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Reductions in functional muscle mass and ability to ambulate in Duchenne muscular dystrophy from ages 4 to 24 years

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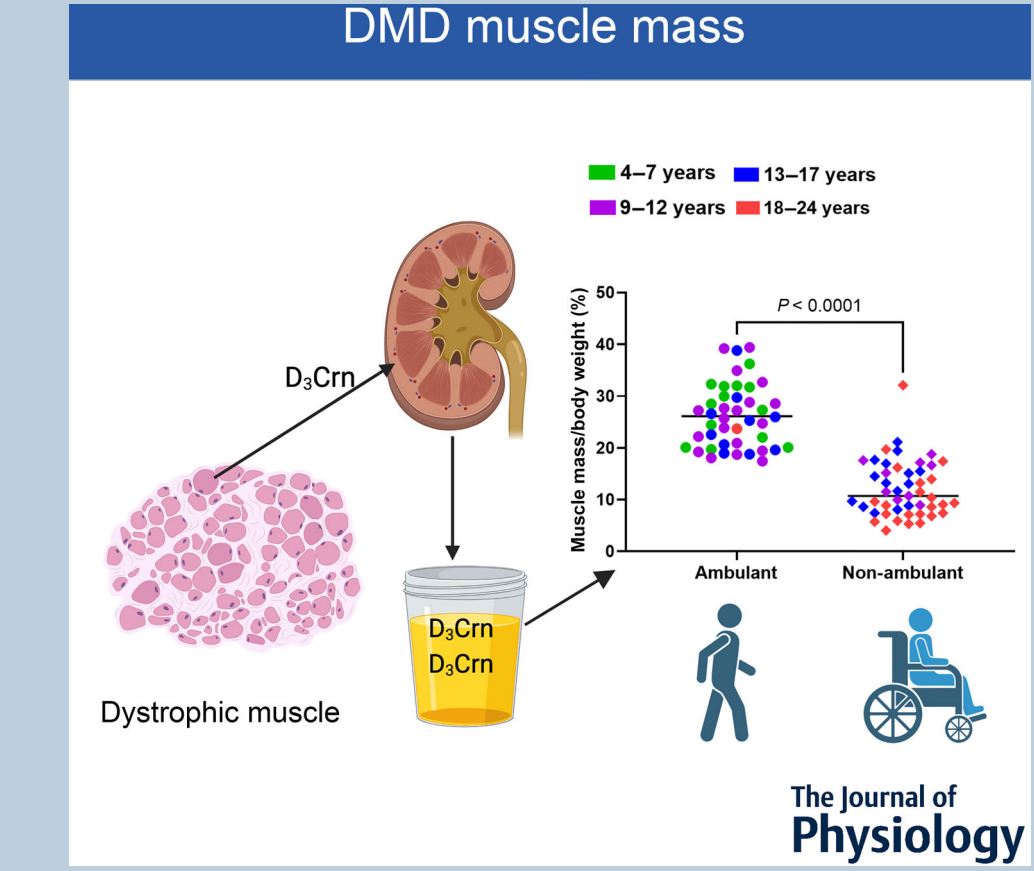

Abstract Duchenne muscular dystrophy (DMD) results in a progressive loss of functional skeletal muscle mass (MM) and replacement with fibrofatty tissue. Accurate evaluation of MM in DMD patients has not previously been available. Our objective was to measure MM using the D_3creatine (D_3Cr) dilution method and determine its relationship with strength and functional capacity in

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patients with DMD over a wide range of ages. Subjects were recruited for participation in a 12 month, longitudinal, observational study. Here, we report the baseline data. A 20 mg dose of D_3Cr dissolved in water was ingested by 92 patients with DMD (ages 4–25 years) followed later with a fasting urine sample. Enrichment of D_3creatinine was determined by liquid chromatography-mass spectrometry analysis. The North Star Ambulatory Assessment (NSAA) total score was determined for ambulatory participants, and the Performance of Upper Limb (PUL 2.0) total score and grip strength for all participants. We observed a significant age-associated increase in body weight along with a substantial decrease in MM/body weight (%MM). MM and %MM were associated with PUL score ($r = 0.517$, $P < 0.0001$ and $r = 0.764$, $P < 0.0001$ respectively). The age-associated decrease in MM and %MM was strongly associated with ambulatory status. We observed very little overlap in %MM between ambulant and non-ambulant subjects, suggesting a threshold of 18–22% associated with loss of ambulation. MM is substantially diminished with advancing age and is highly related to clinically meaningful functional status. The D_3Cr dilution method may provide a biomarker of disease progression and therapeutic efficacy in patients with DMD or other neuromuscular disorders.

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Abstract figure legend D_3creatine (D_3Cr) dilution was used to non-invasively determine total body muscle mass (MM) in subjects with Duchenne muscular dystrophy. After oral ingestion of urinary D_3creatinine (D_3Crn) enrichment is measured and used for the calculation of MM. A clear difference in MM/body weight was observed between ambulant and non-ambulant subjects with a potential threshold for loss of ambulatory ability of close to 20%.

Key points

- The non-invasive D_3creatine dilution method provides novel data on whole body functional muscle mass (MM) in a wide range of ages in patients with DMD and reveals profoundly low functional MM in older non-ambulant patients.
- The difference in %MM between ambulant and non-ambulant subjects suggests a threshold for loss of ambulatory ability between 18 and 22% MM.
- The data suggest that as functional MM declines with age, maintaining a lower body weight may help to conserve ambulatory ability.

Introduction

Individuals with Duchenne muscular dystrophy (DMD) experience a progressive loss of muscle strength, functional capacity and functional muscle mass (MM) along with an accumulation of fibrosis and fat in skeletal muscle (Liu et al., 1993). Magnetic resonance imaging (MRI) has characterized these changes with DMD and shown an increasing proportion of non-contractile tissue in skeletal muscle that is associated with diminished strength and function (Akima et al., 2012) with a large degree of heterogeneity among individual muscle groups (Chrzanowski et al., 2017). MRI provides estimates of regional muscle size, but unless whole-body MRI

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is performed, it does not provide an assessment of whole-body functional MM.

The D_3creatine (D_3Cr) dilution method measures total body creatine pool size, about 98% of which is sequestered in skeletal muscle (Fitch & Shields, 1966; Fitch et al., 1968). By this method, an oral tracer dose of D_3Cr is absorbed and transported against a large concentration gradient into muscle cells. Creatine is turned over in muscle through the non-enzymatic and physically constant conversion of creatine to creatinine, which is rapidly excreted into urine. The D_3 enrichment of urinary D_3creatinine (D_3Crn) thereby reveals the enrichment of intramyocellular D_3Cr and thereby the dilution of administered D_3Cr by endogenous creatine, providing a non-invasive measurement of total body creatine pool size and MM. Importantly, creatine and phosphocreatine are collocated with the contractile components of muscle (Hill, 1962). As a result, the measurement of total body creatine provides a measure of 'functional' MM that is undiluted by fat and fibrotic tissue. This method has been previously validated in rodents (Stimpson et al., 2012, 2013), adult humans (Clark et al., 2014; Shankaran et al., 2018) and premature infants (Evans et al., 2020). In older people, reduced D_3Cr MM is strongly related to strength and functional capacity, and prospectively identifies risk of disability, hip fracture and mortality (Cawthon et al., 2019, 2020, 2022). We recently reported findings in 10 subjects with DMD, ages 7–17 years, and showed that the D_3Cr dilution method provides a non-invasive measurement of functional MM. Compared to healthy, age-matched controls, individuals with DMD showed a strikingly lower muscle mass (Evans et al., 2021). In addition, we observed no relationship between dual-energy X-ray absorptiometry (DXA) lean mass and MM in subjects with DMD.

In the present study, we extended our assessment of functional MM and its relationship to strength and functional status, including ambulation, in a broad age range (4–25 years) of subjects with DMD.

Methods

Ethical approval

This study was approved by the Duke University Institutional Review Board (Pro00107809-INIT-1.0), University of Utah Institutional Review Board (IRB 00140851), and Newcastle University Institutional Review Board (HRA and HCRW approval IRAS # 297878). Parents and/or adult subjects signed an approved informed consent form and all subjects provided assent. A total of 92 subjects (4–24 years old) with DMD were enrolled in a 12 month longitudinal, observation study. This study was performed in compliance with recognized international standards, including the International Conference on Harmonization (ICH), the Council for International Organizations of Medical Sciences (CIOMS) and the principles of the *Declaration of Helsinki*. The study conformed to the standards set by the *Declaration of Helsinki*, except for registration in a database. All procedures and measurements described below were repeated on all subjects 6 and 12 months after baseline. Here, we describe the baseline data. Subjects participated in this study during normally scheduled clinic visits. Out of the 92 subjects enrolled, 48 (52%) were non-ambulant. In total, 79 of the subjects were receiving steroid treatment: Deflazacort ($n = 35$), Prednisone ($n = 24$), Prednisolone ($n = 14$) and Vamorolone ($n = 6$). Subject characteristics are shown in Table 1.

Patients and families were contacted with details of the study and provided a consent form prior to a scheduled clinic visit. After a physical examination, each subject was provided and ingested the dose of D_3Cr. During this visit, all functional tests were performed, as described below. Subjects (families) were provided instructions for collection of two urine samples at home at least 48 and 72 h after ingestion of the dose of D_3Cr. During this period whole body D_3Cr and urinary D_3Crn are at isotopic steady state (enrichments are equivalent). Urine samples were collected in the morning after an overnight fast (from 20.00 h the previous night) prior to eating breakfast or any food. Subjects were also provided an extensive list of creatinine-free foods to consume *ad libitum* in the evening after dinner.

D_3Cr dosing and sample collection

All subjects drank 20 mg of D_3Cr dissolved in 5 ml H_2O. After swallowing the dose, the vial was filled with distilled water and the rinse was also ingested to ensure that the entire D_3Cr dose was consumed. Fasting urine samples were collected at home on a strip of filter paper on days 2 and 3 after ingestion of D_3Cr and stored in a freezer. Frozen urine samples were brought to the clinic in a cooler with ice at each subject's subsequent clinic visit. All samples were stored at −20°C for shipment to the University of California, Berkeley Laboratory for analysis.

Assessment of urinary D_3Cr enrichment was completed by liquid chromatography-mass spectrometry (LC-MS/MS). Then, 100 µl aliquots of urine extracted from filter paper strips were processed for Cr/Crn concentration and D_3Crn enrichment. Mass spectrometry was performed on a Sciex 6500 QTRAP operating in the multiple reaction monitoring (MRM) mode. For determination of the enrichment of D_3Crn, each sample was prepared twice to inject separately for enrichment and concentration with duplicate injections on the LC/MS. The enrichment 9-point standard curve ranged from 0 to 0.6% and all the samples were within this

Table 1. Mean body weight, BMI, Cr/Crn ratio, MM and %MM by age

Age (years)	BMI (kg/m^2)	Body weight (kg)	Cr/Crn ratio	Muscle mass (kg)	Per cent muscle mass (%)
4–7 years (n = 13)	19.1 ± 5.3 (n = 13)	25.9 ± 10.7 (n = 13)	0.7 ± 0.4 (n = 13)	6.8 ± 2.2 (n = 13)	27.4 ± 5.6 (n = 13)
9–12 years (n = 29)	27.0 ± 7.7 (n = 28)*	49.4 ± 19.1 (n = 28)*	0.7 ± 0.5 (n = 28)†	10.0 ± 3.1 (n = 29)*†	22.2 ± 8.2 (n = 28)†
13–17 years (n = 25)	28.2 ± 6.8 (n = 25)*	61 ± 20.4 (n = 25)*	1 ± 0.8 (n = 25)†	10.1 ± 3.3 (n = 25)*†	17.9 ± 7.6 (n = 25)*†
18–24 years (n = 25)	24.8 ± 5.7 (n = 19)	62.6 ± 22.1 (n = 24)*	2.2 ± 1.4 (n = 24)*	6.3 ± 3.0 (n = 25)	11.1 ± 6.7 (n = 24)*
ANOVA P-value			$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
4–7 years *vs.* 9—12 years	$P = 0.0016$ $P = 0.0048$	$P < 0.0001$ $P = 0.0028$	$P = 0.9942$ $P = 0.6049$	$P = 0.0098$ $P = 0.0109$	$P = 0.1570$ $P = 0.0015$
4–7 years *vs.* 13–17 years	$P = 0.0012$ $P = 0.0986$	$P < 0.0001$ $P < 0.0001$	$P < 0.0001$ $P = 0.6112$	$P = 0.9648$ $P > 0.9999$	$P < 0.0001$ $P = 0.1463$
4–7 years *vs.* 18–24 years	$P = 0.9298$ $P = 0.7094$	$P = 0.1426$ $P = 0.0773$	$P < 0.0001$ $P < 0.0001$	$P = 0.0001$ $P = 0.0002$	$P < 0.0001$ $P = 0.0083$
9–12 years *vs.* 13–17 years	$P = 0.3889$	$P = 0.9911$			
9–12 years *vs.* 18–24 years					
13–17 years *vs.* 18–24 years					

*$P < 0.05$ from 4–7-year-olds.
†$P < 0.05$ from 18–24-year-olds. One-way ANOVA P-values as well as adjusted P-values of pairwise Tukey's test corrected for multiple comparisons are shown.

range. Enrichment is measured by MRM transitions (116.1/46.1) corresponding to the M2 peak of Crn and 117.1/47.1 which corresponds to D_3Crn. Samples were run in duplicate and average values are reported. Samples with coefficients of variance (CVs) greater than 10% were subjected to re-analysis. The total body Cr pool size was calculated as retained (delivered) D_3Cr dose divided by D_3Crn enrichment and MM as described previously (Clark et al., 2014). Triplicate analyses were performed on all urine samples.

Creatine pool size was calculated as previously described (Clark et al., 2014) using the following formula:

$$\text{Creatine pool size} = \frac{(131.1/134.1) \times \text{amount of } D_3\text{—creatine dosed } (20\text{ mg})}{(\text{steady} - \text{state } D_3\text{—creatinine enrichment } (\text{from urine sample})}$$

where 131.1/134.1 is the ratio of the molecular weights of unlabelled creatine to D_3Cr.

We used a value of 4.3 g of Cr/kg of muscle to calculate a value for MM. The 4.3 g/kg wet weight is derived from rodents and humans (Kreisberg et al., 1970; Meador et al., 1968) and is based on an average estimated muscle fibre composition of 50% type I and II fibres; %muscle mass = muscle mass/body weight.

Functional assessments

The North Star Ambulatory Assessment (NSAA) (Mazzone et al., 2009) was measured in ambulant subjects, and the Performance of Upper Limb (PUL) v.2.0 (Mayhew et al., 2020) and grip strength in both upper extremities was measured in all subjects.

D_3Cr dilution

We have previously reported that a small amount of the orally ingested D_3Cr is 'spilled' into urine in some human subjects (Shankaran et al., 2018). As a result, an algorithm has been created to correct for D_3Cr loss in urine using

the urine creatine/creatinine ratio measured in the fasting condition (Shankaran et al., 2018). However, such an algorithm to determine potential spillage in patients with DMD is not available. The urine creatine/creatinine ratio was substantially higher in older subjects (Table 1) than that seen in the younger subjects due to elevated urine creatine levels in the older subjects. The determination of MM data presented in this paper did not use an algorithm to determine potential spillage of the D_3Cr dose. The net effect of not including a correction for potential D_3Cr spillage is discussed below.

Statistics

Body weight, body mass index (BMI), creatine/creatinine ratio, MM and %MM were compared across four age groups of subjects (4–7, 9–12, 13–17 and 18–24 years) by one-way ANOVA followed by Tukey's *post hoc* test corrected for multiple pair-wise comparisons. Pearson correlation analysis was performed across various pairs of measurements, including age, body weight, MM, %MM, NSAA, PUL and grip strength. Age, body weight, MM and %MM were compared between ambulant and non-ambulant subjects by an unpaired *t* test. All statistical analyses were performed using GraphPad Prism 10 software and considered significant at $P < 0.05$.

Results

We observed a striking age-related reduction in MM and %MM when comparing DMD subjects of different age groups (Table 1). MM in the oldest group (18–24 years) was significantly lower than that of the 9–17 year cohort and similar to that of the youngest cohort (4–7 years old). %MM in the 18–24 year cohort was significantly lower than that of all other age cohorts, and %MM of the 13–17 year cohort was significantly lower than that of the 4–7 year cohort. There was a significant negative correlation ($r = -0.6258$, $P < 0.0001$) between %MM and age of DMD subjects (Fig. 1). Age, body weight, MM and %M were significantly different between ambulant and non-ambulant subjects (Fig. 2). Despite a 55% higher body weight in non-ambulant subjects, %MM was 54% lower than that of ambulant subjects. While the total body MM was significantly different, there was considerable overlap between the two groups. However, there was very little overlap in %MM between these two groups (ambulant and non-ambulant subjects).

We assessed the relationships between body weight, MM, %MM and functional assessments (PUL2.0, NSAA and grip strength) in subjects with DMD and compiled a correlation matrix of all measured parameters (Table 2). As can be seen in Fig. 3*A*, for all subjects, we observed a strong positive relationship between total PUL total score and %MM ($r = 0.7642$, $P < 0.0001$) (12 subjects scored a maximum of 42 total points). There was also a significant positive correlation between NSAA total score and %MM ($r = 0.6143$, $P < 0.0001$) in 43 ambulatory subjects (Fig. 3*B*). As shown in Table 2, there was a significant relationship of age with body weight and MM. There was a positive relationship between total PUL score and MM ($r = 0.5170$, $P < 0.0001$) and a significant negative relationship ($r = -0.4503$, $P < 0.05$) between rise from floor time and MM for the 26 subjects who were able to complete this test. We also observed a positive relationship between grip strength (both hands) and MM [$r = 0.6163$ (right) and 0.6154 (left), $P < 0.0001$] as well as grip strength *vs.* %MM [$r = 0.3319$ (right) and 0.3251 (left), $P < 0.005$].

Discussion

Here we describe, for the first time, the use of the D_3Cr dilution method to measure total body MM in a wide range of ages in subjects with DMD, including many who were older and non-ambulant. We observed a decrease in functional MM and a striking reduction in %MM with advancing age that was strongly associated with functional status. Although there was a statistically significant difference between ambulant and non-ambulant subjects in age (oldest ambulant subject was 18 years old, and the youngest non-ambulant boy was 9 years old), body weight and total muscle mass, importantly, there was very little overlap in %MM between ambulant and non-ambulant subjects. This finding suggests a critical amount of muscle per kg of body weight that is necessary for standing and walking, leading to loss of ambulation (LOA). Previous research in DMD patients has demonstrated that peak score on the NSAA was associated with older age at LOA (Zambon et al., 2022). The present data support the importance of strength and function to prevent LOA. Our data show that the trans-

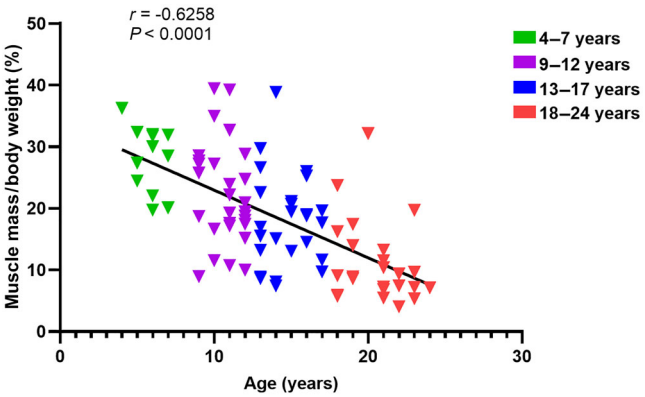

Figure 1. % muscle mass vs age
Negative relationship between %MM and age ($r = -0.6491$, $n = 84$).

Table 2. Correlation matrix of age, body weight, muscle mass, muscle mass/body weight (%) and functional assessments

	Age	Body weight (KG)	Muscle mass (KG)	%MM	Pul total score	Right hand grip strength (KG)	Left hand grip strength (KG)	NSAA Total Score	NSAA 10 M Run time (S)
BODY WEIGHT (KG)	$r = 0.5210$ $P < 0.0001$								
MUSCLE MASS (KG)	$r = -0.1607$ $P = 0.1259$	$r = 0.2549$ $P = 0.0153$							
%MM	$r = -0.6258$ $P < 0.0001$								
PUL TOTAL SCORE	$r = -0.7081$ $P < 0.0001$	$r = -0.4208$ $P < 0.0001$	$r = 0.5170$ $P < 0.0001$	$r = 0.7642$ $P < 0.0001$					
Right HAND GRIP STRENGTH (KG)	$r = -0.2134$ $P = 0.0472$	$r = 0.0786$ $P = 0.4748$	$r = 0.6163$ $P < 0.0001$	$r = 0.3319$ $P = 0.0019$	$r = 0.5676$ $P < 0.0001$				
LEFT HAND GRIP STRENGTH (KG)	$r = -0.2117$ $P = 0.0503$	$r = 0.0937$ $P = 0.3965$	$r = 0.6154$ $P < 0.0001$	$r = 0.3251$ $P = 0.0025$	$r = 0.5747$ $P < 0.0001$	$r = 0.9555$ $P < 0.0001$			
NSAA TOTAL SCORE	$r = -0.2996$ $P = 0.0482$	$r = -0.4307$ $P = 0.0039$	$r = -0.1683$ $P = 0.2749$	$r = 0.6143$ $P < 0.0001$	$r = 0.6946$ $P < 0.0001$	$r = 0.0726$ $P = 0.6517$	$r = 0.1223$ $P = 0.4462$		
NSAA 10 M RUN TIME (S)	$r = -0.0034$ $P = 0.9854$	$r = 0.1910$ $P = 0.2950$	$r = -0.0197$ $P = 0.9148$	$r = -0.2492$ $P = 0.1690$	$r = -0.5401$ $P = 0.0021$	$r = -0.2373$ $P = 0.1985$	$r = -0.2381$ $P = 0.1971$	$r = -0.7828$ $P < 0.0001$	
NSAA RISE FROM FLOOR TIME (S)	$r = -0.3844$ $P = 0.0525$	$r = -0.2466$ $P = 0.2246$	$r = -0.4503$ $P = 0.0210$	$r = -0.2038$ $P = 0.3181$	$r = -0.5161$ $P = 0.0098$	$r = -0.4948$ $P = 0.0119$	$r = -0.4833$ $P = 0.0144$	$r = -0.7777$ $P < 0.0001$	$r = 0.7639$ $P < 0.0001$

ition from ambulant to non-ambulant appears to occur at a relative MM between 18 and 22%. Longitudinal measurements in ambulant patients in this range of %MM may provide more definitive information on the importance of relative MM for ambulation. However, these data suggest that maintaining a lower body weight in the face of diminishing muscle may help to preserve ambulation. The assessment of longitudinal changes in MM in ambulant DMD patients with %MM close to 20% may help to further define this relationship. MM corrected for body weight provides a comparator for all subjects based on body weight. Previous research in a cohort of older men similarly demonstrated a threshold of %MM that was associated with mobility disability (Zanker et al., 2020). Remarkably, several of the non-ambulant subjects had a relative MM of less than 10% of body weight. The data presented here are the baseline values of a 1 year longitudinal examination of changes in muscle mass, strength and function. There are no data that describe muscle mass in healthy children for comparison to the present data, but our results are in stark contrast to that reported age-associated increases in fat free mass (FFM) in children (McCarthy et al., 2014).

MRI images of skeletal muscle in patients with DMD are characterized by not only a decreased muscle size with advancing age, but also by markedly increasing amounts of connective tissue and fibrofatty replacement (Akima et al., 2012; Barnard et al., 2020). However, these assessments are difficult to obtain and do not convey any information with regard to muscle function. The PUL assessment was developed specifically to determine upper extremity function in both ambulant and non-ambulant patients with DMD (Han et al., 2016; Mayhew et al., 2020) and provides a functional score that is associated

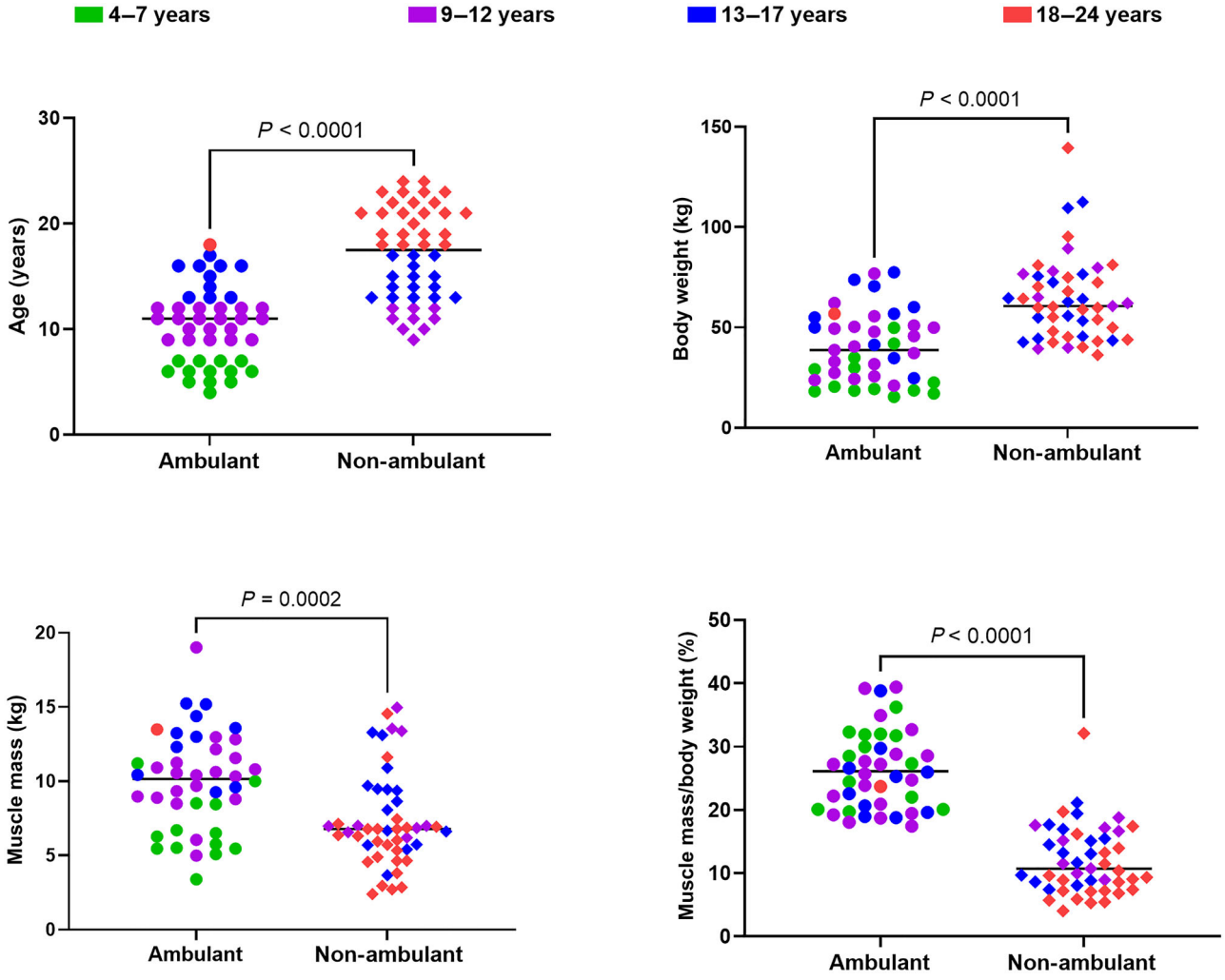

Figure 2. Comparison of ambulant and non-ambulant subjects with DMD
A, age; *B*, body weight; *C*, D_3Cr muscle mass (MM); *D*, MM/body weight ($P < 0.0001$). While there was considerable overlap of values for age, body weight and D_3Cr muscle mass, very little overlap was observed for %MM between ambulant and non-ambulant groups.

with the severity of the disease. Grip strength in patients with DMD, as a percentage of healthy normal values, declines with advancing age and is associated with disease progression (Hogrel et al., 2020). Because of its unique metabolic role for ATP synthesis, creatine and creatine phosphate are co-localized with the contractile apparatus of muscle (Hill, 1962). As stated above, D_3Cr muscle mass probably represents the functional or contractile component of muscle that is undiluted by lipid and connective tissue. The strong relationship between functional MM and PUL total score, as well as grip strength, for ambulant and non-ambulant subjects suggests that D_3Cr MM may provide a non-invasive indicator of functional status for all ages of patients with DMD.

Previous assessments of body composition and estimates of MM (or size) in DMD patients have used DXA and MRI. While MRI provides a cross-sectional analysis of skeletal muscle size, and whole-body MRI can provide an estimate of total muscle volume, DXA provides a measure of lean body mass (LBM) and not muscle exclusively. Importantly, lean mass as assessed by DXA is a measurement of everything in the body that is not fat and bone. In a cohort of healthy older men (>80 years old), DXA lean mass and D_3Cr MM were moderately related, but DXA lean mass was unrelated to functional status or health-related outcomes (Cawthon et al., 2019, 2020). DXA assessment of LBM has been used in previous studies in ambulant patients with DMD and was demonstrated to be associated with functional status. However, in a previous study (Evans et al., 2021) measuring total body water (TBW, LBM), DXA lean mass and D_3Cr MM in subjects with DMD, DXA was significantly associated with LBM by TBW but unrelated to MM. This study also showed that when compared with healthy age-matched controls, %LBM was reduced and MM of DMD subjects had significantly lower %LBM. In subjects with DMD, the relative amount of MM/LBM was reduced with age. In the present study, total body MM was not significantly associated with total NSAA but was significantly (negatively) associated with rise from floor time in the 26 subjects who could complete this task ($r = -0.4503$, $P < 0.05$). %MM was significantly associated with NSAA ($r = 0.6143$, $P < 0.0001$), providing more evidence that the relative amount of MM is an important component of functional status in patients with DMD.

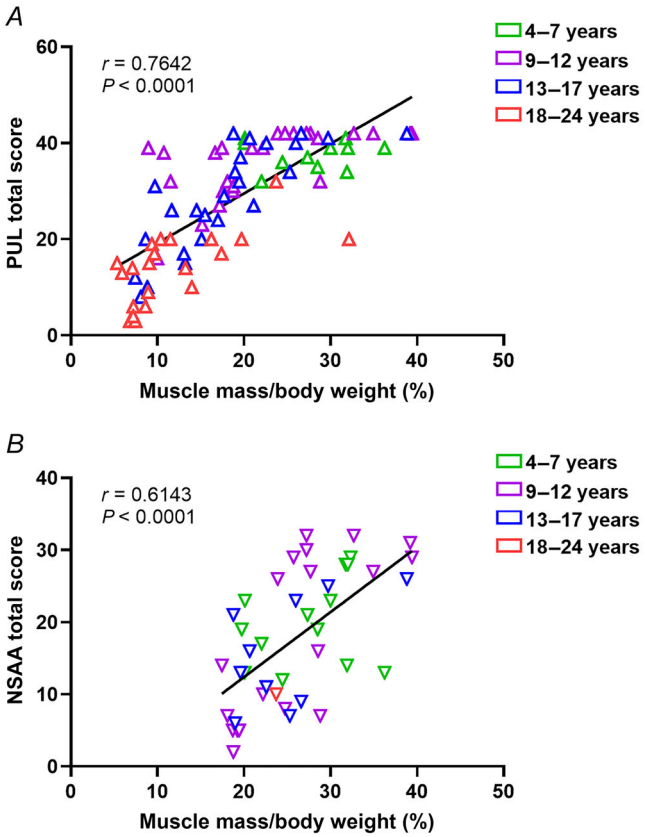

Figure 3. Functional performance vs % muscle mass
A, Performance of Upper Limb v.2.0 (PUL) *vs.* D_3Cr MM/body weight for all subjects ($r = 0.7821$, $P < 0.0001$; several subjects had a score of 42, the maximum score for this test). *B*, NSAA score *vs.* D_3Cr MM/body weight in ambulatory subjects ($r = 0.5600$, $P < 0.0002$).

Potential limitations

As has been described, in some subjects a small percentage of the oral dose of D_3Cr is 'spilled' into urine and not transported into muscle cells (Clark et al., 2014). For this reason, an algorithm has been formulated to correct for this spillage of label using the fasting urine creatine/creatinine ratio (Shankaran et al., 2018). In the present study, we observed a significant age-associated increase in the Cr/Crn ratio ($r = 0.5261$, $P < 0.001$) due to increased urine Cr levels, while urine Crn levels did not change with age. Higher urine Cr levels suggest a higher rate of Cr production than can be transported into the greatly reduced muscle mass. For most of the younger subjects in this study, the urine Cr/Crn ratio was not dramatically high, but the age-associated increase in fasting urine Cr levels has not been previously reported and the use of an algorithm to predict spillage of label based on values in healthy adults is not appropriate. For this reason, we did not apply a correction algorithm in this population. It is important to note, however, that in the context of the present study, lack of correction for spillage will tend to overestimate MM (D_3Cr delivery to muscle will be less than 100% of the administered dose, resulting in lower D_3Crn enrichments and overestimation of dilution). Not correcting for spillage therefore works against our primary finding of markedly

reduced functional MM in DMD and does not explain our findings.

We acknowledge that this value for determination of MM (kg) from the total body creatine pool size has not been validated in patients with DMD and may not be accurate in this patient group. However, because D_3Cr muscle mass calculation is a monotonic transformation of the creatine pool size, effect estimates for standardized variables would be identical for either creatine pool size or D_3Cr muscle mass (analysed with or without adjustment for body size). For example, in a cohort study of older men, each standard deviation decrement in D_3Cr MM/body mass was associated with a 1.9-fold increased risk [hazard ratio, 1.9 (95% CI, 1.2, 3.1)] of incident activities of daily living (ADL) disability 2.2 years later; the same size association is seen when this is expressed as risk per SD decrement in creatine pool size/body mass [hazard ratio, 1.9 (95% CI, 1.2, 3.1)] (Cawthon et al., 2020).

Conclusions

We report here, for the first time, that functional MM and, in particular %MM, is substantially reduced with advancing age in DMD subjects. This reduction in muscle is strongly associated with functional status in both ambulant and non-ambulant subjects. Our data suggest that MM relative to body weight is a critical factor during the transition from ambulant to non-ambulant status. Longitudinal measurements of MM in DMD patients will be necessary to further characterize the effects of loss of functional muscle on strength, functional and ambulant status. Moreover, these data strongly suggest that the D_3Cr dilution method provides an easy to administer, non-invasive and clinically important assessment of MM for patients with DMD across a wide age and functional status range.

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Additional information

Data availability statement

Data will be made available upon completion of all aspects of the longitudinal study and publication of results. Access of data may be made through contact with William Evans (william.evans@berkeley.edu)

Competing interests

W.J.E. participates on scientific advisory boards for BioAge, Veru Pharma and Aliance for Aging Research. He receives grant support from the National Institutes of Health. W.J.E. and M.H. are listed as co-inventors on patents for the D_3creatine dilution method and work with MyoCorps, Inc. M.S. is a consultant for MyoCorps, Inc. R.B. has received investigator-initiated grants from the FSHD Society, Centers for Disease Control, National Institutes of Health and Ionis Pharmaceuticals; and has served on a scientific advisory board for Sarepta Pharmaceuticals, Scholar Rock, Avexis, Pfizer, Biogen, Reata, LocanaBio and AAavanti. N.K. is an advisor for Fulcrum Therapeutics. M.G. participates in advisory boards for Pfizer, NS Pharma and Dyne. She is a member of the DSMB for Antisense Therapeutics. She has research collaborations with ReveraGen and PTC Therapeutics. She is or has been Principal Investigator for clinical trials with Dyne, Pfizer, Roche, Italfarmaco, Edgewise, Santhera, ReveraGen and Dynacure. M.G. has received speaker honoraria from Italfarmaco, Sarepta, Roche, Novartis. E.C.S. has participated on advisory boards for Sarepta, Solid, Entrada, Biomarin, Catalyst and Italfarmaco. He is a member of the DSMB for Solid. He is or has been Principal Investigator for clinical trials with Pfizer, Edgewise, Santhera, ReveraGen, Capricor and Avidity. E.C.S. has received speaker honoraria from Catalyst.

Author contributions

W.J.E., M.S., E.S. and M.H. designed the study and analysed the data. T.J.F. prepared samples and H.M. performed LC-MS

analysis. W.J.E., M.S. and M.H. wrote the manuscript, are the guarantors of this work, have full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors critically revised the drafts and approved the final manuscript. Additional critical contributors from the D_3 team include the following: Melissa McIntyre, Amelia Wilson, Robert Muni Lofra. Jassi Sodhi, Dionne Moat, Karen Wong, Emma Grover, Emma-Jayne Robinson.

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Keywords

ambulatory status, D_3creatine dilution, functional capacity, muscle mass, muscular dystrophy

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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