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Is Exercise the Right Medicine for Dystrophic Muscle?

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Abstract

Introduction: Duchenne muscular dystrophy (DMD) is a neuromuscular disease caused by a dystrophin protein deficiency. Dystrophin functions to stabilize and protect the muscle fiber during muscle contraction, thus the absence of functional dystrophin protein leads to muscle injury. DMD patients experience progressive muscle necrosis, loss of function, and ultimately succumb to respiratory failure or cardiomyopathy. Exercise is known to improve muscle health and strength in healthy individuals as well as positively impact other systems. Because of this, exercise has been investigated as a potential therapeutic approach for DMD.

Methods: This review aims to provide a concise presentation of the exercise literature with a focus on dystrophin deficient muscle. Our intent was to identify trends and gaps in knowledge with an appreciation of exercise modality.

Results: After compiling data from mouse and human studies it became apparent that endurance exercises such as a swimming and voluntary wheel running have therapeutic potential in limb muscles of mice and respiratory training was beneficial in humans. However, in the comparatively few long-term investigations the effect of low intensity training on cardiac and respiratory muscles was contradictory. In addition, the effect of exercise on other systems is largely unknown.

Conclusion: In order to safely prescribe exercise as a therapy to DMD patients, multi-systemic investigations are needed including the evaluation of respiratory and cardiac muscle.

Key words: mdx, DMD, dystrophin, running, swimming

Introduction

Duchenne muscular dystrophy (DMD) affects one in every 5,000 boys (1) and is caused by a deficiency of the protein, dystrophin. It is a muscle wasting disease that leads to impaired mobility, wheel chair confinement, and ultimately patients succumb to respiratory or cardiac failure. Diagnoses of DMD typically occur around 4-5 years of age, driven largely by parental concerns about missed or delayed achievement of developmental milestones, frequent falls, and in some instances a distinct gait. Dystrophin is a 2.3 Mb gene with 79 exons that is translated into a 427 kDa protein (2). Mutations to the dystrophin gene resulting in a frame shift or nonsense mutation cause a deficiency of functional dystrophin protein. Dystrophin acts as the anchor for the dystrophin-glycoprotein complex, functions in related signaling, and serves a critical role in membrane stability during muscle contraction and eccentric contractions, in particular. In the absence of dystrophin, muscle cells are sensitive to eccentric injury resulting in physical disruption of the sarcolemma, loss of Ca^{2+} homeostasis, increased proteolysis, free radical injury, and widespread cellular dysfunction, among other maladies (3-6). DMD is most commonly modeled by the mdx mouse which has a nonsense mutation in exon 23. On the whole, the disease phenotype is relatively mild with only slight reductions in longevity (7) and impaired cardiac function only apparent after approximately 8 months (8, 9) likely due to increased utrophin protein abundance (10), increased capacity for repair (11), and differences in locomotion (12). Limb muscles experience an acute necrotic bout from approximately 3-8 weeks of age followed by relative stability before declining again following 12 months of age (13). The diaphragm undergoes a progressive disease and most accurately recapitulates many aspects of disease progression including impaired function, fibrosis, and necrosis, among others (14).

The absence of functional dystrophin not only affects skeletal and cardiac muscle cells, but also the skeletal, endocrine, and central nervous systems (15). Kyphosis, due in part to osteoporosis, is prevalent in DMD patients. Indeed, vertebral and leg fractures are common in DMD patients and resultant immobilization from leg fractures can lead to permanent loss of ambulation (16). The use of glucocorticoids, which is the standard of care for DMD patients, may exacerbate bone loss and contribute to fractures directly and indirectly, and it prolongs ambulation, which increases the opportunity for fractures due to falls (17). DMD patients tend to be obese with excess weight gain beginning early in disease progression due to decreased mobility and weight gain is further driven by increased appetite with steroid treatments (18). Obesity and increased weight gain result in increased rate of hyperinsulinemia and insulin resistance leading to increased occurrence of type 2 diabetes and cardiovascular disease for DMD patients (19). In addition, and perhaps due, at least in part, to these physical effects, DMD patients suffer increased risk of depression and anxiety (20). Importantly, in healthy populations exercise has been widely shown to counter many of these DMD-associated effects. At the very core of endurance training is increased cardiorespiratory function while overload training increases hypertrophy serving to combat two significant problems encountered by DMD patients. In addition, exercise has been shown to decrease the severity of depression, maintain bone density, and increase overall muscle health, among other benefits (21). Thus, exercise would appear to be a well-suited intervention for DMD patients. Indeed, exercise serves as the basis of physical therapy and utilizes multiple exercise modalities to maintain muscle flexibility and health in DMD patients.

As a premise of this review it is assumed that patients are receiving appropriate nutritional support and effective physical therapy. This review will focus on exercise beyond

that what is generally performed during physical therapy. The role of exercise on DMD has been previously reviewed, i.e. Gianola et al. (22), Grange et al. (23) and Markert et al. (24), though in many of these previous studies exercise was broadly considered. Here, we will independently explore exercise modality and consider independent muscle groups, with a particular focus on functional and histological outcomes, with the intention that this more refined approach will allow us to better identify emerging patterns of exercise benefits for dystrophic muscle (see Glossary, Supplemental Digital Content 1, description of functional measures, http://links.lww.com/MSS/B265).

Endurance Exercise. Researchers have investigated the effects of a variety of exercise modalities in mouse and human studies. The breadth of exercise regimens, measures, durations, and disease states makes comparisons between these investigations difficult. To better elucidate the effects of low intensity exercise in dystrophic muscle, data were collected from studies using swimming, voluntary wheel running, and treadmill running interventions (Figure 1). To visualize these data we plotted exercise modality, age of intervention initiation, duration of treatment, and effect of exercise on a single set of axes. The effect of exercise on a dependent variable was estimated from published investigations in order to calculate a percent change caused by exercise compared to sedentary dystrophic controls. In this manner, values less than 100% indicate a detrimental effect of exercise while values larger than 100% indicate a dependent variable was improved with exercise. To appropriately report and plot measurements such as fibrosis, necrosis and creatine kinase (CK) activity, in which an increase indicates a negative effect, an inverse calculation was used to appropriately place these data on the figures. Hind limb muscles were divided into two categories defined as anterior [i.e.. tibialis anterior

(TA) and extensor digitorum longus (EDL)] and posterior compartments [i.e.. gastrocnemius (GAST), soleus, and plantaris].   In total, the literature provides contradictory information regarding the impact of exercise on disease severity (Figure 1; see also Tables, Supplemental Digital Content 2, all data reviewed, http://links.lww.com/MSS/B266). Duration, intensity and modality of exercise complicate interpretation of data supporting the use of or contraindication of exercise as an intervention for DMD patients. To better identify the effect of exercise modality on disease severity the effects of voluntary wheel running, swimming and treadmill/rota-rod exercises were considered in more depth.

Voluntary wheel running.   In the investigations considered, voluntary wheel running maintained or positively affected all measures of relative tension and fatigue resistance in muscles of the forelimb and in anterior and posterior compartments of the hind limb in dystrophic mice regardless of age and duration of wheel running (Figure 2). Forelimb strength increased by over 25% after four weeks of exercise starting at 12 weeks of age (25). Fatigue resistance of the EDL *in vitro* and TA *in situ* was improved by 45% and 48% after sixteen and eighteen weeks of exercise, respectively, starting at 4 weeks of age (26, 27). In addition, relative tension in the EDL improved while other measures of EDL function were preserved (28) and maximum torque in the plantarflexor muscles were increased with voluntary wheel running (29). Similarly, EDL fatigability decreased by 65% following forty-eight weeks of exercise started at 24-week-old mice (30). Following nine weeks of exercise 12-week old mice maintained soleus function and fatigue resistance was significantly increased (31). Relative tension and specific eccentric force of the soleus in 16-week-old mice also increased 25% following twelve weeks of exercise and tetanic force was increased 45% after sixteen weeks of exercise, while other

measures of soleus and EDL function were maintained (25, 26). Following one year of voluntary wheel running EDL relative tension and soleus function, such as relative tension, fatigue resistance and half relaxation time were maintained compared to unexercised controls (31-33). While these data seem to provide compelling evidence supporting the use of exercise to maintain limb muscle health conflicting results were reported in the diaphragm (Figure 2). In three independent studies 3 to 4-week-old mice ran on a wheel for one year. In one investigation diaphragm relative tension was impaired by nearly 3-fold (33), however, a 30% increase in active tension, a 14% increase in fatigue resistance, and preservation of a variety of respiratory function and fatigue measures were reported in the other investigations (31, 32). In a shorter experiment, mice exercised for nine weeks starting at 3 weeks of age had increased diaphragm contraction time, while maintaining other measures of diaphragm function (31). The effect of voluntary wheel running on cardiac function is also unclear (Figure 2). After just four weeks of exercise, 11-week-old mice had over 25% thinning of the left ventricle wall thickness and increased left ventricle dilation similar to dystrophin-related cardiomyopathy (34). In one investigation left ventricle ejection fraction decreased by 30% after fourteen weeks of exercise (27), but in another, after one year of exercise left ventricle ejection fraction was similar between sedentary and exercised mice while cardiac output was increased 2-fold and stroke volume increased by 80% with exercise training (33).

Integrity of the skeletal system is also compromised in DMD patients both as a function of dystrophin deficiency and common use of glucocorticoids. Kyphosis is a spinal deformity that is a hallmark of dystrophin deficiency and a negatively impacts respiratory function (35). Brereton et al. (36) indicated that after one month of voluntary wheel running kyphosis increased by 25% in 4-month-old mice. In addition to increased kyphosis, and perhaps contributing to it,

fibrosis of the erector spinae increased by 40% (36). Kyphosis and injury to the erector spinae is not routinely reported in the dystrophic literature making comparisons difficult. Notably, these findings are in contrast to the bulk of data that show younger mice after a similar duration of exercise and 4-month-old mice following a longer duration of exercise have improved limb muscle function (25, 28, 29) However, given its function, the erector spinae may experience increased, damaging stress during exercise.

Swimming. Swimming interventions were beneficial for most skeletal muscles in mdx mice (Figure 3). Forelimb strength increased by 30% at 8 weeks of age following four weeks of exercise training (37). Similarly, EDL half relaxation time decreased in 20-week-old mice after fifteen weeks of swimming, indicating improved Ca^{2+} sequestration, fatigability decreased by over 20% in EDL, and EDL relative tension was sustained (38). In addition, soleus half relaxation time was maintained and relative tension increased by 60% following fifteen weeks of swimming (38). In older animals, eight weeks of exercise begun at 44 weeks of age increased relative tension in the soleus and EDL and sustained half relaxation time suggesting improved limb muscle health with exercise in aged animals (39). Importantly, however, cardiac and respiratory muscle had increased fibrosis in exercised mdx mice compared to sedentary mdx mice after ten weeks of swimming in 19-week-old mice (Figure 3). In addition, inflammation was increased 2-fold as was the heart wall to lumen ratio (40).

The intensity of exercise in these studies and how this may affect interpretation of findings above make the impact of swimming unclear. Playing in a swimming pool is a low intensity exercise recommended by physical therapists for a variety of muscle dysfunctions

including DMD. It is likely that the intensity of exercise, as a percent VO2max, in mice from these studies greatly exceeded that of DMD patients playing in a pool.

Forced running. Unlike voluntary wheel running, treadmill and rota-rod running are forced exercises that generally led to increased muscle damage and impaired function (Figure 4). Following four weeks of training 8-week-old mice had a 20-50% reduction in forelimb strength (41-45), increased plasma CK activity, increased fibrosis in the quadriceps and heart, and increased oxidative stress in the quadriceps and abdominal muscles (44, 46, 47). In the TA the abundance of necrotic muscle cells and inflammatory cells increased 2-fold, further supporting increased damage and decreased muscle function with treadmill training (41). Interestingly, damage and muscle regeneration were similar in TA, gastrocnemius and diaphragm muscles following four weeks of treadmill running compared to unexercised controls. Also, diaphragm relative tension was maintained (44). Animals that began training at 8 weeks of age also had reduced forelimb strength after four weeks of training (48) and decreased force production in the gastrocnemius after twenty-four weeks (49). Twelve weeks of training starting at 12 weeks of age decreased tetanic force and increased fibrosis 2-fold in the gastrocnemius (50). Similarly, following six weeks of rota-rod training, forelimbs fatigue was similar between exercised and sedentary age-matched controls, but the gastrocnemius and quadriceps of 14-week-old mdx mice had over 2-fold more necrosis than sedentary age-matched controls (51), further supporting increased muscle damage and decreased muscle function caused by forced training. In some contrast, training for ten weeks starting at 10 weeks of age decreased soleus and gastrocnemius necrosis by over 40%, but, consistent with previous findings, dramatically increased plantaris necrosis (52). The authors suggest (52) the degree of fiber loss may be related to the increased

contractile activity, though it seems likely the increased relative activity of the plantaris in combination with the fast fiber type is accountable for increased plantaris necrosis while the soleus and gastrocnemius are protected. Finally, following four weeks of treadmill training, oxidative stress was increased in quadriceps and abdominal muscles, but not the heart (47). In the same animals, fibrosis was increased in the heart and quadriceps, but the abdominal muscles were protected.

In most studies mice were forced to run for 30 min at 12 m/min (360 m/day) 2-3 times/wk, and in one investigation mice ran at 9 m/min for 60 min, 5 times/wk. By comparison, with volitional wheel running young mdx mice ran an estimated peak of 14 km/day (100 km/wk), 28-week-old mice ran nearly 3 km/day (20 km/wk), and 52-week-old mice ran approximately 2 km/day (14 km/wk) (33). The role of distance (daily or weekly) is unclear, though data suggest that these shorter, forced bouts with treadmill running are not as efficacious as larger daily running volumes seen during wheel running. This interpretation is complicated, however, by the intermittent nature of mouse wheel running, which has typical bouts of 1-10 minutes in mdx mice (53).

Downhill running. Downhill running has been used as a method for deliberate induction of contraction-induced injury in dystrophic muscle (54, 55). Our analysis of these investigations strongly supports this conclusion (Figure 5). For example, after only three days of downhill running, there was a 40% increase in damage to the biceps brachii, triceps brachii, soleus, gastrocnemius and diaphragm in 2-month-old mice (55). Interestingly, damage was not increased in the TA or EDL (55). Following seven weeks of training, fibrosis increased 3-fold in the TA of 31-week-old mice. In addition, plasma CK activity and fibrosis of the biceps

increased over 2-fold, further supporting increased damage (56). Forelimb strength also decreased by 15% in these same animals (56). Fibrosis of the diaphragm and heart following two weeks of downhill running were similar to sedentary controls (56), but after ten weeks of downhill running dystrophic lesions increased in the heart by 3-fold with a 17% increase in heart mass (57). The only improvement was the soleus in which twitch tension increased 50% after three weeks of downhill running, though half relaxation time increased (58).

Human studies. Conservative exercise protocols have been investigated in the context of both ambulatory and non-ambulatory DMD patients. Despite the advent of mechanical respiratory support, respiratory failure remains a significant cause of death in DMD patients. Given this, inspiratory and expiratory muscle training as well as resistive training protocols have been utilized in ambulatory patients. Generally, these interventions increased respiratory endurance by 46% with training after six weeks (59) and increased respiratory pressure and markers of respiratory strength after twenty-four, thirty-six, forty and ninety-six weeks of training (60-63) (Figure 6). In non-ambulatory patients, six and a half weeks of respiratory exercise increased vital capacity and airway pressure over 70% making clear that non-ambulatory patients can respond favorably to a therapeutic intervention (64).

In addition to respiratory function, range of motion and ability to complete tasks of daily living are important to the quality of life of DMD patients. To identify exercise protocols to prolong independence, non-ambulatory patients were provided jaw stretching and strengthening interventions (65, 66). Collectively, Nozaki et al (65) and Kawazoe et al. (66) discovered that masticatory performance and occlusal force and the degree of mouth opening were improved following twenty-four weeks of jaw exercises. In ambulatory patients, training by an assisted

cycle or arm ergometer resulted in increased or maintained endurance and range of motion in targeted muscles (67, 68). These data suggest that stretching and exercise increased range of motion and maintain strength of skeletal muscles. However, given the concerning respiratory and cardiac data presented above more information regarding the interplay between limb muscle exercise and impact on respiratory and cardiac function is necessary.

Acute Exercise. Performance of activities of daily living in DMD patients requires consideration of acute bouts of exercise. This seems particularly urgent considering few patients are part of regular exercise training regimens and regular play, not to mention occasional, high intensity activities, such as the violent forces experienced on inflatable playground equipment (i.e. a bouncy castle or the like), could certainly be considered an acute exercise bout. Indeed, activities like this can lead to agonizing decisions as competing parental roles are in conflict. Despite its importance, the literature does not provide fertile ground or necessary nuance for a rich discussion of acute exercise and we recognize this as an important gap in the literature. Nevertheless, summation of limited available evidence points to increased susceptibility to acute injury in dystrophic muscle.

In mdx mice, less than 30 min of swimming appeared damaging, despite being a non-weight bearing activity, as the number of damaged fibers in the TA were increased by up to 5-fold (69). Further, Evan's blue dye penetration into dystrophic muscle was dramatically elevated compared to healthy following a single 20 min swimming bout (70). Of interest, in this investigation fibers expressing a microdystrophin construct were also resistant to swimming-induced injury, while fibers from within the same section lacking microdystrophin expression had robust penetration of Evan's blue dye (70). These sections are particularly important as they

clearly demonstrate increased damage in dystrophin-deficient muscle compared to dystrophin-expressing muscle when subjected to the same acute exercise bout. In addition, a single bout of treadmill running increased serum CK activity 7-fold (71) while in healthy mice only a 2 to 3-fold change might be expected following either treadmill running or even eccentric exercises (72, 73). In the quadriceps, necrosis was increased over 2-fold following 30 min of treadmill running (71), and in the EDL force was decreased 2.5-fold and membrane permeability was increased following 45 minutes of downhill running in mdx mice (74). While direct comparison to injury in healthy muscle is rare, our expectation, based on the sensitivity of dystrophic muscle to contraction-induced injury, demonstrated elevations in serum CK activity, and increased Evan's blue dye penetration, is that this degree of damage in dystrophic muscle surpasses that anticipated in healthy muscle following performance of the same activity. To that point, and while not a single acute running bout, 3-days of downhill running increased membrane permeability in dystrophic limb muscle by 10% while in healthy muscle no permeability was noted (55). Lastly, when mdx mice ran voluntarily for twenty-four hours, damaged fibers in the gastrocnemius were increased 18% and 6-fold in the TA and quadriceps (75). This later study is of note as long-term volitional wheel running generally improved outcomes while shorter treadmill bouts exacerbated markers of injury. Collectively, these data suggest that dystrophic skeletal muscle is capable of exercise-mediated adaptations despite increased damage early in a training regimen (i.e. acute exercise) though the degree to which it may be trained and how closely the training response of dystrophic muscle follows that of healthy muscle is unclear.

Importantly, and consistent with the animal literature, acute or single bouts of exercise result in increased serum CK activity in DMD patients (76). While certainly increased serum CK activity can occur as a result of exercise in healthy patients the effect, and thus presumptive

underlying muscle injury, is greater in DMD patients following similar activities. For example, eight hours after a 15 minute bout of physical therapy in the water, serum CK activity increased nearly 1,000 U/l from baseline in DMD patients compared to an increase of less than 10 U/l in healthy subjects (76). Supporting this notion of increased susceptibility to acute injury, circulating myoglobin increased 1,114.8 ng/ml from baseline in DMD patients but only 46.2 ng/ml in healthy controls eight hours post-exercise (76).

While dystrophic muscle appears capable of exercise-mediated adaptations under the right training conditions (25, 26, 28-31, 39), it is associated with increased injury following acute exercise, which may suppress exercise-mediated adaptations compared to healthy. Further, despite these data seeming to make clear an acute bout of exercise is more damaging to dystrophic muscle compared to healthy muscle we cannot rule out the possibility that because DMD patients ostensibly have a lower VO2max than healthy controls, DMD patients or dystrophic muscle was operating at a higher relative workload and therefore, may be expected to have a greater degree of muscle injury. Moreover, as injury following acute exercise may be necessary for adaptation in healthy muscle, we cannot rule out the possibility that increased damage following acute exercise is necessary for a training adaptation in dystrophic muscle, though the degree to which disease and disease-related injury may alter this process, either directly or indirectly, is unknown.

Exercise mimetics. Exercise has the potential to provide a robust therapeutic effect to dystrophic muscle, however, the forces produced during exercise and its multi-systemic involvement make its use tenuous. To harness the effects of exercise without associated complications several groups have used pharmaceutical and nutraceutical approaches to mimic

the effects of exercise. For example, myostatin inhibition has repeatedly resulted in increased muscle mass and function in a dystrophic muscle (77, 78), which ostensibly would translate into improved tasks of daily living. Indeed, several clinical trials are currently making use of myostatin inhibition as a therapy for muscular dystrophy (NCT02310763, NCT02515669, NCT02907619), however the impact on cardiac function will need to be carefully monitored. In addition, induction of oxidative metabolism and the oxidative muscle phenotype has been successfully accomplished using the AMPK-activator, AICAR (79, 80), and activation of mitochondrial biogenesis pathways has been attempted via PGC-1α over-expression and gene transfer (6, 81, 82) and supplementation with resveratrol and quercetin (83-87). While generally further testing is needed, including long-term investigations and clinical trials, these approaches have the promise to afford DMD patients with some benefits of exercise while minimizing risk.

Conclusion. When considering exercise as a therapy for DMD patients it is essential to consider the totality of effects on patients and particularly to vital muscles. Duchenne muscular dystrophy patients typically succumb to respiratory or cardiac failure, thus exercises that protect or improve limb muscle function while impairing respiratory or cardiac function provide little benefit to the patient. In mouse studies, swimming generally improved limb muscle function, but caused histopathological damage in cardiac and respiratory muscles, suggesting that caution should be used when considering swimming as an exercise for DMD patients until the role of exercise intensity is made clearer. Similarly, voluntary wheel running provided contradictory information regarding the impact on cardiac and respiratory function, though was consistently beneficial to limb muscles. The multi-systemic nature of DMD and the impacts of exercise on these systems are also of interest for DMD patients. Increased kyphosis (36) paired with

conflicting diaphragm (31, 33) and heart function data in mice (27, 33, 34) brings to light the need to further investigate and balance the benefits of exercise while minimizing detrimental side effects. Concurrent investigation of respiratory and cardiac function as well as other systems are necessary to effectively prescribe exercise as a therapy for DMD.

Conflict of Interest and Source of Funding

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Figure Captions

Figure 1: Effect of low intensity exercise on health outcomes in dystrophic mice. Low intensity exercise provides both beneficial and detrimental health outcomes. The breadth of initiation age, duration, and modality complicate an appreciation for the effects of exercise on disease severity. Data were obtained from the literature and the effect of exercise normalized to sedentary mdx mice. Additional statistical analyses were not performed. For any given variable age of initiation is shown at 100% (Y-axis) and the deviation from 100% and the duration of the intervention can be determined. The figure is constructed such that beneficial changes are above 100% and detrimental changes are below 100%, including those where an elevation would indicate increased pathology. Non-significant findings were not plotted. Data point shapes represent activities as indicated. Colors represent muscles as follows: black = whole body, gray = forelimb, purple = anterior hind limb, blue = posterior hind limb, red = heart, and green = diaphragm

Figure 2: Effect of voluntary wheel running on health outcomes in mdx mice. All measures of hind limb muscle function were maintained or improved by voluntary wheel running. Conflicting results emerged concerning the effect of voluntary wheel running on cardiac and respiratory function. Values above 100% are measures positively affected by exercise. Nonsignificant data were omitted from the figure. Data point shapes/colors represent measures as indicated.

Figure 3: Effect of swimming on health outcomes in mdx mice. Exercise improved forelimb function and hindlimb muscle function but was detrimental to cardiac muscle function. Values above 100% are measures positively affected by exercise. Nonsignificant data were omitted from the figure. Data point shapes/colors represent measures as indicated.

Figure 4: Effect of treadmill running/rota-rod on health outcomes in mdx mice. Forced running caused a variety of responses in dependent variables. Values above 100% are measures positively affected by exercise. Nonsignificant data were omitted from the figure. Data point shapes/colors represent measures as indicated.

Figure 5: Effect of downhill running on health outcomes in mdx mice. Downhill running was largely detrimental hind limb (anterior and posterior compartments), forelimb, and cardiac muscles. Values above 100% are measures positively affected by exercise. Nonsignificant data were omitted from the figure. Data point shapes/colors represent measures as indicated.

Figure 6. Effect of exercise on DMD patients. Exercises designed to improve range of motion and respiratory endurance improved health outcome measure in DMD patients. Starting ages were not provided in this figure as the range of starting ages within a study varied as did ambulatory status. Values above 100% are measures positively affected by exercise. Nonsignificant data were omitted from the figure. Data point shapes/colors represent exercises as indicated.
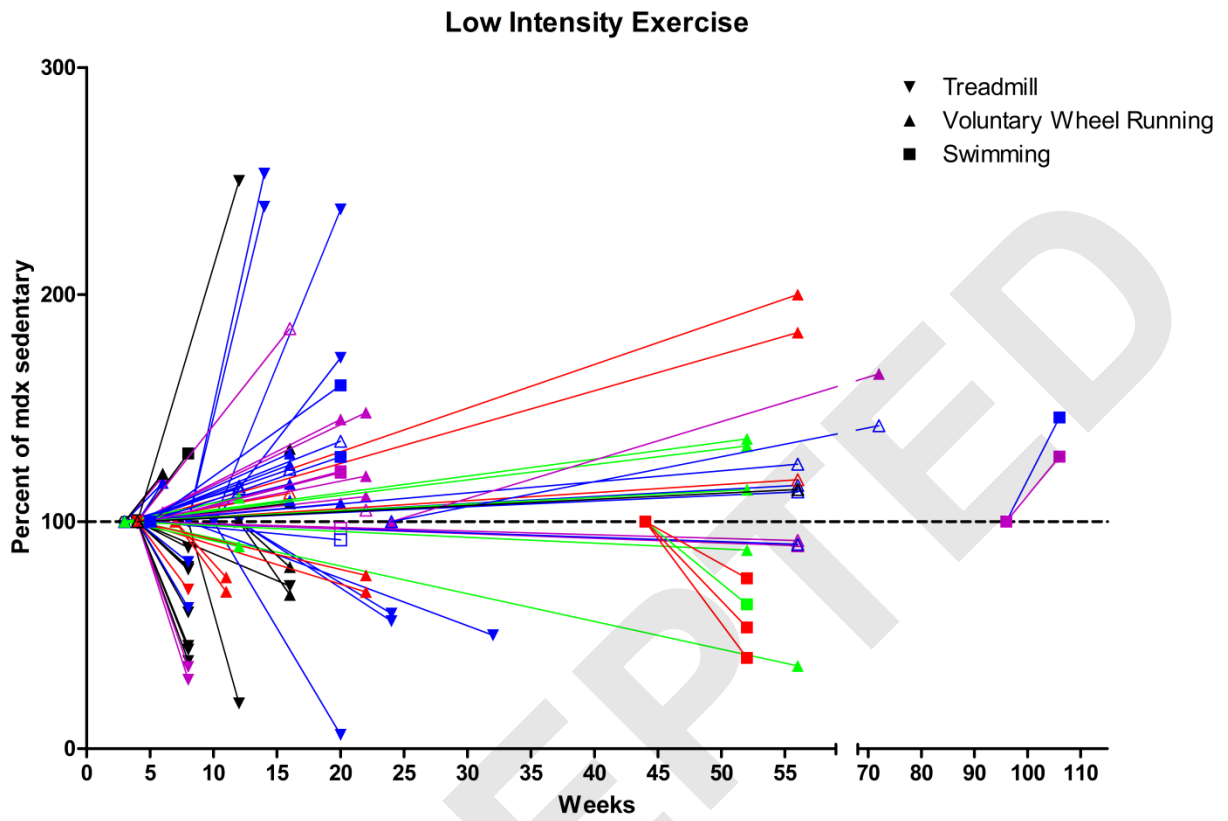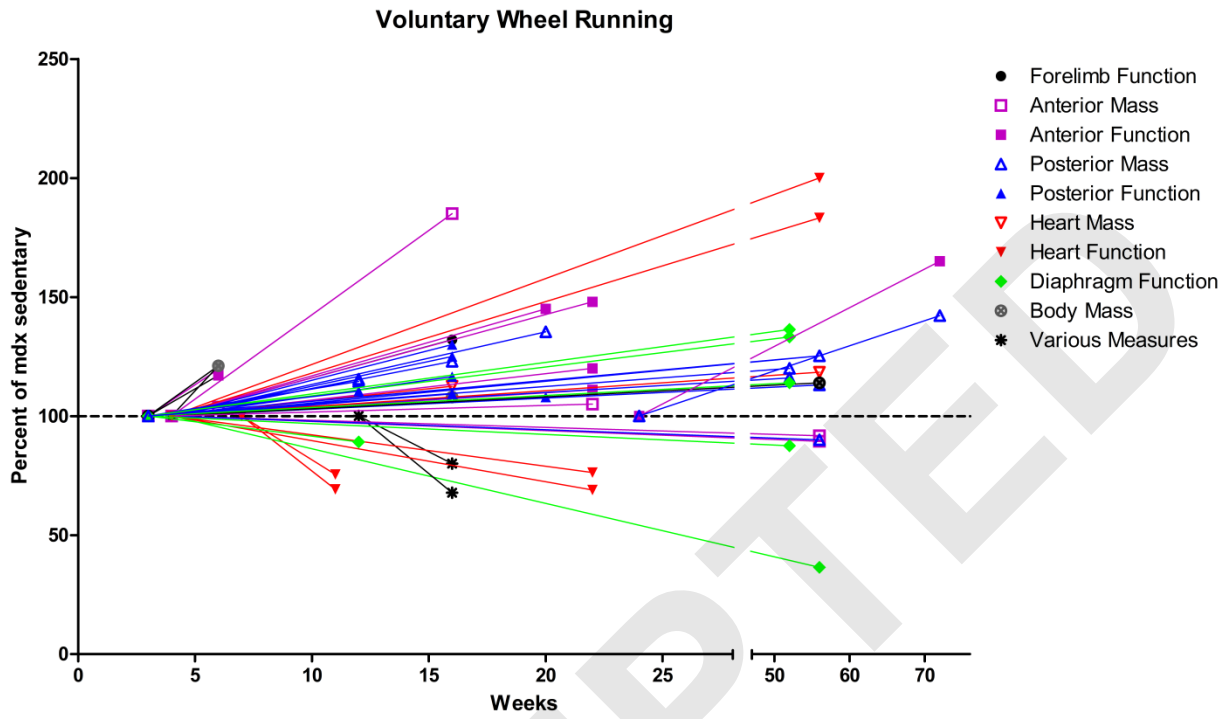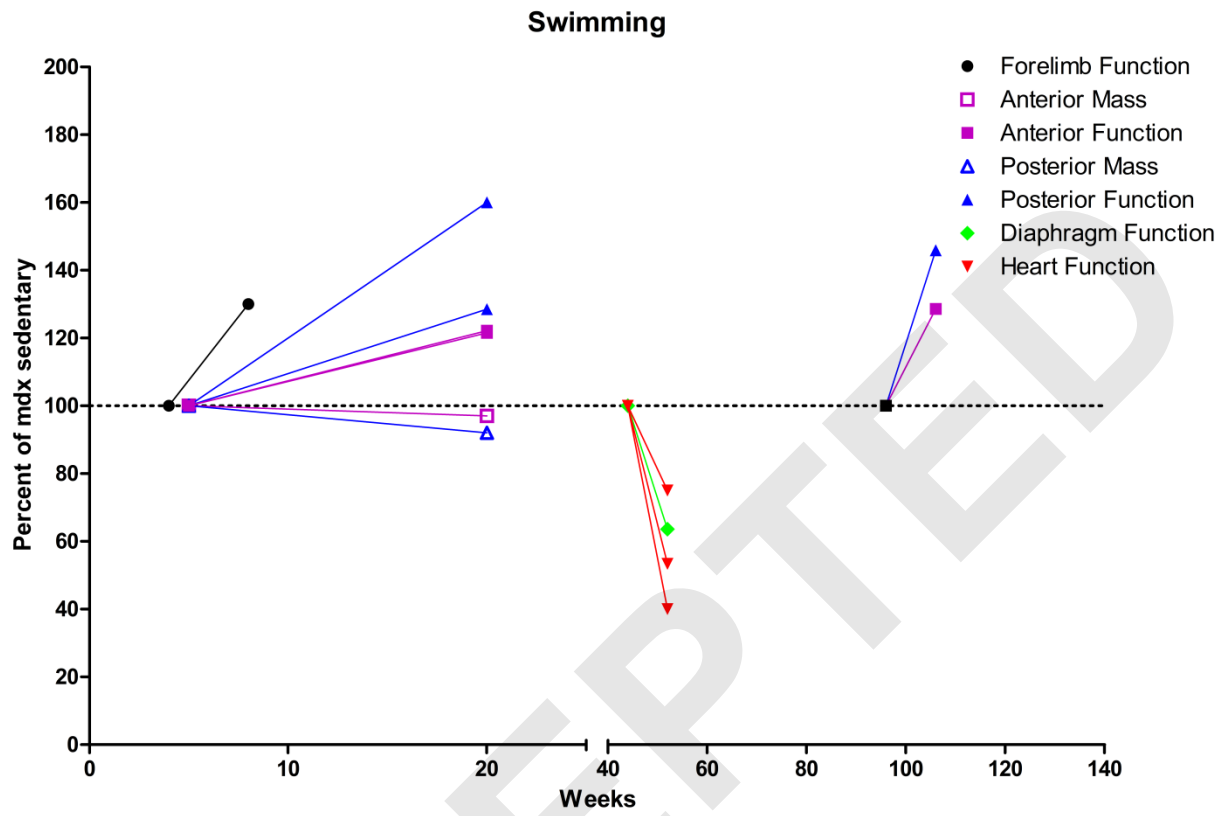
Figure 1

Low Intensity Exercise

Legend:
▼ Treadmill
▲ Voluntary Wheel Running
■ Swimming

Y-axis: Percent of mdx sedentary (0, 100, 200, 300)
X-axis: Weeks (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 70, 80, 90, 100, 110)

Figure 2

Voluntary Wheel Running

Figure 3

Swimming

Figure 4

Treadmill/Rota-Rod

Legend:
- ● Forelimb Function
- ■ Anterior Function
- ▲ Posterior Function
- ▼ Heart Function
- ✱ Circulating Injury Markers

Y-axis: Percent of mdx sedentary (0–300)
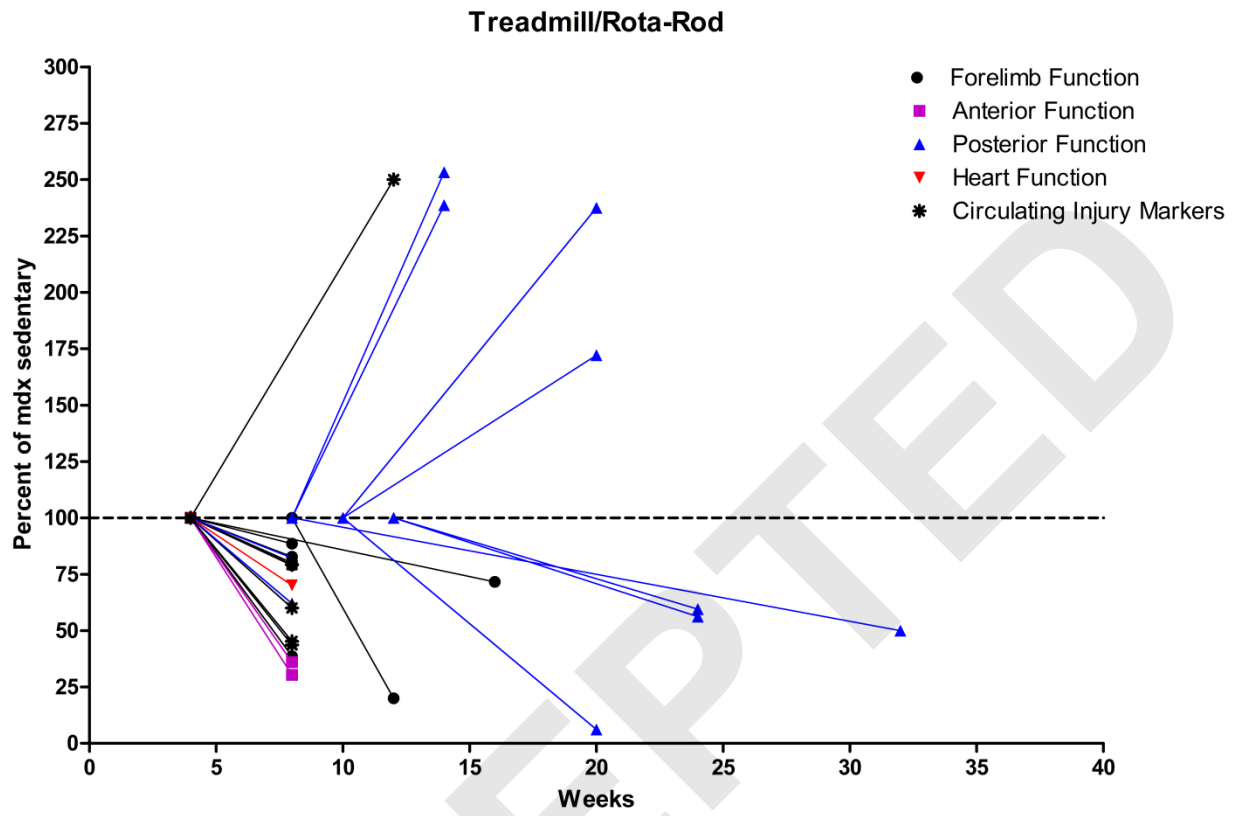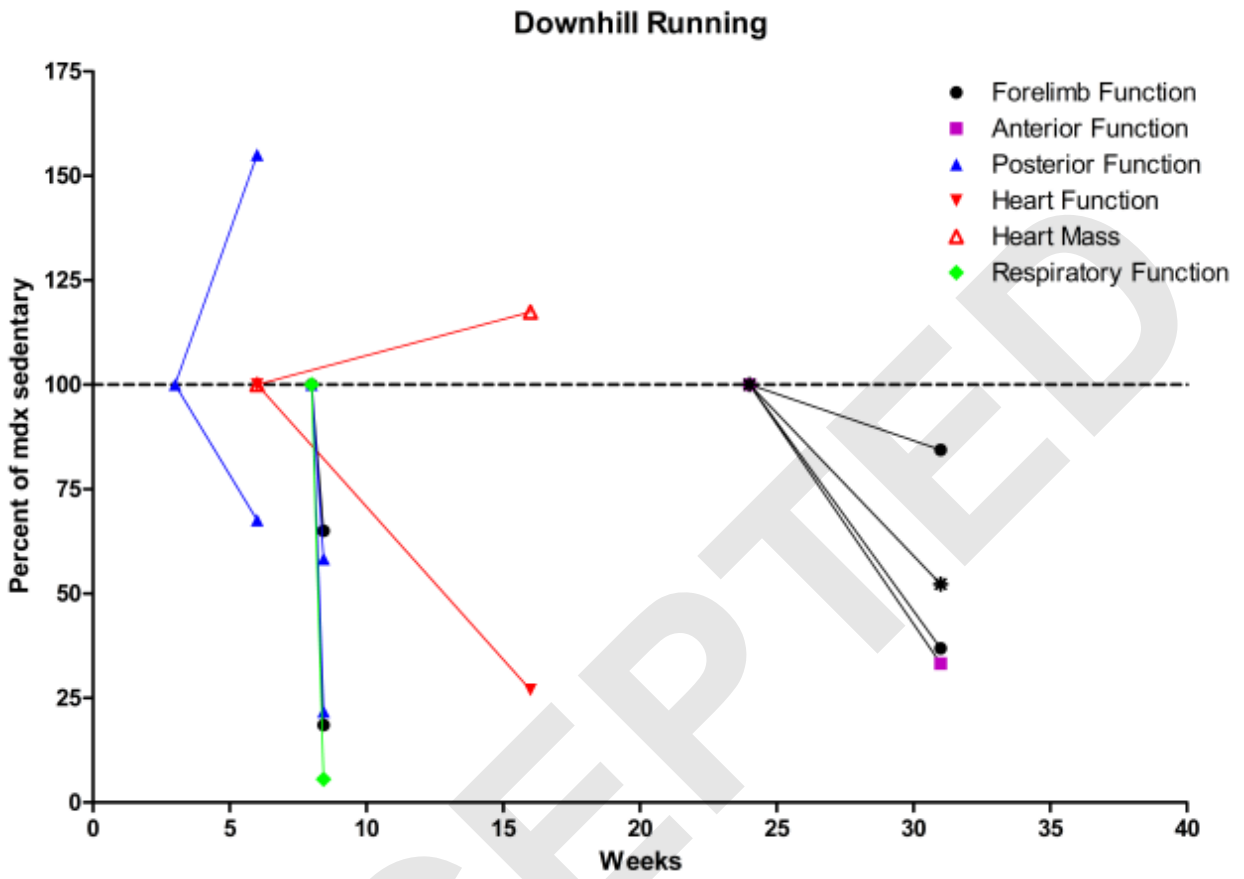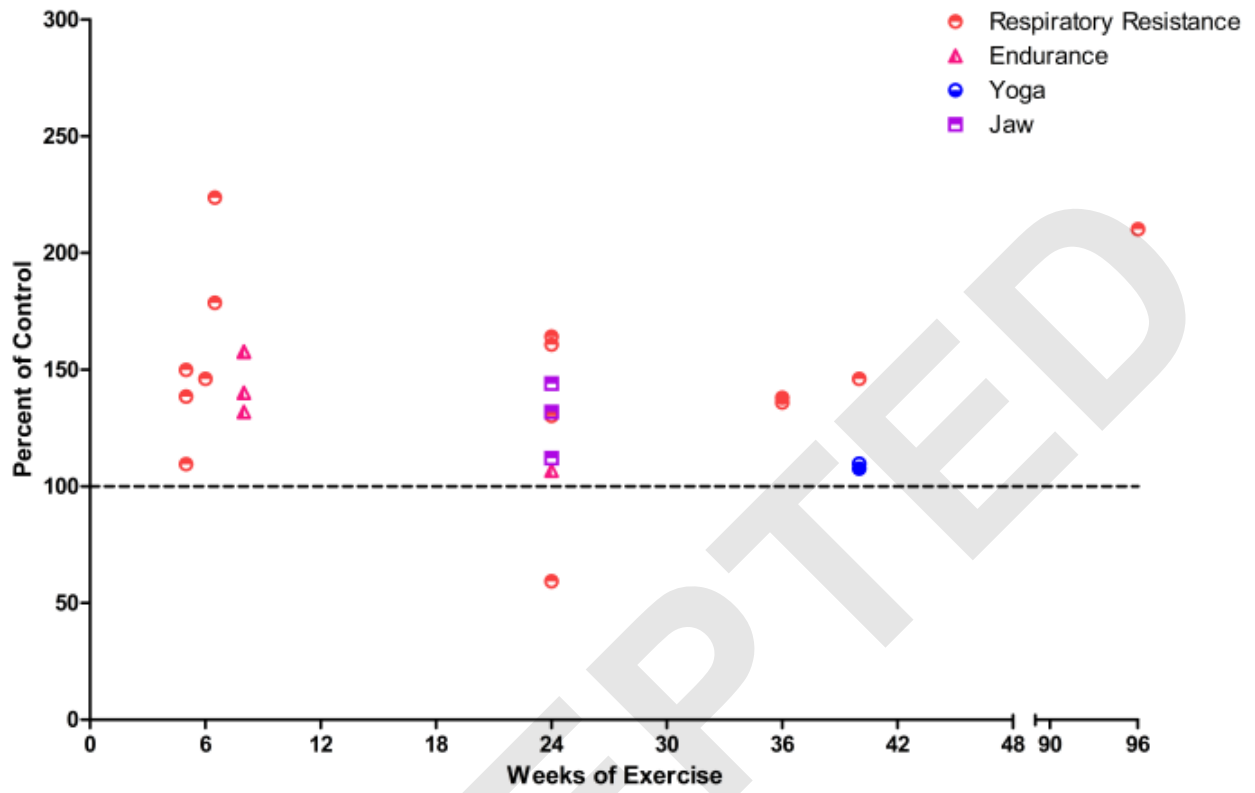X-axis: Weeks (0–40)

Figure 5

Downhill Running

Figure 6

Supplemental Digital Content

Supplemental digital content 1.docx—Glossary

Supplemental digital content 2.xlsx—Human and mice data

Glossary

Contraction Time (ms): Contraction time measured *in vitro* from time of force production to peak tension.

Forelimb strength (N): *In vivo* forelimb strength measured by gently pulling on a mouse holding a fix bar attached to a force transducer.

Half relaxation time (ms): *In vitro* measurement of the time required for the excised muscle to decline from peak tension to 50% of peak tension.

Max Torque (N*mm/g): *In vivo* force generated following stimulation of the plantarflexors at predetermined joint angles normalized to body mass.

Rate of relaxation (N/sec): The rate at which force declines following peak tension.

Relative Tension (N/cm^2 or N/mg): Tension generated from a contraction that is normalized to either body mass when functional tests are performed *in situ* (1) or calculated cross-sectional area of the muscle when functional tests are performed *in vitro* (also called active tension, specific tension and specific isometric force, among others, in the literature) (2-5).

Tetanic Force (N): Absolute maximal isometric force generated from a stimulated muscle. Also called isometric force *(in vitro)* (6) or absolute force *(in situ)* (1).

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	Weeks	Age	Author	Measure	Perce	Treatment
2	1 bout	3 boys (age 5 to 8)	Garrood (2007)	increased myoglobinuria (presence of myoglobin in the urine - muscle degradation)	12.2	one bout of physical exercise (ex. Football, bouncy house)
3	1 bout	6 to 16	Poche (1987)	increased CK	0.8	physical therapy exercise in water (15 minutes)
4				increased myoglobin	4.14	
5	3	9 to 14	Rodillo (2016)	no change in diaphragm measures	100	Triflow II inspirometer (20 inspirations per day) vs placebo training with a mini peak flow meter (10 expirations per day)
6	5	recently immobilized/still walking	Vilozni (1994)	increased MVV (maximum voluntary ventilation)	109.4	ventilatory effort of 46 + 6% MVV, for 10+/- 3 min day
7				increased Emax (maximal achieved ventilation)	149.8	
8				increased duration of PIHV (progressive isocapnic hyperventilation maneuver)	138.5	
9	5	immobilized	Vilozni (1994)	no change MVV (maximum voluntary ventilation)	100	ventilatory effort of 46 + 6% MVV, for 10+/- 3 min day
10				no change Emax (maximal achieved ventilation)	100	
11				no change duration of PIHV (progressive isocapnic hyperventilation maneuver)	100	
12	6	14.7	Topin (2002)	increased endurance in trained DMD patients	146	breathed twice a day for 10 min through a valve with either 30% (trsining group) or less than 5% (control group) of their maximum inspiratory pressure (Pimax).
13	6.5	18 (n=1)	Aldrich (1987)	increased vital compacity	223.7	Inspiratory muscle training (IMT) and intermittent mandatory ventilation (IMV)
14				increased inspiratory airway pressure	178.6	
15	8	8 to 12	Alemdaroglu (2014)	increased arm movement quality	140	Arm ergometer vs range of motion exercises (ROM - control)
16				increased AREA quality score	157.5	
17				increased standing from supine position	157.7	
18				increased endurance	131.8	
19	24	12	Gozal (1999)	increased maximal inspiratory and expiratory pressures	130.2	respiratory muscle training (RMT)
20				decreased respiratory load perception	59.26	
21	24	10.5	Jansen (2013)	stable motor function measure (increased compared to declining controls)	106.7	bicycle leg and arm training (15 minutes, 5 days a week)
22				unchanged assisted 6-minute cycling test	100	
23	24	16-24	Kawazoe (1981)	increased latency of jaw-jerk reflex	112.1	5 min jaw clench, open jaw 5 times, move tongue 5 times
24				increased masticatory performance of masseter	144	
25	24	21.1	Nozaki (2010)	increased greatest occlusal force	131.8	Jaw ROM exercise: therapist assisted 2x a week, every day did other exercises
26				no change in maximum degree of mouth opening	100	
27	24	14.5	Wanke (1994)	increased maximal sniff assessed esophageal pressure values	164.1	training the Inspiratory muscles
28				increased maximal sniff assessed trans diaphragmic pressure values	160.7	
29	36	8 to 23	Winkler (2000)	increased Pimax	137.9	inspiratory training
30				increased12s- MVV test	135.9	
31	40	12.5	Koessler (2001)	increased PI max until 10 months, then plateau	146	IMT
32	40	9.5	Rodrigues (2014)	increased FVC- forced vital capacity (total amount of air exhaled during the FEV test)	109.7	yoga breathing exercises on pulmonary function (3 times a day for 10 months)
33				increased FEV(1) (amount of air exhaled may be measured during the first second of forced breath) forced expiratory volume	107.5	
34	48	various (5-12)	De Lateur (1979)	no effect on quad isokinetic strength	100	Quad submaximal exercise
35	36	12.5	Koessler (2001)	increased PI Max until 10 months, then plateau	210.1	IMT