Swimming Improves Memory and Antioxidant Defense in an Animal Model of Duchenne Muscular Dystrophy

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Abstract

Duchenne muscular dystrophy (DMD) is a genetic disease associated with progressive skeletal muscle degeneration. In humans, DMD has an early onset, causes developmental delays, and is a devastating disease that drastically diminishes the quality of life of young individuals affected. The objective of this study was to evaluate the effects of a swimming protocol on memory and oxidative stress in an animal model of DMD. Male \textit{mdx} and wild-type mice aged \( \geq 28 \) days were used in this study. The animals were trained for a stepped swimming protocol for four consecutive weeks. The swimming protocol significantly reduced the levels of lipid peroxidation and protein carbonylation in the gastrocnemius, hippocampus, and striatum in the exercised animals. It also prevented lipid peroxidation in the diaphragm. Moreover, it increased the free thiol levels in the gastrocnemius, the diaphragm, and all central nervous system structures. The results showed that the protocol that applied swimming as a low-intensity aerobic exercise for 4 weeks prevented aversive memory and habituation in \textit{mdx} mice.

Keywords Duchenne muscular dystrophy · Animal · Swimming · Oxidative stress · Mice

Introduction

Duchenne muscular dystrophy (DMD) is a well-described clinical disease caused by an amendment of the X chromosome, inherited as a recessive trait, which mainly affects males [1, 2]. Affected animals such as \textit{mdx} mice showed the same mutation as that in humans. They are of limited use as they do not develop the early dilated cardiomyopathy observed in humans; however, they are the best option for experimentation [1, 3].

Patients with DMD require a multidisciplinary treatment approach that must be carefully implemented while always focusing on the patient’s well-being. Therefore, the role of physical therapy is important for a successful treatment once
good outcomes have been achieved in the short term, such as maintaining the autonomy of patients [4, 5]. Physical exercises are also used to treat patients with DMD [6, 7]. At the molecular level, exercise reduces frailty by decreasing muscle inflammation and increasing anabolism and muscle protein synthesis [8]. Studies have used physical exercises in patients with DMD to decrease muscle deterioration, muscular contraction, and bone fractures and increase the duration of functional independence [9]. Low-intensity exercise is known to decrease the values of certain parameters associated with muscular degeneration in animal models of progressive muscular dystrophies [2].

Swimming is an extremely popular aerobic exercise modality. Aerobic physical exercise in water is a viable alternative to land-based exercise because water, through its physical properties such as thrust, hydrostatic pressure, and viscosity, increases well-being and quality of life [10]. Swimming, as well as hiking and running, has many health benefits as compared with a sedentary lifestyle [10]. Swimming is generally indicated to avoid impact and facilitate compliance because of better adaptation to an environment with heated water [10]. The literature also shows that swimming has beneficial effects on patients’ cognitions by modulating memory and learning processes and on oxidative stress by increasing antioxidant defenses [10]. Therefore, the purpose of this study was to examine the effects of swimming on memory and oxidative stress in an animal model of DMD by evaluating changes in the skeletal muscle and encephalic tissue.

Materials and Methods

1 Animals

Male 

mdx

mice and wild-type C57BL/6 mice, kindly provided by University of São Paulo (USP), Brazil, were used in this study. The animals were 28 days old, and they weighed 18–23 g. The animals were kept in the Vivarium of the Experimental Neuroscience Laboratory (Lanex), in Unisul, Palhoça, Santa Catarina, Brazil. Each box contained five animals, in a 12-h light–dark cycle (6 am to 6 pm), with food and water provided ad libitum. The temperature of the environment was maintained at 22 ± 2 °C. The study was conducted at Lanex and at the Laboratory of Biochemistry and Molecular Biology of Unisul, Palhoça, Santa Catarina, Brazil, during 2016 and 2017. Approval for conducting this study was obtained from the Animal Use Ethics Committee (CEUA) of Unisul under the number 16.003.4.01.IV.

2.2 Sample Calculation

To calculate the number of animals required for each experimental group, we have to take the following:

\[ n = \frac{[(z\alpha/2 + z\beta)^2 \cdot s^2]}{(\mu — \bar{x})^2} \]

where \( z\alpha/2 \) is the value on normal curve according to \( \alpha \) value; \( z\beta \) = beta error; \( s^2 \) = variance; \( (X — \mu)^2 \) = estimated maximum difference between the sample mean (\( X \)) and the true population mean (\( \mu \)). It is the error margin or maximum estimation error.

Therefore, the following values are assigned to calculate the number of animals: The alpha value will be set at 0.05. Hence, the \( z\alpha/2 \) value, based on the table of \( z \)-values for two-tailed distribution, is 1.96. The beta value will be set at 0.20. Hence, the \( z \) beta value, based on the table of \( z \)-values (single-tailed distribution), is 0.84. The value of the standard deviation (\( s \)) is on average 30% of the means’ value (based on experimental data from our laboratory): The difference between the groups’ means is at least 40% (based on experimental data from our laboratory). Biological experiments have an embedded error of about 10–15% (resulting from individual variations, surgical procedure errors, dosage errors, etc.).

Assigning the values to the above formula, we have

\[ n = \frac{[(1.96 + 0.84)2.302]}{(0.4 — 0.1)^2} = 7.84 \text{ animals.} \]

It is therefore understood that a minimum of eight animals should be used in each experimental group to ensure that the experiments’ conclusions are valid, within an acceptable risk of not observing differences where they exist and not seeing differences where they do not exist. This study used 10 animals per group to guarantee data safety and reliability.

Experimental Design

As illustrated in Fig. 1, the animals were divided into the following four groups, each one consisting of eight animals: (1) non-exercised wild-type, (2) exercised wild-type, (3) non-exercised 

mdx

, and (4) exercised 

mdx

. Groups 2 and 4 were subjected to the low intensity aerobic exercise protocol of the swimming type for 4 weeks. After 24 h of the last day of training, the tests for assessing aversive memory and habituation were conducted. Afterwards, the animals were sedated and submitted to the assisted painless death procedure, and the following structures were removed for determining lipid peroxidation, protein carbonylation, and thiol grouping: the gastrocnemius and quadriceps muscles, diaphragm, prefrontal cortex, cerebellum, hippocampus, striatum, and cerebral cortex.
Aerobic Exercise Protocol of Swimming Type

The groups of exercised animals were submitted to a protocol of swimming aerobic exercise in a plastic container adapted for this purpose (170 × 100 mm), with 35 L of water at 28 to 30 °C, divided into eight lanes. The protocol consisted on four consecutive weeks of exercise, four times a week, with daily 15-min sessions in the first week, 20 min in the second week, and 30 min in the third and fourth weeks (adapted from [11]). It used 1 mL of baby’s shampoo all over the container to decrease the surface tension of the water (adapted from [12]). After the protocol, the animals were gently dried. The groups of non-exercised animals did not perform any type of physical exercise, remaining in their housing boxes during the entire study period.

The determination of the exercise intensity was performed on the fourth week of protocol in the wt e mdx animals. Blood samples were collected before the test, on the 10th and 30th minutes of exercise for subsequent analysis of lactate concentration. The criterion for considering intensity was the increase in concentration of no more than 1 mmol/L between the 10th and 30th minutes of physical exercise. The blood collection was performed in an alcohol (70%)-sanitized place. After this procedure, the distal portion of the animal’s tail was slightly sectioned with surgical scissors and 25 μL of a drop of blood was inserted in the lactate collection tape, and through a portable lactimeter, the blood lactate level was measured. Before each test, the equipment was calibrated according to the manufacturer’s instructions [13].

Aversive Memory Test

Aversive conditioning provides an association between a conditioned stimulus, such as a sound, and an unconditioned stimulus, such as an electric shock. This test consists of an acrylic box whose floor is formed by parallel metal bars. A platform 7 cm wide and 2.5 cm long is placed near the left wall of the appliance. At the training session, the animals are placed on the platform, and the time taken by the animal to go down the platform with the four legs is measured, in seconds. This time is called latency. Immediately after going down the platform, the animal receives a 0.2 mA shock for 2 s. In the test session, the animal is placed on the platform again, and the time it takes to go down (latency) is measured; however, the shock is not administered. The test is also finalized if the animal does not go down within 3 min [14].

Habituation Test

This test was performed in a 40 × 60-cm open field, delimited by four 50 cm high walls—three made of wood and one of transparent glass. The open field floor is divided into 16 equal squares, marked by black lines. In the training session, the animals were carefully placed in the square of the rear left corner of the appliance, from which they freely explored the environment for 5 min. Immediately afterward, the animals returned to the housing box. The test session was held 24 h after the training, in which the training procedure is repeated. The number of four-legged crossings (crossings, motor activity) through the black lines and the number of times the animals rested on their back legs (rearings, exploratory activity) were evaluated in both sessions [15].

Oxidative Stress Measures

Measurement of Thiobarbituric Acid Reactive Substances (TBARS)

This method is used to evaluate the oxidation state of hydroperoxides in biological systems. Damage to membrane lipids is determined by the formation of lipoxidation by-products (such as MDA or malondialdehyde), which are reactive substances to thiobarbituric acid heating, formed during peroxidation in membrane systems. MDA reacts with thiobarbituric acid (TBA), generating a pinkish-colored
product, read in a 535-nm microplate reader. The technique consists of the following: first of all, the dilution value was calculated so that in the TBARS reaction tube, there are 100 µL of tissue protein in 500 µL of BHT buffer. Afterwards, 500 µL of the 0.67% TBA solution were added. The tubes were placed in a dry bath at 96 °C for 30 min. To stop the reaction, the samples were placed on ice for 5 min. Finally, 200 µL of the reaction were placed in 96-well microplates and read in the microplate reader at 535 nm.

Measurement of Oxidative Damage in Proteins

This method was used for protein oxidation dosing. It is based on the principle that several ROS attack protein residues, such as amino acids to produce products with the carbonyl group, which can be measured though the reaction with 2,4-dinitrophenylhydrazine. The carbonyl content is determined by a microplate reader at 370 nm, as described by Levine et al. [16]. First, the tissue was homogenized in 1-mL BHT buffer.

The samples were centrifuged for 15 min at 4 °C at 14,000 rpm. A total of 200 µL of the sample were separated for the blank and 200 µL for the test. A total of 100 µL of 20% trichloroacetic acid (TCA) were placed in all Eppendorfs. They were centrifuged for five min at 14,000 rpm. The supernatant was discarded. The pellet was redissolved in 100 µL of 0.2 molar NaOH. A total of 100 µL dinitrophenylhydrazine 2.4 (DNTP) were placed in the sample and were left to rest for 1 h. A total of 100 µL 20% TCA were placed on all Eppendorfs, which were centrifuged for 3 min. The supernatant was discarded. The pellet was washed three times with 500 µL ethanol and ethyl acetate (1:1). For each wash, it was centrifuged for 3 min at 14,000 rpm and the supernatant discarded. After discarding the last wash, 1 mL of 3% sodium hydroxide (NaOH) was placed on all Eppendorfs. The samples were taken to water bath at 60 °C for 30 min and read in the microplate reader at 370 nm.

Thiol Groups

Sulfhydryl radicals represent all groups of thiols found in proteins such as albumin and low molecular weight compounds, such as glutathione. These groups can be oxidized when oxidative stress is high. The determination of total sulfhydryl groups, protein-linked sulfhydryl groups, and sulfhydryl groups in low molecular weight compounds (free sulfhydryl) can be performed by using Ellman’s reagent (2,2-dinitro-5,5-dithiobenzoic acid—DTNB). The thiol groups react with DTNB forming a light-absorbing complex at 412 nm. The technique consists of adding 10% TCA to the same sample volume (1:1 dilution). White was prepared, containing 100 µL TCA by adding 100 µL PBS. It was centrifuged for 15 min at 3000 rpm (temperature 4 °C); the supernatant was collected, and 30 µL DTNB (1.7 mM) and 300 µL hydrochloric acid (TRIS–HCl) were added to 75 µL of this supernatant. It was left to react for 30 min and transferred to a 96-well plate. The samples were read in a 412 nm microplate reader.

Protein Dosages

The proteins were determined by the BCA method and the bovine serum albumin was used as standard. The method is based on the reaction of the copper with the proteins in basic medium. The samples were analyzed by a plate reader at 562 nm.

Statistical Analysis

The data were entered into a database, developed in electronic media, in IBM SPSS Statistics 24.0 software (@ copyright IBM Corporation and its licensors 1989, 2016). The Shapiro–Wilk normality test was applied to verify the behavior of the data. The data referring to the open field habituation test were expressed in average and standard deviation because they are parametric data. For differences between groups, the two-way ANOVA test with post hoc Bonferroni was used. For differences between training and testing in the same group, the Student’s t test was used for paired samples. The data of the inhibitory elusive test were expressed in median and interquartile range because they are non-parametric data. Wilcoxon test was used for analysis between training and test in the same group. The data from the biochemical tests were expressed as mean and standard deviation because they were parametric data. The two-way ANOVA test was used with post hoc Bonferroni for analysis between groups. Data were considered statistically significant when \( p < 0.05 \).

Results

Lactate Measurement

Figure 2 shows the results obtained after the lactate measurement made in the blood taken from the animals during the performance of the swimming protocol.

Figure 2 shows that in both mdx and wt animals submitted to the swimming protocol, there were no changes in blood lactate above 1 mmol/L between the 10th and 30th minute of physical exercise in relation to the rest values (the average lactate values were 2.31 mmol/L for wt animals and 2.21 for mdx animals); in other words, they remained below the
Lactate measurement of mdx and wt animals submitted to the swimming protocol

Fig. 2 Lactate measurement of mdx and wt animals submitted to the swimming protocol

habituation memory evaluation, through the open field test, can be observed. It can be remarked that there was no difference in the number of crossings and rearings (p > 0.05), between groups, during the training phase, demonstrating that there was no difference in locomotive activity between the groups. The animal wt (wild) non-exercised and exercised demonstrated significant changes, between training and testing, both in the number of crossings and the number of rearings (p < 0.05); in other words, there was no impairment of the evaluated memory. The group of mdx (DMD) non-exercised animals did not show significant changes between training and testing in the number of crossings and rearings (p > 0.95), evidencing a memory impairment. In contrast, the group of mdx animals that were submitted to the protocol showed a decrease in the number of crossings and rearings between training and testing (p < 0.05), suggesting a possible prevention to the impairment of aversive memory in mdx mice.

The results of the aversive memory assessment through the inhibitory avoidance test are shown in Fig. 3B. In the group of non-exercised and exercised wt animals, there was a statistically significant difference in latency time between training and testing, showing no impairment in the aversive memory (p < 0.05). In the non-exercised mdx group of animals, there was no statistically significant difference between training and testing, evidencing an impairment of aversive memory (p > 0.05). The mdx animals submitted to the experimental protocol showed a statistically significant difference between training and testing; in other words, there was no impairment of aversive memory in these animals (p < 0.05).

Oxidative Stress Assessment in the Gastrocnemius Muscle

Figure 4 shows the results of using a swimming protocol on lipid peroxidation (Fig. 4A), protein carbonylation (Fig. 4B), and free thiols (Fig. 4C) in the gastrocnemius muscle.

Figure 4A shows the result of evaluation of lipid peroxidation in gastrocnemius. It was observed that the non-exercised mdx animals presented significantly elevated levels of lipid peroxidation in gastrocnemius, when compared to untrained wild animals (p < 0.05). The mdx animals submitted to the experimental protocol presented a significant reduction of these levels, when compared to the non-exercised mdx animals (p < 0.05), evidencing that the experimental protocol used in this study has protected against the increase in lipid peroxidation in gastrocnemius in mdx animals. It can be observed that the non-exercised mdx animals showed a significant increase in protein carbonylation in gastrocnemius when compared to the group of non-exercised wild animals (p < 0.05). After the experimental protocol, the mdx animals presented significantly lower protein

Learning and Memory Evaluation

Figure 3 shows the results obtained after the application of the swimming protocol on aversive memory (Fig. 3A) and habituation (Fig. 3B). In Fig. 3A, the results of the lactate threshold. Therefore, the swimming protocol used in this study can be considered of moderate intensity.
carbonylation levels when compared to the non-exercised mdx animals ($p < 0.05$), demonstrating that the swimming protocol, for 4 weeks, was able to prevent the carbonylation increase in proteins, observed in the gastrocnemius muscle of mdx animals (Fig. 4B). The quantification of free thiols in gastrocnemius is shown in Fig. 4C. It can be observed that there was a significant increase in the number of free thiols in gastrocnemius of wild animals group submitted to the experimental protocol, when compared to the non-exercised wild animals ($p < 0.05$). There was also a significant decrease in the non-exercised mdx animals when compared to the non-exercised wild animals ($p < 0.05$). When the mdx animals were submitted to the experimental protocol, there was an increase in the number of free thiols, when compared to the mdx non-exercised animals.

Oxidative Stress Assessment on the Diaphragm Muscle

Figure 5 shows the results of using a swimming protocol on lipid peroxidation (Fig. 5A), protein carbonylation (Fig. 5B), and free thiols (Fig. 5C) in the diaphragm muscle. It is observed that the non-exercised mdx animals showed a significant increase in diaphragm lipid peroxidation when compared to the group of non-exercised wild animals ($p < 0.05$). After the experimental protocol, the mdx animals showed significantly lower lipid peroxidation levels when compared to the non-exercised mdx animals ($p < 0.05$), demonstrating that the swimming protocol, for 4 weeks, was able to prevent the increase in lipid peroxidation observed in the diaphragm muscle of
mdx animals (Fig. 5A). Figure 5B shows the result of the assessment of protein carbonylation in diaphragm. It was observed that the non-exercised mdx animals have presented significantly higher levels of protein carbonylation in diaphragm when compared to the non-exercised wild animals ($p < 0.05$). However, the mdx animals that were submitted to the experimental protocol did not present a significant reduction of these levels when compared to the non-exercised mdx animals, evidencing that the experimental protocol used in this study did not protect against the increase in protein carbonylation in diaphragm in mdx animals. The quantitation of the diaphragm free thiols is shown in Fig. 5C. It can be observed that there was a significant increase in the number of free thiols in diaphragm of the group of wild animals submitted to the experimental protocol, when compared to the non-exercised wild animals ($p < 0.05$). There was also a significant decrease in non-exercised mdx animals when compared to non-exercised wild animals ($p < 0.05$). When the mdx animals were submitted to the experimental protocol, there was an increase in the number of free thiols when compared to the non-exercised mdx animals, showing that swimming was able to protect this change.

**Oxidative Stress Assessment in Central Nervous System Structures**

Figure 6 shows the results of using a swimming protocol on lipid peroxidation in the prefrontal cortex (Fig. 6A), hippocampus (Fig. 6B), striatum (Fig. 6C), and cortex (Fig. 6D).

It is observed that the non-exercised mdx animals showed a significant increase on lipid peroxidation in hippocampus and striatum when compared to the group of non-exercised wild animals ($p < 0.05$). After the experimental protocol, the mdx animals showed significantly lower lipid peroxidation levels in the hippocampus and striatum when compared to the untrained mdx animals ($p < 0.05$), demonstrating that the swimming protocol, for 4 weeks, was able to prevent the increase in the lipid peroxidation observed in the hippocampus and striatum structures of mdx animals (Fig. 6B and 6C). Figure 6A and 6D show that in the prefrontal cortex and cortex structures,
there were no significant changes between the groups analyzed. Figure 7 shows the results of using a swimming protocol on protein carbonylation in the prefrontal cortex (Fig. 7A), hippocampus (Fig. 7B), striatum (Fig. 7C), and cortex (Fig. 7D).

It can be observed that the non-exercised $mdx$ animals showed a significant increase in protein carbonylation in prefrontal cortex, hippocampus, and striatum when compared to the group of non-exercised wild animals ($p < 0.05$). After the experimental protocol, the $mdx$ animals showed significantly lower protein carbonylation levels in the prefrontal cortex when compared to the untrained $mdx$ animals ($p < 0.05$), demonstrating that the swimming protocol, for 4 weeks, was able to prevent the increase in protein carbonylation, observed only in the prefrontal cortex structure of $mdx$ animals (Fig. 7A). Figure 7B and 7C show that the swimming protocol, for 4 weeks, was not able to prevent the increase in protein carbonylation in the hippocampus and striatum. Figure 7D shows that in the cortex structure, there was no significant change between the analyzed groups.

Figure 8 shows the results of using a swimming protocol on free thiols in the prefrontal cortex (Fig. 8A), hippocampus (Fig. 8B), striatum (Fig. 8C), and cortex (Fig. 8D).

It can be observed that the non-exercised $mdx$ animals showed a significant decrease in free thiols in prefrontal cortex, hippocampus, striatum, and cortex when compared to the group of non-exercised wild animals ($p < 0.05$). After the experimental protocol, the $mdx$ animals showed significantly higher free thiol levels in prefrontal cortex, hippocampus, striatum, and cortex when compared to non-trained $mdx$ animals ($p < 0.05$), demonstrating that the swimming protocol, for 4 weeks, was able to increase the antioxidant defenses in all central nervous system (CNS) structures of $mdx$ animals (Figs. 8A, 8B, 8C and 8D).

**Discussion**

The effects of swimming on memory and oxidative stress on skeletal muscle and encephalic tissue were evaluated in this study using a DMD animal model.
For this purpose, a moderate-intensity swimming protocol was implemented for four consecutive weeks, four times a week. The results showed that the swimming protocol prevented impairments of aversive memory and habituation in the mdx mice. By analyzing the parameters associated with oxidative damage, we found that parallel to its preventive effect, the protocol also increased protein carbonylation in the prefrontal cortex, hippocampus, striatum, diaphragm, and gastrocnemius, and lipid peroxidation in the hippocampus, striatum, diaphragm, and gastrocnemius muscle, concomitantly decreasing the free thiol levels in non-exercised mdx animals, which indicates oxidative stress. Low-intensity swimming prevented oxidative stress in the gastrocnemius and hippocampal and striatum structures of the animals. This exercise protocol also increased the free thiol levels in the gastrocnemius, diaphragm, and central nervous system (CNS) structures analyzed in this study.

In this study, swimming (aerobic exercise) was considered of moderate intensity on the basis of the lactate measurements of the animals, and 1 mL of shampoo was used in the whole container prepared for swimming. With regard to exercise intensity, besides the classification based on VO2max and maximum heart rate, which classifies exercise as mild/low-, moderate-, or high-intensity [17], another model was proposed by Gaesser and Poole [18] that indicates three domains in relation to effort intensity as follows: moderate, heavy, and severe. The moderate domain is comprised of all the effort intensities that can be performed without modifying blood lactate levels in relation to resting values, that is, below the lactate threshold. The heavy domain ranges from the lowest effort intensity where the lactate level increases to the intensity corresponding to a mean of 4 mM of lactate as upper limit. The severe domain does not have a stable phase of blood lactate level, which increases during all effort times until the individual is exhausted [19].

DMD is characterized by an absence of dystrophin protein in the skeletal muscle [6]. However, the literature also reported the absence of dystrophin in encephalic tissue, and this change is associated with other changes such as oxidative stress [19]. The absence of dystrophin and presence of oxidative stress in the CNS make cognitive impairment part of the pathophysiology of the disease. This study evinced...
that the swimming protocol protected against impairments of aversive memory and habituation in the mdx mice subjected to the protocol.

The protocol of the present study was started when the animals were 28 days old and completed at 56 days old. At this age, animals could be expected to present memory and learning impairments, which did not occur in the animals subjected to the swimming protocol. Thus, we can conclude that the exercise prevented memory and learning deficits. Although no studies have been conducted that related swimming directly to the prevention of memory and learning impairments related to DMD, benefits of swimming practice in cognitive aging have been reported [20]. Swimming is an aerobic exercise. At the molecular level, aerobic exercise reduces frailty by decreasing muscle inflammation, increasing anabolism, and increasing muscle protein synthesis [8]. Aerobic exercise improves cognitive and motor functions [21].

Another study with middle-aged animals showed benefits of swimming when combined with dietary supplementation in object recognition memory tests, demonstrating short- and long-term memory improvements [22].

Besides preventing cognitive impairment, another objective of this study was to verify if swimming can change oxidative stress in neuronal and skeletal muscle tissues. Studies have shown that oxidative stress is present in the pathophysiological process of DMD because of an imbalance between the formation of oxidizing agents and antioxidant activity [23–25]. Oxidative stress is present in DMD, the skeletal muscle, and the CNS [22]. One of the effects of exercise is increased antioxidant activity. Exercise produces ORS, which act as signals of molecular events that regulate muscle cell adaptations such as the regulation of antioxidant enzymes [26]. In this study, we observed that swimming increased free thiol levels, demonstrating that physical exercise increased glutathione antioxidant levels, in agreement with previous studies that demonstrated the protective role of exercise [27, 28]. Free thiol levels are an indirect parameter of the activity of glutathione, an antioxidant present in greater numbers in the CNS [29].

In addition to verifying the influence of swimming on encephalic impairment, evaluation of some skeletal muscle tissues is necessary. As DMD is an essentially neuromuscular disease, it is necessary to include skeletal muscle assessments (gastrocnemius and diaphragm), as the main characteristic of this disease is related to the involvement of these structures with calf pseudohypertrophy, frequent falls, gait loss, and cardiorespiratory dysfunctions [27]. Evidence suggests that oxidative stress is associated with the aggravation of both respiratory and muscular pathologies in these patients [20]. In addition, studies have shown that exercise can protect against oxidative stress in the skeletal muscles of mdx mice [4, 14]. The protocol used in this study protected against the increase in lipid peroxidation in the gastrocnemius muscles in mdx mice. Swimming for 4 weeks prevented the increase in protein carbonylation observed in the gastrocnemius muscles of the mdx mice. This finding is in line with a study conducted in 2015 that used a swimming protocol and found a decrease in protein carbonylation [12]. The same occurred with the diaphragm muscle, where the mdx mice showed significantly higher lipid peroxidation levels than the non-exercised mdx mice, which demonstrates that the 4-week exercise protocol prevented the increase in lipid peroxidation. However, swimming did not reverse the increase in protein carbonylation in the diaphragm [4]. From these findings, we can suggest that swimming as a moderate-intensity aerobic exercise can reduce oxidative stress by increasing antioxidant defenses such as the glutathione level, considering the increase in the formation of free thiols shown in the results of this study. However, as there is still no consensus in the literature on exercise volume, frequency, and intensity in the treatment of DMD, this study can help to propose new perspectives for the therapeutic use of exercise in the treatment of the disease.

In this study, only one of the modulating pathways of the CNS (learning and memory) was analyzed. Future studies are suggested to verify other pathways that can modulate memory and learning, such as neurotrophins, for example. It is expected that data from this study can serve for future pre-clinical and clinical research in order to standardize the use of exercise in the treatment of DMD.

Conclusions

On the basis of the lactate measurement classification, the swimming protocol used in this study was considered of moderate intensity. This protocol, applied to mdx mice, prevented memory impairment and oxidative stress in the gastrocnemius muscle and in most of the analyzed structures of the CNS, with significant increase in antioxidant activity.

Author Contribution Priscila Mantovani Nocetti, Viviane Freiberger, and Leticia Ventura planned the experiments and wrote the paper. Clarissa Martinelli Comim managed the project and performed most of the experiments and analysis and provided input on statistical analysis and interpretation. Adriano Alberti provided substantial input for the manuscript and wrote the paper. Daniel Fernandes Martins, Leoberto Ricardo Grigollo, Cristina Salar Andreau, and Rudy José Nodari Junior provided technical guidance and supervision. All authors read and approved the final manuscript.

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Data Availability Data will be made available upon request.
Declarations

Conflict of Interest  The authors declare no competing interests.

Consent to Participate  Not applicable.

Consent for Publication  Not applicable.

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