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STUDY OF THE HIND LIMB GRIP STRENGTH OF X-CHROMOSOME-LINKED MUSCULAR DYSTROPHY (MDX) MOUSE NOT AFFECTED BY DAILY VOLUNTARY WHEEL RUNNING

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Duchenne muscular dystrophy (DMD) is the most common, severe, and lethal pediatric neuromuscular disorder in the world. Mutations in the dystrophin gene result in absence or dysfunction of dystrophin protein, leading to rapid and subjugating progression of muscle degeneration and necrosis along with gradual impairment of myofiber regenerative capacity. Children with DMD are confined to a wheelchair by early teens due to severe and progressive muscle atrophy and succumb to death in early adulthood primarily due to cardiopulmonary failure. There is currently no cure or proven effective treatment for this devastating disorder. The use of exercise to decrease the amount and decelerate the progression of muscle wasting in DMD patients remains controversial due to contradictory experimental findings. The purpose of this study was to determine whether strength and endurance training has any beneficial or deleterious impact on dystrophic muscle performance. The X-chromosome-linked muscular dystrophy (mdx) mouse was utilized as it is genetically homologous to human DMD. The results indicate that four-week-long voluntary wheel running had marginal beneficial effect on hind limb grip strength of one mdx mouse and had no deleterious effects in three other mdx mice.

Duchenne muscular dystrophy – dystrophin – mdx mouse – strength and endurance training – voluntary running – grip strength

Դյուշենի մկանային դիստրոֆիան (ԴՄԴ) աշխարհում ամենատարածված, ամենածանր-ընթաց և ամենամահացու մանկական նյարդամկանային հիվանդությունն է: Դիստրոֆին գենի մուտացիաները հանգեցնում են դիստրոֆին պրոտեինի բացակայությանը կամ դիսֆունկցիային, որը բերում է մկանների արագ զարգացող դեգեներացիայի ու նեկրոզի և մկանների ռեգեներացիոն ունակության աստիճանական խախտման: ԴՄԴ-ով երեխաները մկանների խորը և պրոգրեսիվող ատրոֆիայի հետևանքով դեռահաս տարիքում գամվում են հաշմանդամության սայլակին և վաղ պատանեկության շրջանում մահանում են հիմնականում սիրտ-թոքային անբավարարությունից: Ներկայումս չկա ոչ միայն ԴՄԴ-ի վերջնական բուժում, այլև չկան հաստատված, լիովին օգտակար վարման միջոցներ: ԴՄԴ-ով հիվանդների մոտ մկանների ատրոֆիան նվազեցնելու և պրոգրեսիան դանդաղեցնելու համար վարժությունների օգտագործումը մնում է վիճահարույց՝ փորձարարական իրարամերժ արդյունքների պատճառով: Այս գիտահետազոտական աշխատանքի նպատակն էր պարզել, թե ուժային և տոկունության վարժությունները դիստրոֆիկ մկանների գործունեության վրա օգտակար, թե վնասակար ազդեցություն ունեն: Օգտագործվել է X-քրոմոսոմով պայմանավորված մկանային դիստրոֆիայով (mdx) մուկը, քանի որ այն գենետիկորեն համանման է ԴՄԴ-ով մարդուն: Արդյունքները ցույց տվեցին, որ անիվի մեջ 4 շաբաթ կամավոր վազելը հետին վերջույթների բռնելու ուժի վրա մեկ մկան մոտ ունեցել է չնչին դրական ազդեցություն, իսկ մյուս երեքի մոտ չի ունեցել վնասակար ազդեցություն:

Դյուշենի մկանային դիստրոֆիա – դիստրոֆին – mdx մուկ – ուժային և տոկունության վարժություն – կամավոր վազք – բռնելու ուժ

Мышечная дистрофия Дюшенна (МДД) является самым распространенным, тяжелым и смертельным педиатрическим нервно-мышечным расстройством в мире. Мутации гена дистрофина приводят к отсутствию или дисфункции протеина дистрофина, что приводит к быстрому прогрессированию дегенерации мышц и некрозу наряду с постепенным затуханием регенеративной способности мышечных волокон. Дети с МДД прикованы к инвалидной коляске с раннего подросткового возраста из-за серьезной и прогрессирующей атрофии мышц и умирают в раннем юношеском возрасте в основном из-за сердечно-легочной недостаточности. В настоящее время нет лечения или проверенных эффективных методов ведения больных с этим разрушительным расстройством. Использование упражнений для уменьшения степени и замедления прогрессирования атрофии мышц у пациентов с МДД остается спорным в связи с противоречивыми экспериментальными данными. Целью данного исследования было определить имеют ли силовые нагрузки и упражнения на выносливость какое-либо полезное или вредное влияние на производительность дистрофических мышц. Была использована мышь с мышечной дистрофией, связанной с Х-хромосомой (*mdx*), так как она генетически гомологична человеку с МДД. Результаты показали, что четырехнедельный добровольный бег в колесе оказал незначительное положительное влияние на силу захвата задних конечностей одной мыши mdx и не имел вредных эффектов у трех других мышей *mdx*.

Мышечная дистрофия Дюшенна – дистрофин – mdx мышь – силовые нагрузки и упражнения на выносливость – добровольный бег – сила захвата

Duchene muscular dystrophy (DMD) is the world's most prevalent, devastating, and fatal pediatric neuromuscular disorder [5, 7]. It is an X-chromosome-linked recessive disorder caused primarily by frame-shift deletions, duplications or point mutations in the dystrophin gene, resulting in a complete absence or loss of critical functional domains of dystrophin protein [12, 13]. Localized to the cytoplasmic surface of sarcolemma in muscle fibers, 427-kDa dystrophin protein, along with other sarcolemmal proteins and glycoproteins, is part of the large trans-membrane oligomeric dystrophin-glycoprotein complex (DGC), which provides a mechanical link between the subsarcolemmal cytoskeleton and laminin in the extracellular matrix [9, 13]. In DMD patients, dystrophin deficiency leads to disruption of the DGC and thus compromised sarcolemmal integrity, which results in increased muscle susceptibility to damage and degeneration due to contraction-induced membrane stresses [5, 7, 13]. In the early stages of the disease muscles of individuals with DMD undergo continuous cycles of pervasive degeneration of individual dystrophic muscle fibers accompanied by extensive myofiber regeneration, resulting in widespread fiber size variation and substantial number of centrally-nucleated fibers [5, 7]. However, rapid and subjugating progression of muscle fiber degeneration along with gradual diminution of myofiber regenerative capacity result in necrotic muscle fibers and their eventual replacement with adipose and connective tissue. Failure to replace necrotic muscle fibers eventually results in an insufficient number of muscle cells for mobility and respiration [5, 7].

Clinical symptoms of DMD become evident between 2-5 years of age. Severe and precipitously progressive muscle wasting in boys with DMD confines them to a wheelchair by early teens and they succumb to death in their late teens to early 20s primarily from cardiopulmonary failure [5, 7]. There is currently no cure or proven effective treatment for this devastating disorder [2, 3]. Various therapies have been proposed to decrease the amount and decelerate the progression of muscle wasting in DMD patients [4, 17]. Use of strength and endurance training for improving muscle performance in dystrophinopathies remains controversial as contradictory findings have been reported with regards to the impact of exercise on dystrophic muscles [6, 18]. On one hand moderate strength and endurance training have both been reported to either have some beneficial effects [6, 8] or no deleterious effects [18] on the progression of the disease. On the other hand increased

work of dystrophic muscles has been conjectured to be harmful by making muscles more susceptible to injury and accelerated functional impairment due to imbalance between muscle injury and repair processes in dystrophinopathies [10, 16].

The X-chromosome-linked muscular dystrophy (mdx) mouse is the most highly utilized animal model of DMD [1, 9]. It is genetically homologous to human DMD as it has loss-of-function mutation in the highly conserved dystrophin gene, resulting in deficiency of highly conserved dystrophin protein in muscles [1, 14]. The purpose of this study was to study the impact of strength and endurance training on skeletal muscle performance in the *mdx* mouse by determining whether voluntary wheel running exercise has any beneficial or deleterious effects on hind limb grip strength of dystrophic mice.

Materials and methods. All animal experimental procedures were conducted in strict accordance with the United States National Institutes of Health (NIH) Public Health Service Policy on Humane Care and Use of Laboratory Animals and were approved by the Subcommittee on Research Animal Care (SRAC), which serves as the Institutional Animal Care and Use Committee (IACUC) for Massachusetts General Hospital (MGH) and Harvard Medical School (HMS). Adult *mdx* (C57BL/10ScSn-DMD^{mdx}/J) mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA) and housed in a pathogen-free facility, under constant room temperature and humidity, and 12/12 hour light/dark cycle. Both water and food (Prolab IsoPro RHM 3000 by LabDiet) were available *ad libitum*. Upon completion of experiments, all mice were euthanized via CO2 inhalation and cervical dislocation.

Voluntary Running Age-matched *mdx* (n=4) male mice were individually housed in standard rat cages for 28 consecutive days from 16 to 20 weeks of age. All cages were equipped with 4½ inch Mini Run-Around Exercise Wheels (Super Pet). The running wheels were fitted with BC 401 bicycle odometers (Sigma Sport) activated by wheel rotation, and calibrated to the wheel size, as previously described [11]. The mice were allowed free access to the wheels at all times.

Grip Strength & Fatigability Weekly, between the ages of 16 to 20 weeks all *mdx* mice were weighed and tested for hind limb grip strength using a Grip Strength Meter with Single Computerized Sensor with RS-232 interface and with Mesh Hind Limb Pull Bar Assembly (Columbus Instruments) as previously described [11, 15]. Upon being pulled by the tail base parallel to the mesh assembly, maximal force was measured as the force applied to the angled mesh pull bar at the moment the animal's hind limb grip was released. Five consecutive hind limb maximal force measurements were recorded for each session. Mean daily and weekly hind limb grip strength values were calculated along with mean grip strength values per trial. Data were normalized to body weight and expressed as N/g. Student's *t*-test was used for 2 variable comparisons.

Results and Discussion. Hind limb grip strength measurement data for the MDX 1 (fig. 1-1) and MDX 2 (fig. 1-2) mice showed that the overall average grip strength differences among trials 1-3 between the ages of 16 to 20 weeks old were not statistically significant from each other. However, both MDX 1 and MDX 2 mice exhibited gradual fatigability each week after trial 3 of five consecutive hind limb grip strength measurement trials. For the MDX 1 mouse the overall average grip strength at trial 5 ($7.24\pm0.04\cdot10^{-3}$ N/g) was 6.46% less ($p<0.001$) than the overall average grip strength at trial 3 ($7.74\pm0.05\cdot10^{-3}$ N/g) (fig. 1-1F). For the MDX 2 mouse the overall average grip strength at trial 5 ($7.26\pm0.04\cdot10^{-3}$ N/g) was 7.37% less ($p<0.001$) than the overall average grip strength at trial 3 ($7.84\pm0.1\cdot10^{-3}$ N/g) (fig. 1-2F). The MDX 3 mouse (fig. 1-3) demonstrated a 3.73 % increase ($p < 0.05$) in the overall average grip strength between trial 1 ($7.25\pm0.08\cdot10^{-3}$ N/g) and trial 3 ($7.52\pm0.03\cdot10^{-3}$ N/g) followed by a 4.02 % decrease ($p<0.001$) from trial 3 to trial 5 ($7.22\pm 0.02\cdot10^{-3}$ N/g) (fig. 1-3F). For the MDX 4 mouse (fig. 1-4) the difference in overall average grip strength between trials 1 and 2 was not statistically significant, however there was a gradual fatigability after trial 2 as trial 5 ($7.212\pm0.02\cdot10^{-3}$ N/g) was 8.69% less ($p<0.001$) than trial 2 ($7.898\pm0.11\cdot10^{-3}$ N/g) (fig. 1-4F).

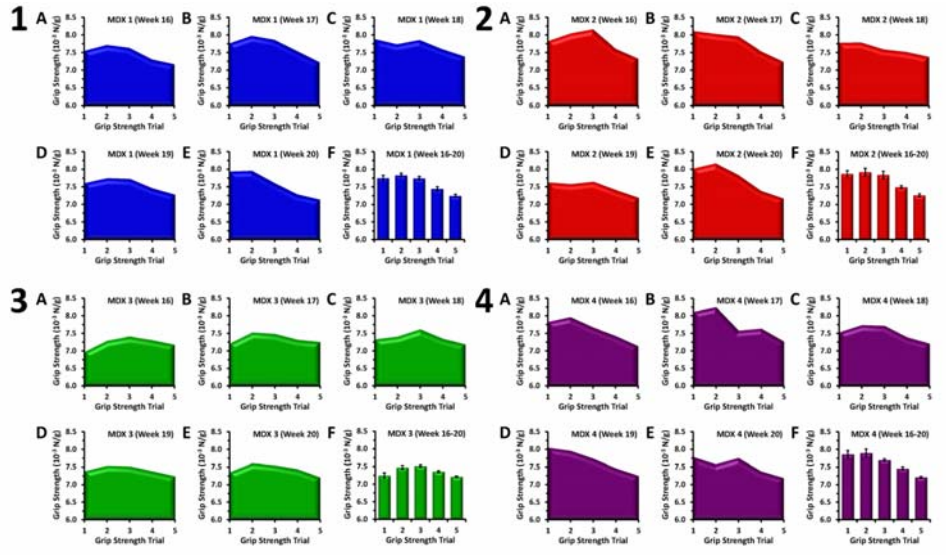

Fig. 1. Hind limb grip strength quantification for the MDX 1 (1-1), MDX 2 (1-2), MDX 3 (1-3), and MDX 4 (1-4) mice. (A-E) Grip strength data corresponding to five consecutive hind limb maximal force measurements recorded weekly during five consecutive weeks between the ages of 16-20 weeks for each mouse respectively. (F) Overall averages of grip strength measurements per trial encompassing weeks 16 to 20 for each mouse respectively. Data are expressed as mean ± SEM.

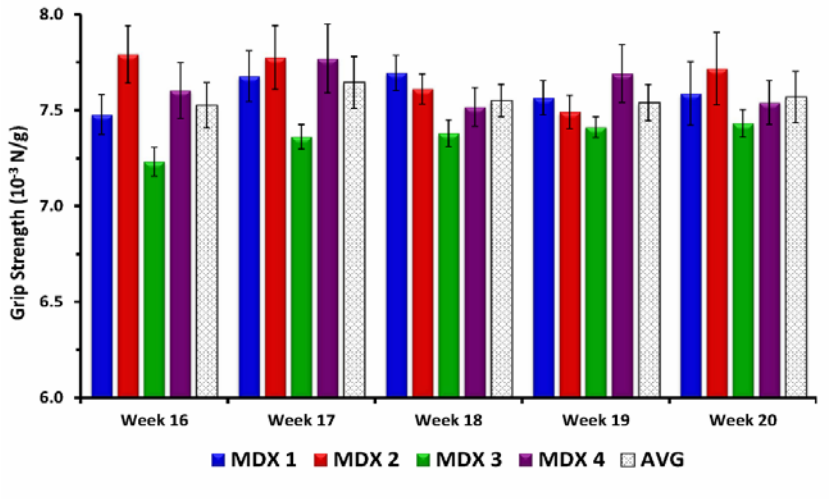

Fig. 2. Averages of hind limb grip strength measurements per week per animal between the ages of 16 to 20 weeks old. Data are expressed as mean ± SEM.

The weekly average hind limb grip strength for the MDX 3 mouse on week 16 ($7.23\pm0.07 \cdot 10^{-3}$ N/g) had gradually increased by 2.77 % ($p=0.089$) by week 20 ($7.43\pm 0.07\cdot10^{-3}$ N/g) (fig. 2). The abovementioned difference did not reach statistical significance. There were no statistically significant differences between the weekly average hind limb grip strength data for the MDX 1, MDX 2, and MDX 4 mice (fig. 2). In summary, the aforementioned findings en masse indicate that all mdx mice exhibited gradual fatigability after the first two or three hind limb grip strength measurement trials during five conse-

cutive trials conducted weekly between the ages of 16 to 20 weeks old. Furthermore, this study demonstrates that strength and endurance training exercise, specifically four-week-long voluntary wheel running, had marginal beneficial impact on dystrophic muscle performance or at minimum was not deleterious to skeletal muscle function as corroborated by hind limb grip strength measurement data.

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