

# T2 Relaxation Time and Activity in Boys with Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is an inherited neuromuscular disorder characterized by progressive muscular degeneration. It is unknown what amount of activity is beneficial or detrimental to dystrophic muscle. This study examined functional mobility, activity, and T2 relaxation time in boys with DMD. DMD subjects were divided into two groups (active vs. inactive), and functional ability was tested using the 10m walk/run test. T2 relaxation times (4 echo times; at 3T) were used as a construct of muscle damage and composition. A trend was noted for the relationship between daily step counts and time for 10 m walk/run in DMD. For DMD subjects, there was a weak, negative relationship between average step count and T2 of the leg muscles ( $r = -0.278$  to  $-0.308$ ). The relationship between time spent in sedentary activity and T2 values of the leg muscles demonstrated a moderately positive, linear relationship ( $r = 0.377$  to  $0.440$ ). Overall, the results show that there may be a relationship between T2 and activity in boys with DMD, and suggest that active DMD boys tend to have better functional abilities than inactive DMD. Further research is needed to better understand how activity impacts functional ability and muscle pathology in this patient population.

## INTRODUCTION

### **Pathology**

Duchenne muscular dystrophy (DMD) is a terminal, X-linked inherited, neuromuscular disorder characterized by progressive muscular degeneration (Tadayoni, Rendon, Soria-Jasso, & Cisneros, 2011). It occurs in about one in every 3,500 live male births and the loss of ambulatory abilities will typically occur at the beginning of the second decade of life (Doglio et al., 2011). The muscle degeneration is caused by a lack of dystrophin, a protein normally located in the muscle membrane. The absence of dystrophin can lead to a series of pathological processes including inflammation, muscle fiber necrosis, and eventual replacement of muscle with adipose tissue (Cirak et al., 2011). A combination of these pathological developments has functional implications for boys with DMD, and may appear by the age of three.

### **Activity**

Boys suffering from DMD will develop a wide range of functional impairments due to their progressive muscle weakness. The most common clinical observations are the presence of Gower's sign, waddling gait, and possible toe walking (Bushby et al., 2010). Previous research has explored the

relationship between functional abilities and the progression of muscle deterioration with non-contractile tissue (Akima et al., 2012). Further exploration of functional assessments and disease progression may contribute to our understanding of the relationship between function and muscle pathology in DMD.

The extent of muscle integrity varies in this patient population, and a wide range of factors contributes to the overall condition of the muscle. It appears that exercise and activity may contribute to the progression of muscle damage in DMD, and research suggests that dystrophic muscle often becomes damaged following episodes of extreme activity (Call, McKeehan, Novotny, & Lowe, 2010). The damage is related to a lack of dystrophin in the muscles, which causes the sarcolemma to become fragile and more susceptible to injury, especially after excessive exercise. Once the muscle is damaged, inflammation occurs in response to the body's reparative process. The muscle continues to engage in this cycle of damage, inflammation, and repair as children with DMD participate in normal daily activities and ambulation (Mathur et al., 2010). Eventually, these recurrent relapses exhaust the regenerative capacity of the muscle and induce additional inflammation, progressive fibrosis, and loss of function as the muscle is replaced with non-contractile tissue (Deconinck & Dan, 2007).

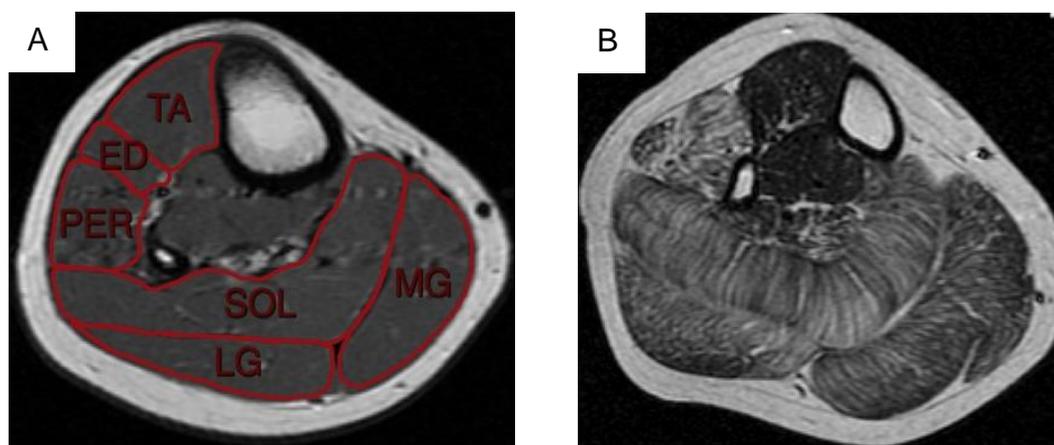
Sufficient participation in physical activity (PA) during childhood is considered essential for the health and development of a child. While it is commonly accepted that exercise is beneficial to the development of non-pathological tissue (Garber et al., 2011), the effects of exercise on dystrophic tissue are not well understood. Exercise may be either beneficial or detrimental to dystrophic muscle and depends on several parameters including frequency, intensity, type, and duration (McDonald et al., 2005; Markert, Ambrosio, Call, & Grange, 2011). Research suggests that exercise may be beneficial for boys with DMD at low intensities (Ansved, 2003), but definitive human trials on the use of exercise as a treatment in neuromuscular diseases have not been conducted. This lack of sufficient, well-controlled human studies has led to a deficiency in our understanding of the role that activity and exercise have on DMD (Grange & Call, 2007). Further investigation may provide information that could help lead to exercise recommendations for children with DMD and other neuromuscular disorders.

Although there is evidence to suggest that children with DMD are, on average, less active than healthy age-matched controls, there is a lack of understanding of the mechanisms that can explain this difference (Shimizu-Fujiwara et al., 2011). The Actigraph GTX3 Accelerometer is used for exploring various aspects of PA, and is designed to measure a wide range of variables, including daily step counts, activity counts,

and activity intensities according to cut point thresholds. Established thresholds are used to determine whether the subject was engaging in activity that was considered sedentary, light, moderate, or vigorous in nature (Evanson et al., 2008). The device has been utilized in a variety of experiments in people with obesity, post-partum depression, and diabetes (Wafa et al., 2011; Sung, 2011), and has been shown to be a reliable device when investigating ambulatory activity among children with neuromuscular disease (Clanchy, Tweedy, Boyd, & Trost, 2011).

### **MRI and T2 Relaxation Times**

While PA may offer some useful information regarding this patient population, the development of magnetic resonance imaging (MRI) provides a sophisticated method of assessing the progression of muscle pathology. MRI data provides information on a number of variables including muscle composition, integrity, and magnetic resonance proton transverse relaxation time (T2). T2 relaxation times may be used as an objective, quantitative method to monitor disease activity, and may be used as a construct of damage and composition of the leg muscles (Figure 1). MRI has been performed on the upper and lower leg muscles, and was determined that, on average, T2 in patients with DMD was increased compared to that of healthy controls (Kim et al., 2010).



**Figure 1.** Figure 1 demonstrates transaxial MR images of the lower leg muscles that are representative for both a healthy control (A) and a DMD subject (B). The muscles of the lower leg are outlined in 1A. Note the pathological changes observed in the dystrophic muscles in figure 1B, relative to the homogenous presentation of the muscles of the healthy control in 1A.

Other investigators have found that T2 relaxation times with DMD subjects rose progressively with age, and they suggested that the increased T2 measurements may be due to the presence of edema within the dystrophic muscle (Wansapura et al., 2010). How each contributing factor influences the progression of muscle pathology, and consequently T2 in boys with DMD, is still unknown. Further research may provide more insight into the specific ways in which T2 and MRI could be of use in understanding the progression of muscle damage.

### **Aims and Hypothesis**

To avoid further damaging highly fragile dystrophic muscle, some researchers recommend avoiding high intensity exercise; yet, without sufficient physical activity, these boys may experience disuse atrophy in the muscles that are still present and functioning (Jansen, de Groot, van Alfen, & Geurty, 2010). In order to provide information that may eventually lead to recommended activity levels in this patient population, the following specific aims were targeted:

- 1) Compare step counts and strength measures between boys with DMD and healthy controls.
- 2) Compare functional abilities of active and inactive boys with DMD to healthy controls.
- 3) Determine the relationships between muscle damage (measured from T<sub>2</sub> weighted MRI), step count, and intensity of activity according to threshold counts.

It was hypothesized that the average daily step count for healthy controls would be higher than for boys with DMD. In terms of functional ability, the hypothesis was that healthy controls would have better functional capabilities than active boys with DMD, and that active boys with DMD would have better functional capabilities than inactive boys with DMD. It was hypothesized that there would be a parabolic relationship between muscle damage and step count, as well as with muscle damage and intensity of activity according to threshold counts. Lastly, it was hypothesized that there would be a positive linear relationship between step count and the intensity of PA according to threshold counts.

## **METHOD**

### **Participants**

A cross-sectional study design was used to assess the relationship between PA and muscle damage.

Twenty-five participants with DMD (9.0±1.8 years) and 10 controls (9.2±2.1 years) volunteered to participate. Subjects had to be able to ambulate 100 meters and ascend four steps independently. DMD was confirmed by muscle biopsies and/or genetic testing. The study was approved by the Institutional Review Board of the University of Florida, and informed written consent was obtained from the parents/guardians of the participants.

### **Procedure**

To measure activity, an Actigraph GTX3 accelerometer was worn on the hip to measure intensities of PA through count thresholds and step count. The Actigraph was to be worn for seven days, with a minimum of ten hours per day, and subjects recorded a summary of their daily activities. The first five consecutive days the monitor was worn (four weekdays and one weekend day) was used to assess daily step counts and PA according to cut point thresholds. The cut points thresholds were broken down into four distinct categories of physical intensity based on previous studies of children with neuromuscular disease: sedentary (0\_499), light (500\_1999), moderate (2000\_2900) and vigorous (3000+); (Evenson et al., 2008).

Functional ability was assessed by using the 10m walk/run test. Subjects performed this test three times, and the fastest time was recorded for analysis. For comparison of the results, DMD subjects were divided into two categories (active vs. inactive) depending on their average daily step counts. Thirteen boys above the mean were considered “active” and had a significantly higher number of steps per day compared to the twelve “inactive” boys. Strength assessments were performed using a Biodex dynamometer to determine isometric muscle strength of the knee extensors (KE) and plantar flexors (PF).

To track the disease progression, T2 weighted MRIs were performed using a whole body MRI scanner (Philips Achieva Quasar Dual 3T). Using transaxial, non-fat saturated images of the soleus (lower leg) and vastus lateralis (upper leg), T2 maps of the leg muscles were produced by using custom written software for four different echo times (40, 60, 80, 100 ms) and 3 second repetition times. For each T2 image slice, a region of interest (ROI) was selected in each muscle to determine a value (in milliseconds) that represented the average T2 relaxation time (Maillard et al., 2004). The ROIs were manually traced on

obtained T2 maps, with special care to avoid including any subcutaneous fat. The three slices with the largest cross-sectional area of the leg muscles were selected, and the average of these three slices provided the mean T2 value used in the analysis.

### **Measures**

An independent t-test was used to compare step count and strength measures between boys with DMD and controls. A one-way ANOVA was used to compare functional abilities of the 10m walk/run test for active boys with DMD, inactive boys with DMD, and controls. Qualitative assessments of various scatterplots were used in evaluating the existence of a parabolic relationship between T2 values and PA, as well as with T2 and daily step count. Pearson correlation coefficients were used to examine the linear relationships between step count and intensity of activity according to count thresholds. The level of significance was set at 0.05.

## **RESULTS**

### **Muscle Strength and Step Count**

Results indicated by the independent t-test demonstrate that peak isometric torque measures of the PFs and KEs were significantly lower in boys with DMD when compared to healthy, age-matched controls (PFs:  $33.2 \pm 9.02 \text{ft} \cdot \text{lbs}$  vs.  $74.5 \pm 25.5 \text{ft} \cdot \text{lbs}$ ; KEs:  $11.1 \pm 9.6 \text{ft} \cdot \text{lbs}$  vs.  $61.9 \pm 27.1 \text{ft} \cdot \text{lbs}$  in boys with DMD vs. controls, respectively;  $P < 0.001$ ). Average daily step count was significantly lower for boys with DMD compared to healthy controls ( $4556 \pm 1655$  vs.  $7918 \pm 2227$  in boys with DMD vs. controls, respectively;  $P < 0.001$ ).

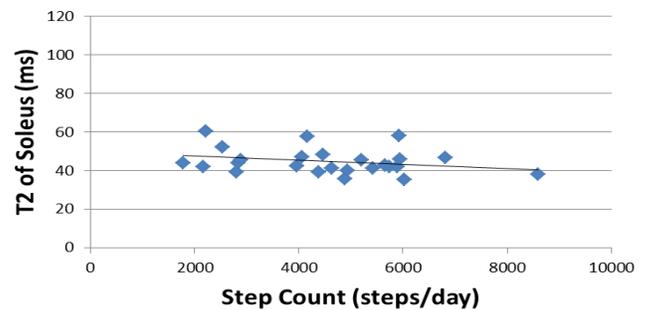
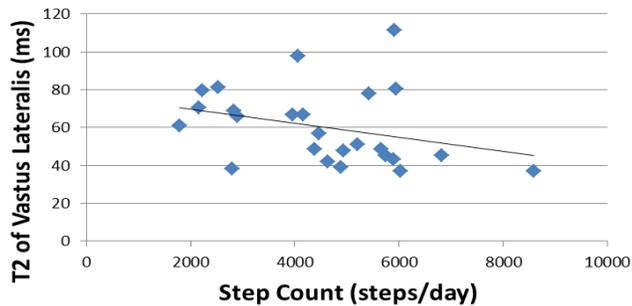
### **Functional Ability**

Participants with DMD were divided into two categories (active vs. inactive) depending on their average daily step counts. The mean daily step count

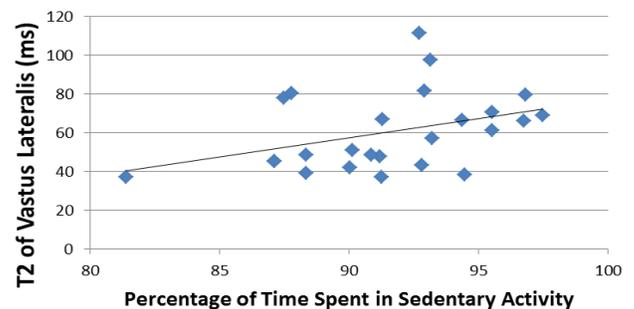
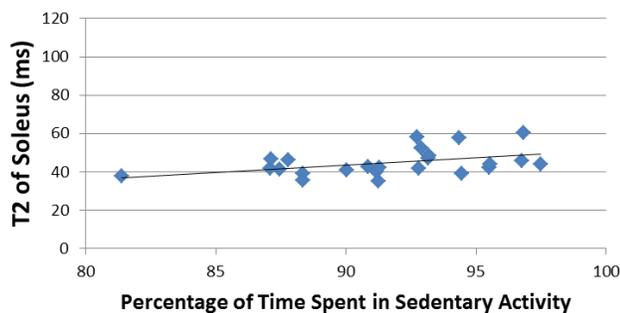
for all participants with DMD was 4556 steps/day. Thirteen boys above the mean were considered “active” and had a significantly higher number of steps per day ( $5820 \pm 1016$ ) compared to the twelve “inactive” boys ( $3187 \pm 958$ ). The amount of time to complete the 10m walk/run test was significantly higher in both active and inactive boys with DMD when compared to healthy, age-matched controls (active:  $5.8 \text{s} \pm 1.6 \text{s}$ ; inactive  $7.1 \pm 1.7 \text{s}$ ; control  $3.0 \text{s} \pm 1.2 \text{s}$ ;  $P < 0.001$ ). When comparing the 10m walk/run completion times in active and inactive boys with DMD, it was noted that, on average, active boys completed the test in a shorter amount of time than the inactive boys ( $P = 0.077$ ).

### **Muscle Damage and Energy Intensities**

The T2 values for both the soleus and vastus lateralis were significantly higher in boys with DMD compared to healthy, age-matched controls (soleus:  $44.7 \pm 6.5$  vs.  $33.5 \pm 2.2$  ms; vastus lateralis  $60.3 \pm 19.9$  vs.  $34.9 \pm 4.3$  ms in boys with DMD vs. controls, respectively;  $P < 0.001$ ). The relationship between step count and intensity of activity showed a negative linear relationship ( $r = -0.834$ ,  $P < 0.001$ ). There was a weak, negative, linear relationship between the average step count and T2 values for both the soleus and vastus lateralis ( $r = -0.278$ ;  $P = 0.178$  and  $r = -0.308$ ;  $P = 0.134$ , respectively) (Figures 2 & 3). The relationship between the time spent in sedentary activity and T2 values of both the soleus and vastus lateralis demonstrated a moderately positive, linear relationship ( $r = 0.440$ ;  $P = 0.028$  and  $0.377$ ;  $P = 0.063$ , respectively). Qualitative visual assessments of the individual scatterplots suggested that there was no clinically meaningful correlation with a parabolic relationship between the T2 values and the average daily step counts (Figures 2 & 3), or between the T2 values and the amount of time spent in sedentary activity (Figures 4 & 5).



**Figures 2 & 3.** Comparison of the average daily step count and T2 values of the leg muscles. Figure 2 (left) specifically examines the vastus lateralis (VL), and the graph demonstrates a weak, negative linear relationship ( $r = -0.308$ ,  $P=0.134$ ) between the variables. Figure 3 (right) examines the soleus (SOL) muscle and also represents a weak, negative linear relationship ( $r = -0.278$ ;  $P=0.178$ ).



**Figures 4 & 5.** Comparison of the percentage of time spent in sedentary activity and T2 values of the leg muscles. Figure 4 (left) specifically examines the vastus lateralis (VL), and the graph demonstrates a moderate, positive linear relationship ( $r = 0.377$ ,  $P=0.063$ ) between the variables. Figure 5 (right) examines the soleus (SOL) muscle and also represents a positive linear relationship ( $r = 0.440$ ,  $P=0.028$ ). Qualitative assessments of the individual scatterplots provided no correlation with a parabolic relationship between the T2 values and the percentage of time spent in sedentary activity.

## DISCUSSION

This is the first study to specifically examine the relationship between T2 values, step count, and intensity of physical activity in DMD, and the results provide novel and pertinent information regarding activity and muscle damage. Overall, the data suggest that increased daily step count may improve functional abilities, and that an increased amount of time spent in sedentary activity may lead to an increase in T2 (as a construct of muscle damage). Further research should build upon these results and specifically examine the duration, type, and frequency of activity in boys with DMD. In order to avoid exacerbating the continuously deteriorating muscle condition associated with this disease, further research should also examine new methods that may help lead to exercise prescriptions for this patient population.

Strength measures for boys with DMD were significantly lower than healthy controls. This is associated with the muscle deterioration experienced by boys with DMD once their body's reparative process becomes exhausted (Mathur et al., 2010). Also, activity levels in boys with DMD showed an increased amount of time spent in sedentary activity as compared to healthy controls. These findings were in accordance with a previous study, and suggest that young boys with DMD have significantly more inactive time each day, and have lower levels of high-intensity activities (McDonald, 2005). The inactivity may be related to physical exhaustion following normal daily activities, such as going to school or doing household chores.

Data for the 10m walk/run test was collected to provide information about the potential impact that PA poses on the status of functional ability in children

with DMD. When examining the results, a trend ( $P=0.077$ ) can be seen in the activity levels and time taken to complete the 10m walk/run, as active boys with DMD tend to complete this test in a shorter amount of time than inactive boys with DMD. Comparing different functional assessments, such as the time to climb four stairs or the six-minute walk test, with various activity levels may provide further information on this matter.

T2 measures reflect the level of muscle damage in boys with DMD and may be used as an important tool in tracking disease progression. Utilizing MRI and T2 measures in this patient population is an innovative technique that hopes to provide a non-invasive procedure for boys with DMD, while also providing accurate data for researchers. The results of this study demonstrate that as average daily step count increases, T2 values for both the soleus and vastus lateralis tend to decrease in dystrophic muscle (Figures 1 & 2). These findings may provide information for researchers to build upon in future studies to examine if PA done in a safe and therapeutic manner could be beneficial in reducing muscle deterioration in boys with DMD.

There were limitations to this study that were recognized and addressed. Completing strength measures and functional tests with a young population can be difficult, as the results depend on participant compliance and enthusiasm (McWhorter et al., 2011). To address this issue, the research team consistently provided verbal motivation to the subjects before, during, and after each strength and functional test to encourage optimal participation and cooperation from the subjects. Also, there were limitations with regards to the interpretation of the T2 MRI measurements, as the value is influenced by muscle damage/edema as well as other pathological changes in the tissue such

as fibrosis and fat infiltration. Edema has been shown to increase T2 while fibrosis has been shown to decrease T2 (Wansapura et al., 2010); therefore, it cannot be determined, precisely, how the T2 value solely correlated with muscle damage. Due to the heterogeneity and changes that occur in the dystrophic muscle, one cannot rule out the possibility that fibrosis and fat infiltration contributed to the T2 MRI values being measured in this study. Current, ongoing, and future research is aimed at utilizing MRI to distinguish between fibrosis, fat, and inflammation of dystrophic muscle with regards to the T2 value.

## CONCLUSION

The results of our study show that there is a relationship between T2 values, step count, and intensity of physical activity. As the amount of time spent in sedentary activity increases, T2 values for both the upper and lower leg muscles also increase, suggesting that a lack of activity may contribute to muscle deterioration. These findings may imply that PA could be beneficial in reducing muscle deterioration in boys with DMD, if prescribed in a safe and therapeutic fashion. The results of the 10m walk/run test may also suggest that longer periods of activity and ambulation could lessen the functional detriments common to boys with DMD. Overall, these results suggest that some level of activity could be beneficial in reducing the progression of muscle pathology, while possibly increasing ambulatory abilities in boys with DMD. The development of exercise prescriptions for this patient population may help parents of boys with DMD better instruct their children in terms of PA intensities and durations (Markert et al., 2011).

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