Citrate synthase catalyzes the first step within the cycle, the condensation of acetyl-coenzyme A with oxaloacetate to form citrate; it is the only enzyme in the cycle that can catalyze the formation of a carbon–carbon bond[12]. IDH occurs in 3 isoforms and is localized to the cytosol as well as the mitochondrion and peroxisome; in addition, this enzyme is responsible for production of 3 NADH molecules. SDH, or mitochondrial complex II, is a multimeric enzyme that is bound to the inner membrane of mitochondria and has a dual role; it serves both as a critical step of the Krebs cycle and as a member of the respiratory chain that transfers electrons directly to the ubiquinone pool[13].

In this context, the dystrophin complex provides mechanical support to the plasma membrane of contracting muscle, such as quadriceps and diaphragm, both muscles required for daily activities such, including walking and breathing, respectively. These 2 skeletal muscle are enriched in the dystrophin-glycoprotein complex[1,2]. Another structure that is similarly enriched is the cortex[4]; it is involved in a variety of mental and neural processes. Based on the hypothesis that metabolic impairment is involved in the pathophysiology

This article is protected by copyright. All rights reserved.

of DMD in skeletal muscle and brain, the participation Krebs cycle enzyme activity is still not clearly understood. Thus, in this study we evaluated the activities of the Krebs cycle enzymes CS, IDH, MDH, and SDH in the cortex, diaphragm, and quadriceps of *mdx* mice. We sought to understand whether alterations in Krebs cycle enzyme activity can be involved in the energy metabolic alterations in these tissues.

METHODS

Animals and experimental design

Adult male dystrophic (mdx) and wild-type (C57BL/10) mice (60 days old, n=8 per group), provided by the Human Genome Research Center, Biosciences Institute, University of São Paulo, were housed 5 per cage with food and water available *ad libitum* and were maintained on a 12h light/dark cycle (lights on at 7:00 am). The Animal Care and Experimentation Committee of UNISUL, Brazil approved all procedures (protocol number 14.019.4.08.VI). After study completion at 60 days, the mice underwent euthanasia by decapitation and had the cortex, diaphragm, and quadriceps immediately dissected, isolated, and stored at −80 ◦C for biochemical analyses.

Tissue and homogenate preparation

Cortex, diaphragm, and quadriceps were homogenized (1:10, w/v) in SETH buffer, pH 7.4 (250 mM sucrose, 2 mM EDTA, 10mMTrizma base, 50 IU/mL heparin). The homogenates were centrifuged at 800 x g for 10 min at 4℃, and the supernatants were used for enzyme activity assay. The maximal

This article is protected by copyright. All rights reserved.

Accepted Article

period between homogenate preparation and enzyme analysis was always less than 5 days. The supernatants were freeze thawed twice at −80℃ to disrupt the mitochondrial membranes,[14] and protein content was determined by the method described by Lowry, et.al.[15] using bovine serum albumin as standard.

Activity of Krebs cycle enzymes

Citrate synthase

CS activity was assayed using the method described by Srere[16]. The reaction mixture contained 100mM Tris, pH 8.0, 100 mM acetyl CoA, 100 mM 5,59-dithiobis-(2-nitrobenzoic acid), 0.1% Triton X-100, and 2-4 μg supernatant protein, and was initiated with 100 mM oxaloacetate and monitored at 412 nm for 3 min at 25ºC.

2.3.2 Isocitrate Dehydrogenase

IDH activity was measured as described by Plaut[17], by following NAD^+ reduction at wavelengths of excitation and emission of 340 and 466 nm, respectively. The reaction mixture consisting of 33 mM Tris, pH 7.4, 1.0 mM rotenone, 24 mM manganese chloride, 13,4 mM ADP, 0.2% Triton X-100, 6.0 mM NAD^+, and 100 mM isocitrate was pre-incubated with 2–4 μg supernatant protein at 30℃ for 20 min.

Malate dehydrogenase

This article is protected by copyright. All rights reserved.

Accepted Article

MDH was measured as described by Kitto[18]. Aliquots (20 μg supernatant protein) were transferred into a medium containing 10 mM rotenone (specific inhibitor of complex I), 0.2% Triton X-100, 0.15 mM NADH, and 100mM potassium phosphate buffer, pH 7.4, at 37 6C. The reaction was started by addition of 0.33 mM oxaloacetate. The absorbance was monitored as described above.

Succinate dehydrogenase

SDH activity was determined as described by Fischer, et.al.[19], measured by following the decrease in absorbance due to reduction of 2,6-di-chloro-indophenol (2,6-DCIP) at 600 nm with 700 nm as reference wavelength (e = 19.1 mM-1 cm-1) in the presence of phenazine methosulphate (PMS). The reaction mixture consisting of 40 mM potassium phosphate, pH 7.4, 16mM succinate, and 8 mM 2,6-DCIP was pre-incubated with 40-80 μg supernatant protein at 30 6C for 20 min. Subsequently, 4 mM sodium azide, 7 mM rotenone (specific inhibitor of complex I), and 40 mM 2,6-DCIPwere added. The reaction was initiated by addition of 1mM PMS and was monitored for 5 min.

Statistical analysis

Results are presented as means ± SEM. Assays were performed in duplicate, and the mean was used for statistical analysis. Data were analyzed by Student *t*-tests for unpaired data. Differences between groups were rated significant at $P<0.05$.

Accepted Article

This article is protected by copyright. All rights reserved.

Accepted Article

RESULTS

Table 1 shows the results of all evaluations listed as mean and standard deviation. Figure 1 shows the CS activity in cortex (A), diaphragm (B), and quadriceps (C). There was an increase in the quadriceps (df=16; t=2.716; P=0.015), without alteration in the cortex (df=18; t=-0.147; P=0.885) or diaphragm (df=18; t=1.073; P=0.298) in the *mdx* group when compared with the control group. Figure 2 shows the IDH activity in cortex (A), diaphragm (B), and quadriceps (C). There was an increase in quadriceps (df=11; t=3.393; P=0.006), without alteration in the cortex (df=12; t=-0.184; P=0.857) or diaphragm (df=8; t=0.473; P=0.649) in the *mdx* group when compared with the control group.

Figure 3 shows the results of MDH activity in cortex (A), diaphragm (B), and quadriceps (C).The was an increase in the cortex (df=21; t=2.710; P=0.013), diaphragm (df=16; t=3.606; P=0.002), and quadriceps (df=21; t=2.151; P=0.043) in the *mdx* group when compared with the control group. Finally, Figure 4 shows the SDH activity in cortex (A), diaphragm (B), and quadriceps (C). There was an increase in the diaphragm (df=22; t=4.635; P=0.0001), without alteration in the cortex (df=22; t=1.858; P=0.077) or quadriceps (df=19; t=0.214; P=0.833) in the *mdx* group when compared with the control group.

DISCUSSION

Mitochondrial dysfunction and oxidative stress may underlie the pathophysiology of DMD. Mitochondria are the main source of reactive oxygen

This article is protected by copyright. All rights reserved.

species, which are produced in the complexes of the electron transport chain[20]. Moreover, a shift in the antioxidant/pro-oxidant balance toward oxidative stress may inhibit Krebs cycle and electron transport chain complexes, leading to decreases in ATP production and cellular dysfunction[8].

In the CNS, dystrophin is enriched in the postsynaptic densities of pyramidal neurons. These are specialized regions of the subsynaptic cytoskeletal network critical for synaptic transmission and plasticity[21]. The lack of dystrophin in the brain has been correlated with impaired cognitive function[22]. Mitochondria presumably produce much of the ATP essential for excitability and survival of neurons, and the protein phosphorylation reactions that mediate synaptic signaling are related to long-term changes in neuronal structure and function. Impairment in energy production caused by mitochondrial dysfunction has been found in some neurodegenerative diseases such as dementia, Alzheimer, and Parkinson diseases, and all culminate in some level of cognitive impairment[23]. Recently, our group showed an alteration in energy metabolism and an increase of creatine kinase activity in brain tissue of *mdx* mice[7]. In this study, we showed that *mdx* mice have increased MDH in the cortex. MDH catalyzes the final reaction; that is, the last oxidation step and hence production of another NADH[24]. Alterations in the Krebs cycle would greatly alter the rate of brain metabolism and the production of free radicals[25,26]. In this context, oxidative stress was also found in the brain tissue in the *mdx* mice[6]. Having that in mind, we suggest that the increases in the activity of enzymes of the Krebs cycle may alter the function of the mitochondrial respiratory chain complexes and, consequently, the rate of brain metabolism. This would explain the

Accepted Article

This article is protected by copyright. All rights reserved.

changes reported in energy metabolism[7] and oxidative stress[6] in the brain previously found by our research group.

DMD is characterized by progressive muscle wasting and weakness. At the cellular level, the loss of dystrophin initiates a complex series of pathophysiological changes that drive skeletal muscle cell to weakness, atrophy, and death. Most prominent is abnormal Ca^{2+} influx and handling that is thought to activate proteases and cause mitochondrial Ca^{2+} overload and dysfunction[27-29]. Despite a potentially important role for mitochondria in this pathogenic cascade, the impact of dystrophin deficiency on fundamental aspects of mitochondrial biology is incompletely understood. In this study, we also found an increase in the activity of MDH and SDH in the diaphragm and increases in the activity of CS, IDH, and MDH in the quadriceps of *mdx* mice. The muscle alterations in DMD are most pronounced in the diaphragm muscle and fast-twitch limb muscles such as quadriceps, which are susceptible to damage, inflammatory cell infiltration, and proinflammatory signaling[30,31]. In *mdx* mouse skeletal muscle, studies indicate dysfunction in mitochondria and changes in mitochondrial protein composition. Enzymatic analysis of skeletal muscle showed a 50% decrease in the activity of all respiratory chain-linked enzymes in quadriceps muscle[5]. Mitochondria in *mdx* mouse muscle had only 60% of maximal respiratory activity and contained only 60% of hemoproteins of the mitochondrial inner membrane[5]. Similar findings were observed in a skeletal muscle biopsy of a DMD patient[3]. Other studies have demonstrated that in isolated mitochondria from quadriceps muscle of the *mdx* mouse there was elevated calcium content and decreased respiratory control ratios with the NAD-linked substrates pyruvate/malate. Recently, it was demonstrated that

Accepted Article

This article is protected by copyright. All rights reserved.

dystrophin deficiency disrupts subsarcolemmal mitochondrial localization, promotes mitochondrial inefficiency, and restricts maximal mitochondrial ATP-generating capacity[32].

In conclusion, this study shows increased activity of Krebs cycle enzymes in cortex, quadriceps, and diaphragm in *mdx* mice. Thus, we suggest an interaction between Krebs cycle enzyme increases and mitochondrial dysfunction in *mdx* mice. These findings will enhance understanding of the pathology of DMD.

Accepted Article

This article is protected by copyright. All rights reserved.

Accepted Article

REFERENCES

1. Emery AE. Population frequencies of inherited neuromuscular diseases: A world survey. Neuromuscul Disord 1991; 1:19 –29.

2. Chelly J, Desguerre I. Progressive muscular dystrophies. Handb Clin Neurol. 2013; 113:1343-66.

3. Scholte HR, Luyt-Houwen IE, Busch HF, Jennekens FG. Muscle mitochondria from patients with Duchenne muscular dystrophy have anormal beta oxidation, but an impaired oxidative phosphorylation. Neurology 1985; 35:1396–1397.

4. D'Angelo MG, Bresolin N. Cognitive impairment in neuromuscular disorders. Muscle Nerve 2006; 34:16-33.

5. Kuznetsov AV, Winkler K, Wiedemann FR, von Bossanyi P, Dietzmann K, Kunz WS. Impaired mitochondrial oxidative phosphorylationin skeletal muscle of the dystrophin-deficient *mdx* mouse. Mol Cell Biochem 1998; 183:87–96.

6. Comim CM, Cassol-Jr OJ, Constantino LC, Constantino LS, Petronilho F, Tuon L, Vainzof M, Dal-Pizzol F, Quevedo J. Oxidative variables and antioxidant enzymes activities in the *mdx* mouse brain. Neurochem Int. 2009; 55:802-5.

7. Tuon L, Comim CM, Fraga DB, Scaini G, Rezin GT, Baptista BR, Streck EL, Vainzof M, Quevedo J. Mitochondrial respiratory chain and creatine kinase activities in *mdx* mouse brain. Muscle Nerve 2010; 41:257-60.

8. Calabrese V, Scapagnini G, Giuffrida Stella AM, Bates TE, Clark JB. Mitochondrial involvement in brain function and dysfunction: relevanceto aging, neurodegenerative disorders and longevity. Neurochem Res 2001 26:739-64.

This article is protected by copyright. All rights reserved.

9. Kelly DP, Gordon JI, Alpers R, Strauss AW. The tissues pecific expression and developmental regulation of two nuclear genes encoding rat mitochondrial proteins. Medium chain acyl-CoA dehydrogenase and mitochondrial malate dehydrogenase. J BiolChem 1989; 264:18921‑18925

10. Blass JP, Brown AM. Lower activity of Krebs cycle enzymesthan of electron transport in human brain: disease implications. Neurobiol Aging 2000; 21:81

11. Minard KI, McAlister-Henn L. Isolation, nucleotide sequence analysis, and disruption of the MDH2 gene from Saccharomy cescerevisiae: evidence for three isozymes of yeast malate dehydrogenase. Mol Cell Biol 1991; 11:370–380

12. Wiegand G, Remington SJ. Citrate synthase: structure, control, and mechanism. Annu Rev Biophys Biophys Chem 1996; 15:97–117

13. Oyedotun KS, Lemire BD. The quaternary structure of the Saccharomyces cerevisiae succinate dehydrogenase. Homologymodeling, cofactor docking, and molecular dynamics simulationstudies, J BiolChem 2004; 279:9424–9431

14. Robinson JB, Jr, Brent LG, Sumegi B, Srere PA. An enzymatic approach to the study of the Krebs tricarboxylic acid cycle. In: Darley-Usmar VM, Rickwood D, Wilson MT, editors. Mitochondria: A Practical Approach. IRL Press; Washington D.C.: 1987. p. 153.

15. Lowry OH, Rosebough NG, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265-75.

16. Srere PA. Citrate synthase. Methods Enzymol 1969; 13: 3-11.

17. Plaut GWE. Isocitrate dehydrogenases from bovine heart. Methods Enzymol 1969; 13:34–42

18. Kitto GB. Intra- and extramitochondrial malate dehydrogenases from chicken and tuna heart, Methods Enzymol. 1969; 13:107-16.

Accepted Article

This article is protected by copyright. All rights reserved.

19. Fischer JC, Ruitenbeek W, Berden JA, Trijbels JM, Veerkamp JH, Stadhouders AM, Sengers RC, Janssen AJ. Differential investigation of the capacity ofsuccinate oxidation in human skeletal muscle Clin Chim Acta 1985; 153:23-36.

20. Mattiasson G, Shamloo M, Gido G, Mathi K, Tomasevic G, Yi S, Warden CH, Castilho RF, Melcher T, Gonzalez-Zulueta M, Nikolich K, Wieloch T. Uncoupling protein-2 preventsneuronal death and diminishes brain dysfunction after stroke and brain trauma. Nat Med 2003; 9:1062–1068

21. Lidov HG, Byers TJ, Watkins SC, Kunkel LM. Localization of dystrophin to postsynaptic regions of central nervous system cortical neurons. Nature 1990; 348:725–728.

22. Anderson JL, Head SI, Rae C, Morley JW. Brain function in Duchenne muscular dystrophy. Brain 2002; 125:4–13.

23. Heales SJ, Bolan˜os JP, Stewart VC, Brookes PS, Land JM, Clark JB. Nitric oxide, mitochondria and neurological disease. Biochim Biophys Acta 1999; 1410:215–228.

24. Scheffler IE. Mitochondria, 2nd edn. Hoboken, USA, 2008

25. Lyubarev AE, Kurganov BI. Supramolecular organization of tricarboxylic acid cycle enzymes. Biosystems 1989; 22:91–102

26. Velot C, Mixon MB, Teige M, Srere PA. Model of a quinary structure between Krebs TCA cycle enzymes: a model for the metabolon. Biochemistry 1997; 36:14271–14276

27. Wrogemann K, Pena SD. Mitochondrial calcium overload: ageneral mechanism for cell-necrosis in muscle diseases. Lancet 1976; 1:672–674.

This article is protected by copyright. All rights reserved.

Accepted Article

28. Millay DP, Sargent MA, Osinska H, Baines CP, Barton ER, Vuagniaux G, Sweeney HL, Robbins J, Molkentin JD. Molkentin, Genetic and pharmacologic inhibition of mitochondrial-dependentnecrosis attenuates muscular dystrophy, Nat. Med. 2008; 14:442–447.

29. Millay DP, Goonasekera SA, Sargent MA, Maillet M, Aronow BJ, Molkentin JD. Calcium influx is sufficient to induce musculardystrophy through a TRPC-dependent mechanism. Proc. Natl Acad. Sci. 2009; 106:19023–19028

30. Kim JH, Lawler JM. Amplification of proinflammatory phenotype, damage, and weakness by oxidative stress in the diaphragm muscle of *mdx* mice. Free Radic Biol Med. 2012; 52:1597-606

31. Glesby MJ, Rosenmann E, Nylen EG, Wrogemann K. Serum CK, calcium, magnesium, and oxidative phosphorylation in *mdx* mousemuscular dystrophy. Muscle Nerve 1988; 11:852–856.

32. Percival JM, Siegel MP, Knowels G, Marcinek DJ. Defects in mitochondrial localization and ATP synthesis in the *mdx* mouse model of Duchenne muscular dystrophy are not alleviated by PDE5 inhibition Hum Mol Genet. 2013; 22:153-67.

Accepted Article

This article is protected by copyright. All rights reserved.

Accepted Article

TABLE LEGENDS

TABLE 1. Activity of citrate synthase, isocitrate dehydrogenase, malate dehydrogenase, and succinate dehydrogenase in the cortex, diaphragm, and quadriceps in *mdx* mice. The results are listed as mean ± SEM; **P*<0.05 vs. wt group, Student *t*-test for unpaired data.

This article is protected by copyright. All rights reserved.

LEGENDS OF FIGURE

Figure 1. Activity of citrate synthase (CS) in the cortex (A), diaphragm (B), and quadriceps (C) in *mdx* mice. Bars represent mean ± SEM; **P*<0.05 vs. control group, Student *t*-test for unpaired data.

Figure 2. Activity of isocitrate dehydrogenase (IDH) in the cortex (A), diaphragm (B), and quadriceps (C) in *mdx* mice. Bars represent mean ± SEM; **P*<0.05 vs. control group, Student *t*-test for unpaired data.

Figure 3. Activity of malate dehydrogenase (MDH) in the in the cortex (A), diaphragm (B), and quadriceps (C) in *mdx* mice. Bars represent mean ± SEM; **P*<0.05 vs. control group, Student *t*-test for unpaired data.

Figure 4. Activity of succinate dehydrogenase (SDH) in the in the cortex (A), diaphragm (B), and quadriceps (C) in *mdx* mice. Bars represent mean ± SEM; **P*<0.05 vs. control group, Student *t*-test for unpaired data.

Accepted Article

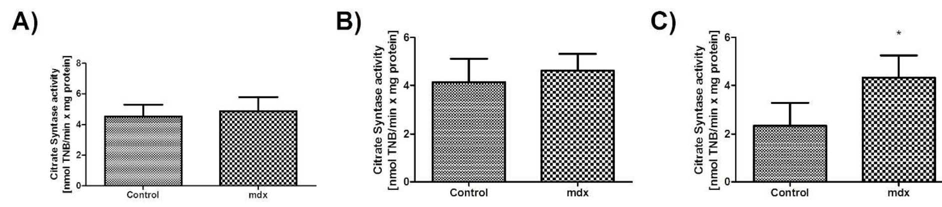
This article is protected by copyright. All rights reserved.

Accepted Article

Groups	Citrate synthase [nmol TNB/min x mg protein]	Isocitrate dehydrogenase [nmol TNB/min x mg protein]	Malate dehydrogenase [nmol TNB/min x mg protein]	Succinate dehydrogenase [nmol/mg protein]
Cortex				
wt	4.55 ± 0.75	382.14 ± 76.16	32.05 ± 6.71	3.21 ± 0.63
mdx	4.88 ± 0.91	372.11 ± 80.24	40.45 ± 6.43*	3.76 ± 0.84
Diaphragm				
wt	4.13 ± 0.99	239.70 ± 48.66	14.26 ± 0.45	5.91 ± 1.38
mdx	4.61 ± 0.71	259.83 ± 21.26	27.01 ± 1.29*	10.44 ± 2.48*
Quadriceps				
wt	2.34 ± 0.94	282.47 ± 78.99	18.78 ± 2.90	4.83 ± 0.83
mdx	4.32 ± 0.94*	549.70 ± 102.85*	31.58 ± 9.37*	4.95 ± 1.46

This article is protected by copyright. All rights reserved.

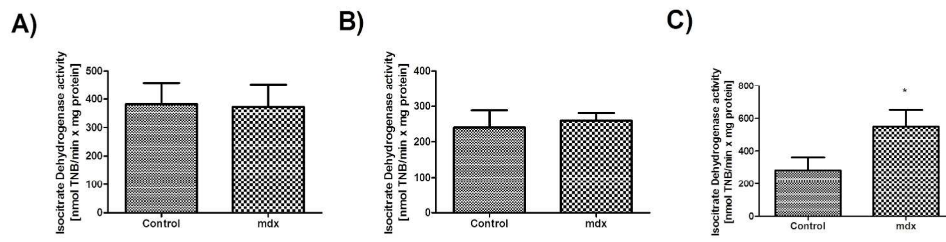
Accepted Article



276x63mm (300 x 300 DPI)

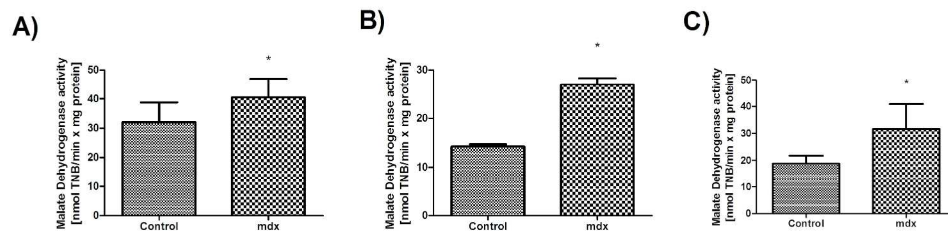
This article is protected by copyright. All rights reserved.

Accepted A



270x77mm (300 x 300 DPI)

This article is protected by copyright. All rights reserved.

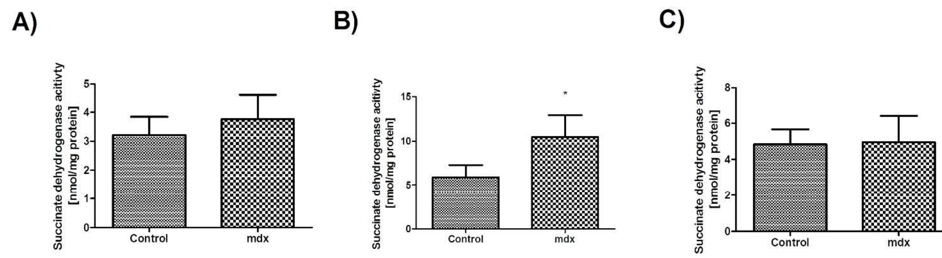


268x75mm (300 x 300 DPI)

Accepted A

This article is protected by copyright. All rights reserved.

Accepted Article



275x83mm (300 x 300 DPI)

This article is protected by copyright. All rights reserved.