

Gastrocnemius medialis muscle architecture and physiological cross sectional area in adult males with Duchenne Muscular Dystrophy.

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Running Head: Muscle size in Muscular Dystrophy

Abstract

Objectives: To describe muscle size and architecture of the gastrocnemius medialis (GM) muscle in eleven adult males with Duchenne Muscular Dystrophy (DMD, age 24.5 ± 5.4 years), and a control group of eleven males without DMD (CTRL, age 22.1 ± 0.9 years).

Methods: GM anatomical cross sectional area (ACSA), volume (VOL), physiological cross sectional area (PCSA), fascicle length (Lf) and pennation angle (θ) were assessed using B-Mode Ultrasonography. GM ACSA was measured at 25, 50 and 75% of muscle length (Lm), from which VOL was calculated. At 50% of Lm, sagittal plane images were analysed to determine GM Lf and θ . GM PCSA was calculated as: VOL/Lf . The ratio of Lf and Lm was also calculated.

Results: GM ACSA at 50% Lm, VOL and PCSA were smaller in DMD males compared to CTRL males by 36, 47 and 43%, respectively ($P < 0.01$). There were no differences in Lf and θ . GM Lm was 29% shorter in DMD compared to CTRL. Lf/Lm was 29% longer in DMD ($P < 0.01$).

Conclusions: Unlike previous data in children with DMD, our results show significant atrophy in adult males with DMD, and no change in Lf or θ . The shorter Lm may have implications for joint flexibility.

Key Words: Duchenne Muscular Dystrophy, Atrophy, Gastrocnemius, Muscle Architecture, Ultrasonography.

Introduction

Duchenne muscular dystrophy (DMD) is characterised by an absence of dystrophin, a cytoskeletal protein that is normally found on the sarcolemma membrane of striated muscle ¹. Although the genetic and molecular basis of DMD is well established, and the consequent decline in physical function is well documented ², there remains no comparison of skeletal muscle morphology in adults with the condition.

Children with DMD present with a “pseudohypertrophy” of the quadriceps ³ and plantarflexors ⁴, an apparent increase in muscle size associated with an accumulation of non-contractile material. Specifically magnetic resonance imaging (MRI) scans reveal a larger Anatomical Cross Sectional Area (ACSA-the area of the muscle compartment at right angles to the longitudinal axis of the muscle), but with a composition that consists of a higher proportion of fat and connective tissue ³. Interestingly however, this increase in ACSA appears to be muscle specific, and occurs to a lesser extent in children over 11 yrs of age ⁴. At present there is only limited age matched comparisons of muscle size between DMD and unaffected counterparts; however, murine muscle with reduced dystrophin (MDX Mice) also shows a characteristic pseudohypertrophy of the limb muscles ^{5, 6}, and an associated impairment in muscle strength ⁷. In humans with DMD, there is currently no data to describe the decline in muscle strength with changes in muscle size; however in other forms of human muscular dystrophy, progression of the disease is associated with reductions in muscle size and an associated worsening of motor disability scores ⁸. It is likely therefore, considering this continuous loss of motor function in DMD, that a smaller contractile mass may be observed in adults with the condition, however there are no direct comparisons of muscle mass between adults with and without DMD.

The assessment of muscle architecture in pennate muscle allows for a more complete measure of contractile area i.e. Physiological Cross Sectional Area (PCSA) considers the pennate arrangement of fascicles within the muscle volume, whereas ACSA may underestimate the contractile area in a pennate muscle ⁹. Furthermore, measurements of fascicle length and pennation angle allows for the consideration of changes to the number of sarcomeres in series, and mechanical efficiency of the fascicles within the muscle, respectively ¹⁰. Consistent with the lack of data on muscle size in adults with DMD, there is no extant data on muscle architecture in adults with DMD. In MDX mice showing pseudohypertrophy, pennation angle is reported to be increased ⁶, but with no difference in fascicle length ⁷; a finding that is consistent with architectural adaptations following hypertrophy, secondary to resistance training ¹¹.

The medial head of the gastrocnemius (GM) muscle, is frequently investigated in regards to muscle size and architectural characteristics (e.g. Fukunaga et al. 1992), and the validity of ultrasound imaging techniques are well established in this muscle¹³. Based on previous reports in children, it is not clear whether adults with DMD will present with larger GM muscle size and architecture resulting from pseudohypertrophy (Jones et al. 1983; Mathur et al. 2010); or whether the lower levels of physical activity in DMD¹⁴, will result in a disuse related atrophy of the GM, as has been observed in otherwise healthy adult males who have experienced disuse following lower limb injury¹⁵. However, as mentioned the muscle architecture and size of the GM is unreported in adults with DMD.

Therefore, the aim of the present investigation was to compare the muscle size and architectural characteristics of the GM muscle in a group of adult males with and without DMD. We hypothesise that based on the observation that although pseudohypertrophy is observed in children with DMD, atrophy may be present in adults with the condition, as a trend towards atrophy has been reported in older children with DMD, compared to younger children⁴.

Materials and Methods

Eleven healthy young men [age: 22.1 ± 0.9 years (range 22-24 years), height: 1.84 ± 0.09 m, mass: 83.8 ± 11.3 kg, mean \pm SD] and 11 non-ambulatory men diagnosed with DMD [age: 24.6 ± 5.43 years (range 20-38 years), height: 1.67 ± 0.10 m, mass: 67.0 ± 17.6 kg] volunteered to participate in this study. All control participants (CTRL) self-reported as being recreationally active (undertaking no more than 1 hour of "moderate" physical activity per week) and not undertaking any structured training regime. Ethical approval was obtained through the Department of Exercise and Sport Science, Manchester Metropolitan University and all participants signed informed consent prior to taking part in the study. All procedures complied with the latest revision of the Declaration of Helsinki World Medical¹⁶.

Procedures

Participants were tested in a single session; the DMD group were recruited and tested at The Neuromuscular Centre (Winsford, UK) and the Control group were tested at MMU (Cheshire, UK). Ultrasound scans were recorded using a portable device for the DMD participants (MyLab25, Esaote Biomedica, Genoa, Italy) and a free standing device for the control participants (AU5 Harmonic, Esaote Biomedica, Genoa, Italy). All tests were taken from the self-reported dominant limb in all participants.

Anthropometric measures

In the control group stature and mass were measured using a wall mounted stadiometer (Harpenden stadiometer, Holtain Crymych, UK) and digital scales (Seca model 873, Seca, Germany) respectively. In the DMD group, stature was assessed using point to point (index finger, elbow, shoulder and across midline) span, and were weighed in a set of seated scales. In order to account for the known discrepancy between standing height and arm span measures, a correction was applied consistent with regression data from adult Caucasian males, the known error of making this correction is 3.5%¹⁷. Participant height is presented as this corrected value.

Muscle Volume

A real-time B-mode ultrasound was used to measure the ACSA at three points along the length of the GM muscle, from which muscle volume and PCSA were calculated. ACSA was measured using transverse plane ultrasound scans (7.5-MHz linear array probe) at 25, 50 and 75% of GM muscle length (Lm). GM Lm was measured using a tape measure over the skin as the distance from the visible origin of the GM at the posterior aspect of the femur to the distal formation of the myotendinous junction, identified using sagittal plane ultrasonography. Due to the limited mobility within the knee and ankle joint of the DMD group, all participants (DMD and Control) remained seated throughout ultrasound testing (both for volume and architecture measurement), with the dominant leg raised and supported so the hip and ankle were in alignment.

Strips of echoabsorptive tape (Transpore, 3M, USA) were placed longitudinally across the GM over the three regions of interest (25, 50 and 75% muscle length), at approximately 3.5 cm intervals. These strips of tape were used as echo absorptive markers that project a shadow onto the ultrasound image to provide a positional reference into the scanned structures. With the probe in a transverse plane, a digital recording of the probe moving from the medial to the lateral border of the GM was obtained. Consistent, minimal pressure was placed on the muscle during scanning to avoid compression of the muscle. The ultrasound was recorded in real time onto a PC at 25 frames per second (Adobe Premier pro Version 6). At each 3.5 cm interval, individual images were acquired using capturing software (Adobe Premier Elements, version 10). The shadows casted by the echo-absorptive markers allowed the images to be aligned by the contour of the muscle and the entire GM ACSA to be recreated in a single image (Adobe Photoshop Elements, version 10, Figure 1). The GM ACSA was then measured using digitising software (ImageJ 1.45, National Institutes of Health, USA). This method for using ultrasound to measure ACSA has previously been accepted as reliable and valid when compared to MRI, with a reported interclass correlation between 0.998 and 0.999, for reliability and validity respectively¹⁸.

GM muscle volume (VOL) was calculated from the three ACSA measurements by adopting the truncated cone method^{18, 19}. Three ACSA measures were taken at 25, 50 and 75% of GM muscle length (ACSA25%, ACSA50%, and ACSA75%, respectively), as previous estimates of muscle volume have been shown to be improved with multiple rather than single ACSAs²⁰. Volumes for the GM regions between 0-25% and 75-100% of GM muscle length (Lm) were calculated assuming a conical volume $VOL = 1/3 \text{ ACSA} \cdot Lm$ 0-25%. The GM volume between 25-50% and 50-75% of GM Lm were calculated using the truncated cone approach e.g:

$$Volume_{25 - 50\%} = \frac{25\% - 50\%Lm}{3} \left(ACSA_{25\%} + \sqrt{(ACSA_{50\%} \times ACSA_{25\%}) \cdot ACSA_{50\%}} \right)$$

GM VOL was then calculated as the sum of the segment volumes along the length of the GM (0-25,25-50,50-75,75-100% Lm).

Muscle architecture

Resting fascicle length (Lf) and pennation angle (θ) were measured using B-Mode, real time ultrasonography, as shown in Figure 2. A scan of the muscle fascicles was obtained for the GM along the mid-sagittal plane at the mid-distance between the proximal and distal tendon insertion and the muscle borders. The head of the probe was held perpendicular to the dermal surface to show both the superficial and deep aponeuroses and a number of clearly visible fasciculi that could be followed between each aponeuroses. Water-soluble transmission gel was placed over the ultrasound probe head to improve acoustic coupling. GM and Achilles tendon muscle-tendon unit (MTU) length was measured using sagittal plane scans to identify the distance from the GM myotendinous junction, to the insertion of the Achilles tendon into the calcaneus²¹, this distance was then added to GM Lm.

GM PCSA was estimated as GM VOL/Lf consistent with Maganaris, Baltzopoulos²².

Statistics

IBM SPSS Statistics 21 software was used to analyse the data. All data are presented as mean \pm SD. All variables showed homogeneity of variance (Levene's test, $P > 0.05$); only pennation angle violated the parametric assumption of normal distribution (Shapiro-Wilk test, $P < 0.05$), all other variables were normally distributed. Differences between the two groups [DMD and healthy controls] were analysed using an independent Students t-test. However, as pennation angle failed to satisfy a normal distribution, differences between the two groups were analysed using an independent Mann-Whitney U test.

In order to assess the impact of height as a covariate, ANCOVA was performed following a regression analysis between all dependent variables and stature.

The critical level of statistical significance was set at 5%.

Results

DMD participants demonstrated significant differences in height (-9.0 %) and mass (-20.0 %) compared to CTRL participants ($P < 0.05$). There was no difference in the age of the participant groups.

GM ACSA was 36.3% smaller in the DMD group compared to the CTRL participants at 50% of GM Lm, ($P < 0.01$, Figure 3). There was no significant difference in GM ACSA between DMD and CTRL participants at 25% or 75% of GM length (Figure 3).

The GM length was 28.8% shorter in the DMD participants ($P < 0.01$, Table 1), and the GM muscle-tendon unit length was 10.3% shorter in the DMD participants compared to control participants ($P < 0.01$, Table 1). The relative contribution of the GM muscle to the MTU length as a whole was significantly less in DMD ($38.1 \pm 3.8\%$) compared to control participants ($47.7 \pm 3.4\%$, $P < 0.01$, Table 1).

GM muscle architectural parameters Lf and θ were not significantly different between the two participant groups (Table 1). However, Lf relative to Lm was 29.4% longer in the DMD participants compared to the control group (Table 1, $P < 0.01$).

GM Volume was 47.0% smaller in the DMD group compared to the CTRL group ($P < 0.01$, Table 1). GM PCSA was 43.1% smaller in the DMD participants compared to the control participants (Table 1, $P < 0.01$).

ANCOVA was performed on the four dependent variables that achieved a significant correlation with participant height (Table 2). All four variables (GM ACSA50%, GM VOL, GM PCSA and GM Lm) remained significantly different between the DMD and CTRL participants when height was included as a covariable ($P < 0.05$).

Discussion

The main findings from this study have shown that adult males with DMD demonstrate significantly smaller GM muscle size (ACSA50%, VOL and PCSA) than age matched males without muscular dystrophy. Furthermore, the adult males with DMD showed no significant differences in absolute GM fascicle length or pennation angle, but a significantly smaller GM muscle length, and as a result, a larger GM fascicle: muscle length ratio.

In the present study, all measures of GM muscle size (ACSA50%, VOL and PCSA) were significantly smaller in DMD compared to controls. This represents the first data on muscle size from an adult population with DMD, and surprisingly, is the first to report atrophy in adults with DMD. This observation of smaller muscle size is in contrast to previous data that has consistently reported larger muscle size in children ³ and MDX mice ^{5, 7}. In children, the “pseudohypertrophy” of DMD muscle is attributed to the accumulation of fat and connective tissue within the muscle compartment ³. Although speculative at present, the current observation of a smaller muscle size in adults with DMD is consistent with the stem cell exhaustion model of muscle regeneration in mdx mice ²³. The previous reports of pseudohypertrophy in children with DMD is likely to correspond with a period of elevated inflammatory cell infiltration ²⁴, cycles of muscle cell degeneration and regeneration in their remaining muscle ²³, and corresponds with an accumulation of fat and non-contractile material within this muscle ²⁵. As the disease progresses with age, there is evidence of muscle wasting in adult mice, which corresponds with a decline in muscle stem cell regenerative capacity ²³. It is possible therefore, that the smaller muscle size observed in the GM in the present study represents the progression of the dystrophic condition, rather than an anomaly when compared to the pseudohypertrophy observed in children with DMD, where the atrophying muscle is offset by the elevated inflammatory response and accumulation of non-contractile material ³.

The architectural data obtained from the present study was unexpected, but as with muscle size there are no comparable data from adult population groups with DMD. In MDX mice, consistent with the fascicle data from the present study, fibre length appears to be unaltered from healthy age matched mice ⁷. It is possible that the pseudohypertrophy from the MDX models contributes to both the maintained fascicle length and the higher pennation angles reported from MDX data ⁶, but not the adult humans in the present study. Indeed, in the present study, there is apparent proportionate atrophy whereby architectural parameters are maintained in the presence of a smaller muscle size and length.

Although there is no known extant data from adults or children with muscular dystrophy, the present observation of no difference in GM Lf or θ , appears to be at odds with data from disuse atrophy in adult humans. Given the known relationship between in series sarcomeres and muscle length, it would be hypothesised that both muscle length and fascicle length would decline in unison²⁶. In contrast, here we have observed a relative shortening of the GM muscle length, with no change in Lf and a relatively longer GM tendon. Similarly, based on previous observations from disuse atrophy, both Lf and θ would be expected to reduce in accordance with a decrease in muscle volume¹⁵. It is likely therefore, that the present observations are as a result of the seated position imposed from the dystrophic condition, where participants have an almost constant knee- and dorsi- flexed posture. It is known that sustained immobilisation with the MTU in a shortened position can result in a shortening of MTU length²⁷ consistent with the observations from the present adult males with DMD. However, in contrast with disuse data¹⁵, the anomaly of GM length being shorter, with no difference in Lf, may be explained by the biarticular nature of this muscle; whereby a knee flexed position may result in a shortening stimulus from the proximal origin, with the extended gastrocnemius tendon absorbing the stretch influence from the seated dorsiflexed ankle posture. Indeed, Herbert and Crosbie²⁷, reported a relative shortening of proximal fascicle length, in the presence of lengthening of the distal fascicles within the shortened MTU (albeit non-significantly over the 14 day immobilisation period).

The speculative nature of the mechanisms of adaptation we have described from these initial observations in the GM highlights the paucity of data at the *in vivo* level in humans with DMD. When compared with the only other published data comparing muscle mass in children with and without DMD^{3, 4}, our findings from the adult GM, suggest that at some point in development there is a transition from pseudohypertrophy to atrophy, likely corresponding with the progression of the dystrophic participants to powered wheel chairs, and a sustained seated posture. However, this remains to be observed experimentally. Considering the progression of pseudohypertrophy towards atrophy in DMD, it should be noted that three of the DMD participants included within our data demonstrated GM ACSA at 25, and 75% of muscle length larger than means for both CTRL and DMD participants. For example, compared to the controls these three DMD participants had 13% larger GM ACSA, in contrast to the remaining DMD participants who's GM ACSA at the equivalent muscle length was 43% smaller. Future research should therefore consider the progression of muscle atrophy into adulthood within DMD.

In the present study, height was 9% less in adult males with DMD compared to controls. This has been consistently reported in children with DMD. For example, others²⁸⁻³⁰ have reported

significantly shorter stature in boys with the dystrophic condition compared to age matched counterparts. In order to account for height as a covariable, ANCOVA was performed, and although height did have a significant correlation with ACSA50%, VOL, PCSA and Lm; the groups differences persisted when included as a covariable. This would suggest that although associated with measures of muscle size, accounting for height differences between the groups does not entirely account for differences in muscle mass between the participants with and without muscular dystrophy in the present study.

The clinical significance of changes in muscle architecture are often discussed considering the functional implications associated with contractile properties of the muscle¹⁰. Given the inability of the adult Duchenne GM muscle to produce measurable forces, it is irrelevant to discuss the functional significance of the present architectural findings as they pertain to muscle contraction, and the production of contractile force. It is of greater relevance perhaps, to consider the implications for joint range-of-motion in DMD. Children with DMD experience a loss of joint range of motion³¹, and undertake surgical interventions for this loss of flexibility in the lower limb³²; these previous observations on a loss of range of motion may be consistent with our observations of a shortened MTU in adult males with DMD, however this remains to be shown experimentally. As previously mentioned, there remains no data on the architectural characteristics from the muscles of adults with DMD. Our observations of a shortening of the MTU, and the GM muscle specifically may provide the first experimental observations of a shortening within the MTU. However, there are no *in vivo* measures of passive stiffness from the Duchenne MTU or the contribution of the altered muscle mass or the in series elastic components of the MTU to limitations in flexibility from a dystrophic population, similar to those we have made in other neuromuscular conditions e.g.³³. Furthermore, the smaller GM muscle mass reported in the DMD adults in the present study is likely to have implications for resting energy expenditure and daily calorific requirements. If the reported atrophy from the present study is indicative of other muscles around the body, this would significantly reduce the daily energy requirements of adults with DMD beyond those expected from a lack of physical activity, consistent with observations from children with DMD³⁴.

In conclusion, we have observed an atrophy in the GM muscle of adult males with DMD that is indicative of the progressive nature of the condition rather than being at odds with previous data reporting pseudohypertrophy in children with DMD. The architectural observations of a shortening in GM muscle length, may provide a mechanism that contributes to the loss of joint range of motion in children with the condition. However, it is apparent that as the first descriptive data from adult

muscle with DMD, a more complete picture of the longitudinal impairments to skeletal muscle is needed from this population.

Acknowledgements: Mr J Smith received a postgraduate studentship from Dream it, Believe It, Achieve it (Northwich, UK. Registered Charity Number: 1153116).

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Tables

Table 1. Gastrocnemius Medialis (GM) muscle morphology and architecture from adult males.

	DMD	Control
GM Lm (cm)	18.6 ± 1.4*	26.2 ± 2.6
MTU Length (cm)	49.1 ± 3.9*	54.7 ± 2.6
GM Volume (cm ³)	124 ± 57 *	234 ± 49
Lf (cm)	5.33 ± 0.72	5.77 ± 1.02
θ (deg)	27.0 ± 7.27	24.9 ± 6.5
GM Lf/Lm	0.29 ± 0.04 *	0.22 ± 0.04
GM PCSA (cm ²)	23.5 ± 11.1*	41.3 ± 9.6

Values are means ± SD. * P < 0.01 significant difference from controls.

DMD, Duchenne Muscular Dystrophy; Lf, fascicle length; θ, fascicle pennation angle; Lm, muscle length; MTU, muscle-tendon unit; PCSA, Physiological cross sectional area.

Table 2. Regression analysis for variables demonstrating a significant correlation with height

	r	P
GM ACSA50%	0.454	0.034
GM VOL	0.563	0.006
GM PCSA	0.440	0.04
GM Lm	0.689	0.001

Gastrocnemius Medialis (GM) Anatomical Cross sectional area at 50% of muscle length (ACSA50%), Volume (VOL), Physiological Cross Sectional area (PCSA), and muscle length (Lm).

Figures

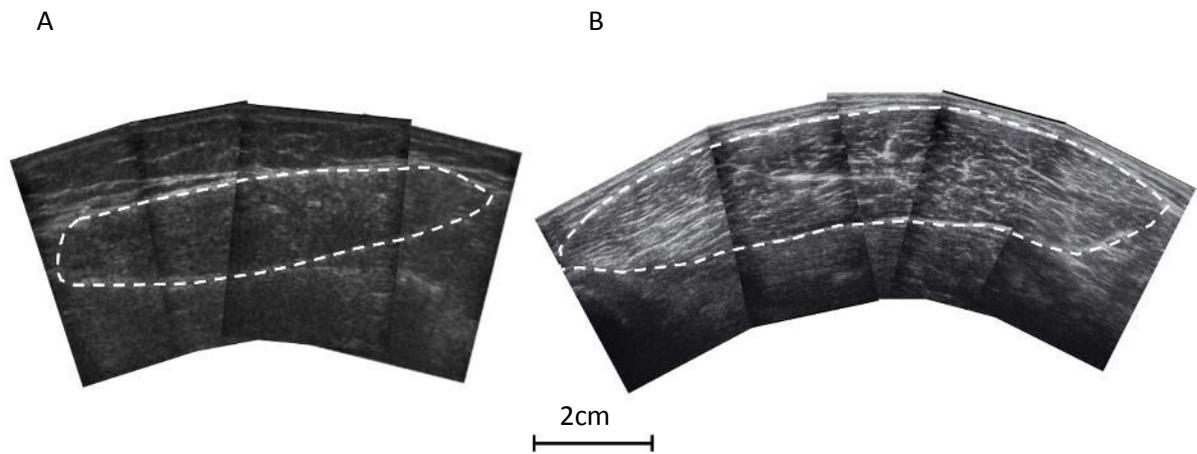


Figure 1: An example of the reconstructed GM ACSA at 50% of muscle length in a participant with DMD (A) and a control participant (B).

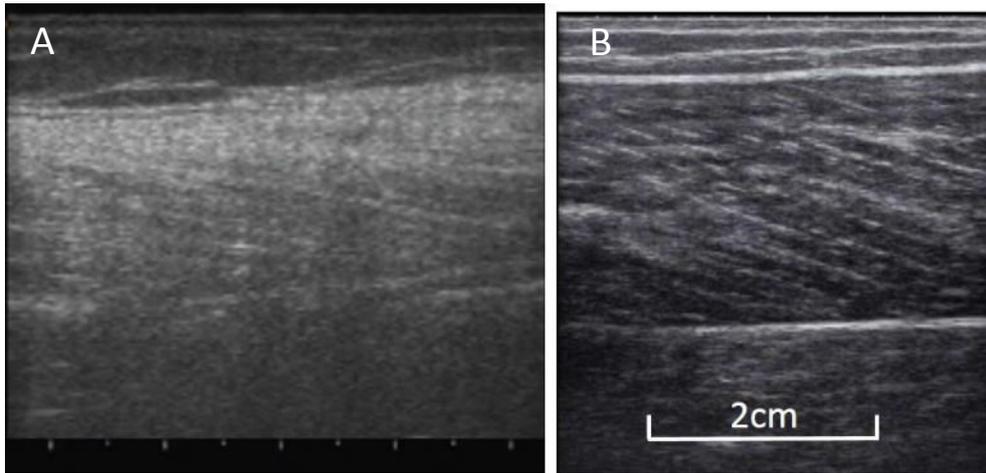


Figure 2: An example of a sagittal plane ultrasound scan at 50% of GM length in an individual with DMD (A) and a control participant (B).

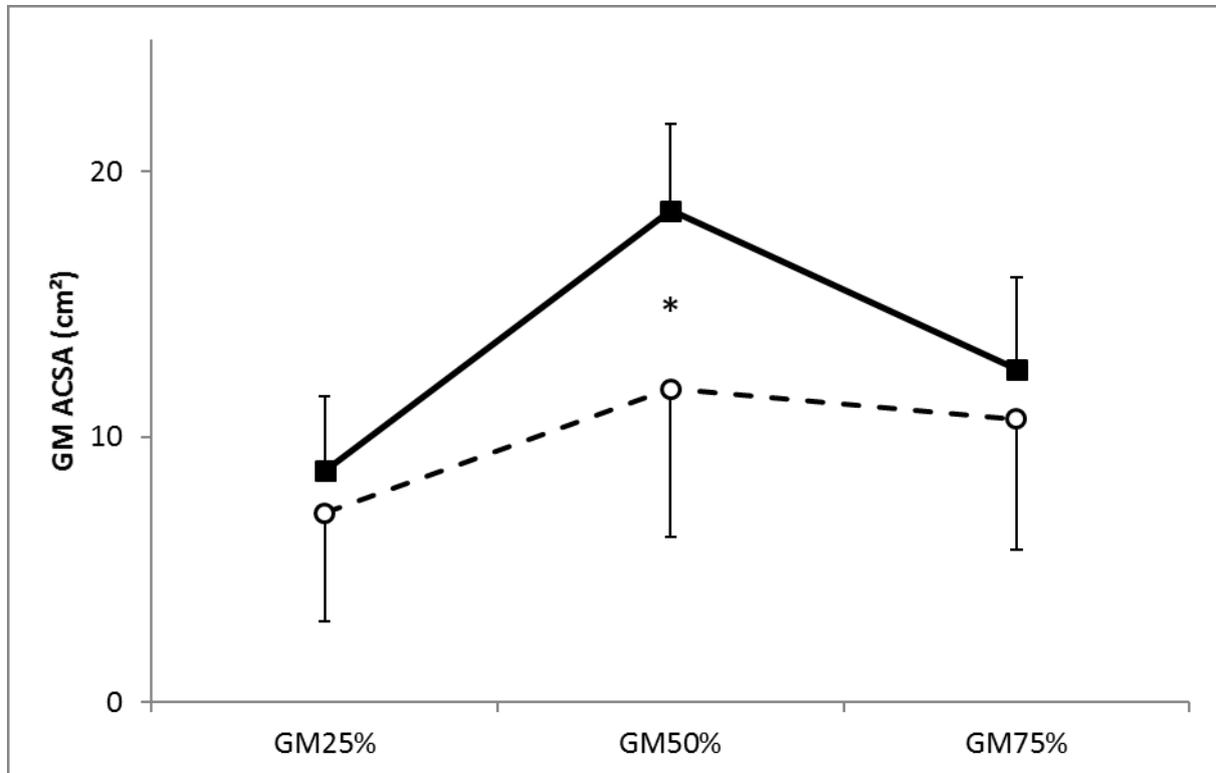


Figure 3: Gastrocnemius medialis Anatomical Cross Sectional Area (GM ACSA) at 25, 50 and 75% of GM length from origin. Adult males with Duchenne Muscular Dystrophy are denoted with open circles, and the control participants are denoted with closed squares. * denotes significant difference from the control group (P<0.05).