Alteration of skeletal and cardiac muscles function in DBA/2J mdx mice background: a focus on high intensity interval training

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SUMMARY Duchenne muscular dystrophy (DMD) is a recessive hereditary myopathy due to deficiency of functional dystrophin. Current therapeutic interventions need more investigation to slow down the progression of skeletal and cardiac muscle weakness. In humans, there is a lack of an adapted training program. In animals, the murine Mdx model with a DBA/2J background (D2-mdx) was recently suggested to present pathological features closer to that of humans. In this study, we characterized skeletal and cardiac muscle functions in males and females D2-mdx mice compared to control groups. We also evaluated the impact of high intensity interval training (HIIT) in these muscles in females and males. HIIT was performed 5 times per week during a month on a motorized treadmill. Specific maximal isometric force production and weakness were measured in the tibialis anterior muscle (TA). Sedentary male and female D2-mdx mice produced lower absolute and specific maximal force compared to control mice. Dystrophic mice showed a decline of force generation during repetitive stimulation compared to controls. This reduction was greater for male D2-mdx mice than females. Furthermore, trained D2-mdx males showed an improvement in force generation after the fifth lengthening contraction compared to sedentary D2-mdx males. Moreover, echocardiography measures revealed a decrease in left ventricular end-diastolic volume, left ventricular ejection volume and left ventricular end-diastolic diameter in sedentary male and female D2-mdx mice. Overall, our results showed a serious muscle function alteration in female and male D2-mdx mice compared to controls. HIIT may delay force loss especially in male D2-mdx mice.

Keywords cardiac function, muscle function, force production, HIIT, cardiomyopathy

1. Introduction

Duchenne muscular dystrophy (DMD) is a chronic and degenerative disease characterized by a progressive weakness of skeletal, respiratory and cardiac muscles due to deficiency of functional dystrophin (1,2). DMD has an incidence rate affecting closer to 1/5000 male births but also females, being asymptomatic carriers of mutations (3,4). Cardiomyopathy is the main cause of death of DMD patients, due to myocardial tissue lesions associated with systolic dysfunction (5). Male DMD patients exhibit cardiomyopathy, and myocardial fibrosis but females mostly develop later dilated cardiomyopathy (6,7). The development of cardiomyopathy is often due to relative physical inactivity and exercise intolerance inherent to their disability and progressive muscle wasting (8). DMD also alters skeletal muscles making them more susceptible to damage caused by high-force contractions like eccentric contractions both in situ and in vitro (9,10). Consequently the disease contributes to a muscle mass loss and leads to progressive loss of locomotion in DMD patients (11,12). These events may be related to several mechanisms such as, failure in neuromuscular transmission, reduced muscle excitability, impaired calcium release and uptake in the sarcoplasmic reticulum, and/or contractile impairment (13,14).

Today, there is no treatment for DMD. In healthy subjects, exercise induces beneficial effects, but its effects on DMD patients are still not well known. A new form of training, namely high intensity interval training (HIIT) has gained in popularity in clinics. Despite its potential beneficial effects in humans (15,16), no data are available on the effect on cardiac and skeletal muscles function in DMD patients and...
mdx mice. Studies focusing on the effect of exercise in mdx mice reported contrasting results (17-19). These discrepancies may be attributed to the type of exercise, the age of animals and their genotype. Concerning this last point, it was recently demonstrated that C57BL/10 mdx mice, the commonly used mdx mouse model for DMD, exhibited a less severe disease progression in skeletal and cardiac muscles than in a DBA/2J mdx (D2-mdx) mice background when both were compared to their control peers (20). D2-mdx congenic mice are generated by backcrossing C57BL/10-mdx mice to DBA/2J inbred mice for several generations to generate D2-mdx mice (20). These mice showed a lower hind limb muscle weight, fewer myofibers, increased fibrosis and fat accumulation, and muscle weakness compared to C57BL/10-mdx mice (20). Thereafter, data on the pathophysiological profile of this mouse model in different genders are still missing. Authors suggested that D2-mdx mice might represent a more robust animal model of DMD that may be used to determine new strategies of treatment.

The goal of the present study was to explore gender differences and functional properties of skeletal and cardiac muscles in 9-month old D2-mdx mice compared to their age-matched DBA/2J control peers and to determine the effects of a 4-week HIIT on skeletal and cardiac muscle function in female/male, sedentary/trained and control/D2-mdx mice.

2. Materials and Methods

2.1. Animals

All experimental protocols were performed in accordance with the national and European legislations and were approved by the institutional Ethics Committee Charles Darwin (project 01362.02). D2-mdx mice used in this study have been described previously and were generously provided by D. Coley (The Jackson Laboratory, Bar Harbor, ME, USA) (20). C57BL/10-mdx mice were backcrossed to DBA/2J inbred mice (Stock No. 000671) for several generations using a marker-assisted, speed congenic approach to generate the D2.B10-congenic strain (also referred to as DBA/2J-mdx or D2-mdx) as Stock No. 013141 (The Jackson Laboratory strain#013141). The DBA/2J background was used for control mice.

The experimental protocols followed the European directives on animal rights (86/609/CEE) and were approved by the institutional Ethics Committee "Charles Darwin" (project 01362.02). Animals were housed under standard conditions (20-22 °C, 12 h-12 h light-dark cycle), in normal cages with ad libitum access to water and food. 9-month old D2-mdx female (n = 12) and male (n = 12) mice were used in this study. They were compared to control DBA/2J female (n = 10) and male (n = 10) mice. Mice were weighed before starting the training protocol and then were divided into sedentary (SED) or HIIT groups after familiarization period and the identification of the most tolerant mice to running (Table 1).

After 4 weeks of training, animals were sacrificed and heart, gastrocnemius (Gastro) and tibialis anterior (TA) muscles were immediately weighed and isolated for further analyses (Table 1).

2.2. Running performance

All mice were tested before and after the training period. They were familiarized during 2 days with a motorized treadmill (LE8710MTS-Bioseb) at a running speed of 5 cm/s during 30 minutes before beginning the HIIT protocol. After this period, control DBA/2J (5 male and 5 female) and D2-mdx (8 male and 8 female) mice groups performed a maximal running speed (MRS) test every week. The remaining mice were divided into sedentary groups. The MRS test was determined with a running test in which the speed was gradually increased. It consisted of running 10 minutes at 10 cm/s followed by speed increments of 5 cm/s every minute until mice could no longer maintain the treadmill pace, then speed was recorded (21). Trials end at exhaustion as defined by the mouse touching the shock grid more than three times.

2.3. High intensity interval training (HIIT) protocol

After a 5-minute warm-up period at 10 cm/s, the HIIT protocol consisted of 10 repetitions of 30 seconds of high-intensity at 40 cm/s (80-90% of MRS) running followed by 1 minute of low intensity 15 cm/s (30-40% of MRS) running, 5 days/week over 1 month. The intensity of training was adapted every week after the MRS test. During the HIIT period, sedentary animals remained in their cages in the treadmill room with water and diet ad libitum.

2.4. Force-generating capacity and fragility of skeletal muscle

Force-generating capacity and fragility were evaluated by measuring the in situ TA muscle contraction in response to nerve stimulation, as previously described (17). The absolute maximal force that was generated during isometric contractions in response to electrical stimulation (frequency of 75 to 150 Hz, train of stimulation of 500 milliseconds) was measured. Absolute maximal force was determined at L0 (length at which maximal tension was obtained during the tetanus). It was normalized to the muscle mass as an estimate of specific maximal force, an index of muscle weakness.

Fragility (i.e. susceptibility to contraction-induced injury in D2-mdx mice) was estimated from the force decrease resulting from lengthening contraction-
induced injury. Nine lengthening contractions (eccentric contractions) of the TA muscles were performed in D2-mdx and control mice, each separated by a 60-second rest period. Maximal isometric force was measured 1 minute after each lengthening contraction and expressed as a percentage of the initial maximal force. After contractile measurements, the animals were sacrificed with cervical dislocation.

2.5. Echocardiography

Echocardiography was performed before and after the training period, on anesthetized mice under isoflurane (induction with 2% and maintained with 0.5%) as previously described (18). Non-invasive measurements of left ventricular (LV) dimensions were done using echocardiography-Doppler (Vivid 7 Dimension/Vivid7 PRO; GE Medical System Co, Vélizy, France) with an ultrasound probe at a 9-14 MHz frequency range. The following measurements were performed: diastolic (IVSd) and systolic intraventricular septal (IVSs) and posterior wall thicknesses (LVPWd and LVPWs), Left ventricle end-diastolic (LVEDD) and end-systolic (LVESD) diameters and heart rate. LV shortening fraction (LVSF) was calculated using the formula: (LVEDD-LVESD)/LVEDD × 100. LV ejection volume (LVEV), LV end-diastolic (EDV), and end-systolic (ESV) volumes were calculated using a half-ellipsoid model of the LV . From these volumes, LV ejection fraction (LVEF) was calculated using the formula: (EDV-ESV)/EDV × 100. The LV thickness/LV radius ratio (h/r) was also assessed in all animals.

2.6. Statistical analysis

All data are presented as the mean ± SEM. A two-way ANOVA followed by the Tukey post-hoc procedure was used to determine the effects of training and the animal genotype. We also evaluated the effects of training and the animal's gender with a two-way ANOVA test. The significance level was set at $p < 0.05$. Data were analyzed using the statistical package GraphPad Prism version 6.02 for Windows (La Jolla, California).

3. Results and Discussion

In this study, we investigated the function of skeletal and cardiac muscles in males and females with a DBA/2J mdx (D2-mdx) background. Then, we evaluated the impact of HIIT on these tissues in dystrophic mice.

3.1. Animals and muscles characteristics

Sedentary male and female D2-mdx mice showed a significant decrease in body weight (BW) compared with sedentary control DBA/2J mice. The decline of BW was especially marked in sedentary D2-mdx mice.
female mice compared to sedentary control females (Table 1; \( p < 0.0001 \)). The decrease in muscle mass of Gastro was observed also in both sedentary D2-mdx gender compared to control groups (\( p < 0.0001 \)) (Table 1). No sex or genotype difference in TA muscle mass was observed in sedentary groups. However, mass loss was also observed in the heart of sedentary D2-mdx mice compared with sedentary control groups (\( p < 0.0001 \)). A significant decrease was especially shown in control and D2-mdx females compared to males in both genotypes (Table 1).

Trained D2-mdx groups remain lighter compared to trained control DBA/2J mice and showed a larger decrease of BW in trained female D2-mdx compared to trained male D2-mdx (Table 1; \( p < 0.001 \)). In addition, HIIT did not improve Gastro and TA mass in trained females and males of both genotypes. Trained D2-mdx males showed a significant reduction in TA mass compared to sedentary D2-mdx males (Table 1; \( p < 0.05 \)). Heart mass was also lower in trained D2-mdx groups especially in females compared to control trained mice (Table 1; \( p < 0.001 \)).

Overall, the decrease of BW in sedentary and trained D2-mdx mice might be linked to the mass loss of cardiac and skeletal muscles (i.e. Gastro). This could be explained by the fact that dystrophin absence in tissue impaired regeneration and muscle growth inducing loss of mass and muscle function (22,23).

3.2. Functional impairment and fragility in the tibialis anterior muscle

A decrease in absolute maximal isometric force (Po) generated by TA muscle was observed in the male and female D2-mdx group compared to control DBA/2J mice (Figure 1A; \( p < 0.001 \)). The TA specific maximal isometric force (sPo), was also significantly lower in D2-mdx mice compared to control groups, without gender difference (Figure 1B; \( p < 0.001 \)).

During repetitive eccentric contractions, a large force decrease was demonstrated in sedentary D2-mdx mice from the third to tenth lengthening contraction compared to the control DBA/2J group (Figure 2A; \( p < 0.001 \)). This decrease was particularly pronounced in male D2-mdx mice compared to female D2-mdx (Figure 2A; \( p < 0.001 \)). In this context, several studies have confirmed that loss of muscle mass is always associated with decrease of force generation in mdx mice (17,18). This last point reinforces the fact that D2-mdx mice are characterized by a loss of BW associated with decline of the Po and sPo as found in DMD patients. However, D2-mdx females showed a better resistance to muscle damage caused by lengthening contraction compared to D2-mdx males in sedentary groups. Although no sex difference was reported in skeletal muscle mass, the male D2-mdx generated a lower force during contractions. These findings are in agreement with the
previous study of Van Putten et al. 2019, indicating that D2-mdx females outperformed compared to D2-mdx males. To sum up, DBA/2J mice are characterized by a loss of BW associated with a deficit in force generation with a greater impact on males.

The sedentary D2-mdx group, trained D2-mdx male and female mice, still generated a lower Po compared to control DBA/2J mice (Figure 1C; \( p < 0.001 \)). Trained males in the D2-mdx and control groups showed a decline of Po compared to their sedentary peers (Figure 1C; \( p < 0.05 \)). Furthermore, TA specific maximal isometric force (sPo) was significantly lower in trained D2-mdx male and female mice compared to control groups (Figure 1D; \( p < 0.001 \)). Contrary to Po, no sex effect was detected in sPo of trained groups (Figures 1D).

The fragility test showed no difference in control male and female mice of sedentary and trained groups. Until the tenth lengthening contraction, all control mice were able to maintain 70%, on average, of their force production (Figure 2B). In the D2-mdx group, females were still stronger in force generation than males independent of training status (Figure 2C, \( p < 0.001 \)). Furthermore, trained D2-mdx males generated greater force especially after the fifth lengthening contraction compared with sedentary D2-mdx male mice (Figure 2C; \( p < 0.05 \)).

These results demonstrated a delayed force production loss of the TA in male D2-mdx mice during repetitive eccentric contractions. However, our protocol training did not significantly increase the TA Po and sPo or muscle mass in D2-mdx or control mice. This is in contrast with other studies using a custom program of HIIT. In these studies, 24-month-old male and female control (C57BL/6J) mice underwent a 10-minute HIIT starting with 3 minutes of warm up at 8 m/min followed by intervals of 1 minute sprint at 13 m/min interspersed with a 1 minute period of relative rest at 8m/min. They performed 3 sessions/week for 2 or 4 months. This training induced an increase in muscle mass, an enlargement of fibers and an improvement of grip strength in trained mice compared to a sedentary group (24,25). In addition, the HIIT program (i.e. treadmill inclination 25°: 10 intervals of 4 minutes at 85-90% of VO2max interspersed with 2 minutes active rest at 5 m/min, 5 days/week for 2 months) improved metabolic dysfunction induced by High Fat Diet (HFD) and decreased the body weight and percentage of fat mass in 10-week old mice with a diet-induced obesity phenotype (26). In contrast, our HIIT program, was more intense (i.e. 10 repetitions of 30 seconds sprint interspersed with 1 minute of low intensity running; 5 days/week) and shorter in total duration (i.e. 1 month). These methodological differences and the age of animals might explain these differences and support the development of a standard training program to fully determine the impact of training in DMD.
3.3. Functional alterations in cardiac muscle of male and female D2-mdx mice

Echocardiography evaluations showed a significant alteration of the LV structure in sedentary male and female D2-mdx mice compared to the DBA/2J control group. A significant decrease of LVEDV, LVEV and LV EDD was observed in D2-mdx mice compared to control group (Figure 3A, B, C; \( p < 0.05 \)). No significant sex difference was observed in either genotype. This finding validated the installation of pathology in this mouse model (21) closer to the one observed in humans.

No significant difference was found between pre and post training for all echocardiographic variables between trained controls and D2-mdx groups vs. sedentary mice. Figure 3D represents two representative electrocardiographs of the myocardium before (white bars) and after (black bars) the HIIT period and a measure of the ejection fraction, which is a global parameter of cardiac function. This is contrary to other studies reporting severe muscle and heart damage (27,28) in trained mdx mice (C57Bl/10ScSn mdx/mdx) compared to their sedentary peers. These discrepancies highlight the importance of the impact of the genetic background onto the phenotype of mice used for studies in the DMD field. Future studies are needed to explore the adaptive potential of DBA/2J mdx mice to other HIIT protocols and determine new potential training programs for DMD patients.

4. Conclusion and Perspectives

The present study underlines functional impairments in skeletal and cardiac muscles of male and female D2-mdx mice with an original evaluation of the impact of HIIT in these tissues. These results might be related to metabolic disorders and to a higher susceptibility to weakness in dystrophic muscle as reported previously (29,30).

This study also demonstrated the potential feasibility, safety and beneficial (i.e. delayed TA generation force loss and no impact on cardiac function) effect of HIIT for DMD care management in the future.

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