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Outcome measures in Duchenne muscular dystrophy

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Chapter 5

Preserved thenar muscles in non-ambulant Duchenne muscular dystrophy patients

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Abstract

background

Clinical trials in Duchenne muscular dystrophy (DMD) focus primarily on ambulant patients. Results cannot be extrapolated to later disease stages due to a decline in targeted muscle tissue. In non-ambulant DMD patients, hand function is relatively preserved and crucial for daily-life activities. We used quantitative MRI (qMRI) to establish whether the thenar muscles could be valuable to monitor treatment effects in non-ambulant DMD patients.

Methods

Seventeen non-ambulant DMD patients (range 10.2-24.1years) and 13 healthy controls (range 9.5-25.4years) underwent qMRI of the right hand at 3T at baseline. Thenar fat fraction (FF), total volume (TV), and contractile volume (CV) were determined using 4-point Dixon and $T2_{\text{water}}$ was determined using multi-echo spin-echo. Clinical assessments at baseline (n=17) and 12 months (n=13) included pinch strength (kg), Performance of the Upper Limb (PUL) 2.0, DMD Upper Limb Patient Reported Outcome Measure (PROM), and playing a video game for 10 minutes using a game controller. Groups differences and correlations were assessed with non-parametric tests.

Results

TV was lower in patients compared to HCs (6.9cm³, 5.3-9.0cm³ versus 13.0cm³, 7.6-15.8cm³, $p=0.010$). CV was also lower in patients (6.3cm³, 4.6-8.3cm³ versus 11.9cm³, 6.9-14.6cm³, $p=0.010$). FF was slightly elevated (9.7%, 7.3-11.4% versus 7.7%, 6.6-8.4%, $p=0.043$), while $T2_{\text{water}}$ was higher (31.5ms, 30.0-32.6ms versus 28.1ms, 27.8-29.4ms, $p<0.001$). Pinch strength and PUL decreased over 12 months (2.857kg, 2.137-4.010kg to 2.243kg, 1.930-3.339kg, and 29 points, 20-36 to 23 points, 17-30, both $p<0.001$), while PROM did not (49 points, 36-57 to 44 points, 30-54, $p=0.041$). All patients were able to play for 10 minutes at baseline or follow-up, but some did not comply with the study procedures regarding this endpoint. Pinch strength correlated with TV and CV in patients ($\rho=0.72$, $\rho=0.68$) and controls (both $\rho=0.89$). PUL correlated with TV, CV and $T2_{\text{water}}$ ($\rho=0.57$, $\rho=0.51$, $\rho=-0.59$).

Conclusions

Low thenar FF, increased $T2_{\text{water}}$, correlation of muscle size with strength and function, and the decrease in strength and function over one year indicate that the thenar muscles are a valuable and quantifiable target for therapy in later stages of DMD. Further studies are needed to relate these data to the loss of a clinically meaningful milestone.

Introduction

duchenne muscular dystrophy (DMD) is characterized by progressive replacement of muscle with fat and fibrotic tissue due to the absence of full length dystrophin.¹ This leads to a clinical presentation with a proximal to distal gradient of muscle weakness, in which loss of independent ambulation generally occurs years before loss of upper arm function.² Although the first drugs in DMD have now received regulatory approval, there is no cure yet, and ongoing clinical trials focus primarily on ambulant patients.³ Regulators do not support extrapolation of trial results in ambulant patients to later disease stages, because progressive replacement of muscle by fat is considered irreversible and thus leads to a progressive reduction in target tissue.^{4,5}

Hand function is preserved even longer than upper arm function in DMD, and is crucial for daily-life activities.^{6,7} Many of these activities require grasping motions for which the thenar muscles primarily generate the opposition of the thumb.⁸ Since efficacy of new drugs has to be proven separately in non-ambulant patients, preparation for clinical trials in later disease stages is essential. To facilitate the development of these trials, it is necessary to collect natural history data, develop outcome parameters, and study biomarkers that reflect a decline in upper limb function. Quantitative MRI (qMRI) fat fraction (FF) has shown potential as such a biomarker in the lower extremities, and increased FF of the vastus lateralis predicts a decline in ambulatory function.⁹⁻¹¹ Ongoing studies assess the feasibility of qMRI FF in the upper and lower arm, but studies in intrinsic hand muscles are lacking.¹²⁻¹⁴ Another qMRI parameter, the T2 relaxation time of the muscle compartment ($T2_{\text{water}}$), is indicative for early muscle pathology, since it increases due to inflammation, myocyte swelling, edema and necrosis.^{15,16} Here, we aimed to study the thenar muscles for their value to monitor treatment effects in non-ambulant DMD patients by obtaining natural history of hand function over 1 year, and assessing qMRI parameters compared to healthy controls (HC).

Methods

Participants

Between March 2018 and July 2019, DMD patients were recruited from the Dutch Dystrophinopathy Database (DDD),¹⁷ and via Dutch neurologists, rehabilitation specialists and patient organizations. Inclusion criteria were male, non-ambulant genetically confirmed DMD, aged ≥ 8 years. Exclusion criteria were MRI contra-indications (e.g. scoliosis surgery or daytime respiratory support), inability to lie still for 45 minutes, exposure to an investigational drug ≤ 6 months prior to participation, and recent (≤ 6 months) upper extremity surgery or trauma. Healthy age-matched controls were recruited using posters and advertisements in local media. The study was approved by the local medical ethics committee, and written informed consent was obtained from all patients and legal

representatives prior to inclusion. The investigation was conducted according to the 1964 Declaration of Helsinki and its later amendments. The reported assessments were performed as part of a longitudinal study on outcome measures in non-ambulant DMD patients at Leiden University Medical Center (LUMC): 'Upper extremity outcome measures in non-ambulant DMD patients', registered on ToetsingOnline with ABR number NL63133.058.17 (www.toetsingonline.nl). We report results from baseline (DMD and HC) and 12 months follow-up (DMD only). MRI of the hand muscles was only performed at baseline and clinical assessments were performed at every visit.

MRI acquisition

MRI scans of the right hand were acquired on a 3T scanner (Ingenia, Philips Healthcare, Best, The Netherlands) with two 47 mm surface coils placed on the ventral and dorsal side of the thenar muscles. The standard position for participants was on the right side with the right shoulder in 90° flexion, the elbow in 90° flexion and the forearm in maximum pronation, which was supported by holding a handle (Figure S1). If this position was uncomfortable, patients could choose from lying on the right side with the elbow halfway between pronation and supination or lying in supine position with the forearm either in maximum pronation or halfway between pronation and supination.

The protocol consisted of a 4-point Dixon scan (25 contiguous slices of 4mm thickness, repetition time (TR) 310ms, first echo time (TE) 4.40ms, echo spacing (Δ TE) 0.76ms, flip angle 20°, field of view (FOV) 100x100mm, voxel size 1x1mm, reconstructed voxel size 0.89x0.89mm), a multi-echo spin-echo (MSE) scan (5 slices of 4mm thickness with 8mm slice gap, TR 300ms, TE 8.0ms, Δ TE 8.0ms, FOV 100x100mm, voxel size 1x1mm, reconstructed voxel size 0.89x0.89mm), and a B1 map (5 contiguous slices of 12mm thickness, dual-TR gradient echo with TRs of 30ms and 100ms, TE 2.01ms, FOV 100x100mm, voxel size 1.56x2mm, reconstructed voxel size 0.89x0.89mm). The Dixon scans, MSE scans, and B1 maps were aligned perpendicular to the first metacarpal bone and covered the thenar muscles completely.

MRI analysis

Water and fat images were reconstructed from the Dixon data using in-house developed software (Matlab 2016a, The Mathworks of Natick, Massachusetts, USA) assuming a six-peak lipid spectrum.¹⁸ B0 maps were determined from the phase data of the first and last echoes, and the Goldstein branch cut method was used for phase unwrapping.¹⁹ Scans with major movement artefacts, water/fat swaps, insufficient signal or other artefacts in the thenar were excluded. Regions of interest (ROIs) of the thenar muscles were drawn on all slices of the Dixon scans using online available software (www.mipav.cit.nih.gov) (Figure 1A). The thenar muscles consist of the abductor pollicis brevis, flexor pollicis brevis and opponens pollicis muscle. The ROIs were drawn on the border of this muscle group, except at the dorsal side because the fascia between the different intrinsic hand muscles could not be

observed. This dorsal ROI boundary was defined by a straight line on the palmar side of the tendon of the flexor pollicis longus muscle and the first metacarpal bone. All ROIs of the HCs were drawn by one rater (K.J.N.) and of the DMD patients by two raters (K.J.N. and A.J.P.). Since different positioning of the first metacarpal bone could influence the position and size of a single slice, all qMRI values are reported for the whole thenar muscle and used for subsequent analyses. The total volume (TV; cm³) of all thenar ROIs combined was assessed from these raw ROIs. For fat fraction measurements (FF; %), an erosion of two voxels was performed for every ROI to avoid contamination of ROIs with subcutaneous fat. A correction for the T1-weighting in the water and fat images was incorporated in the FF calculation, assuming T1 values of 1420ms (correction factor: 1.25) and 371ms (correction factor: 1.05) for water and fat respectively.²⁰ Thenar FF was then calculated as a weighted mean value by averaging all thenar voxels of all eroded ROIs from the reconstructed fat and water images as:

$$FF = \frac{1.05 \times \text{Fat}}{1.25 \times \text{Water} + 1.05 \times \text{Fat}}$$

Contractile volume (CV) was calculated by subtracting the fat containing area from TV as follows:

$$CV = TV - (TV \times FF)$$

The MSE scans were fitted using a dictionary fitting algorithm with an Extended Phase Graph model in which the FF, $T2_{\text{water}}$ and B1 were fitted, and the flip angle slice profile and through-plane chemical shift displacement were incorporated.^{21,22} This dictionary was created using $T2_{\text{water}}$ values from 10-60ms, $T2_{\text{fat}}$ values from 120-200ms, FF values from 0-100% and B1 values from 50-140%. The $T2_{\text{fat}}$ was calibrated on the subcutaneous fat per slice by creating an automatic fat mask using the last echo of the MSE sequence. The fitted B1 values were visually confirmed to correspond to the B1 map by comparing values from similar locations on the same slices. Uneroded ROIs were copied from the Dixon images, manually checked and adapted in case of movement between scans, and eroded with two voxels before further analysis. Voxels that fitted on the physiological boundaries of the dictionary were excluded. If less than 100 thenar voxels remained for a particular scan, that scan was excluded. Thenar $T2_{\text{water}}$ was estimated over all slices as a weighted mean of all thenar voxels (Figure 1B), and for all intrinsic hand muscle voxels (supporting information).

Clinical assessments

All clinical assessments were performed after the MRI examination. Weight was measured using a patient lift with inbuilt scale (Maxi Move™, ArjoHuntleigh, Sweden). Height was calculated from the ulna length for both DMD patients and HCs using the formula proposed by Gauld et al. with 18 as maximum age.²³

Pinch strength of the right hand was assessed with MyoPinch and grip strength with MyoGrip (Institute of Myology, Ateliers Laumonier, France).²⁴ Specific strength was defined as the

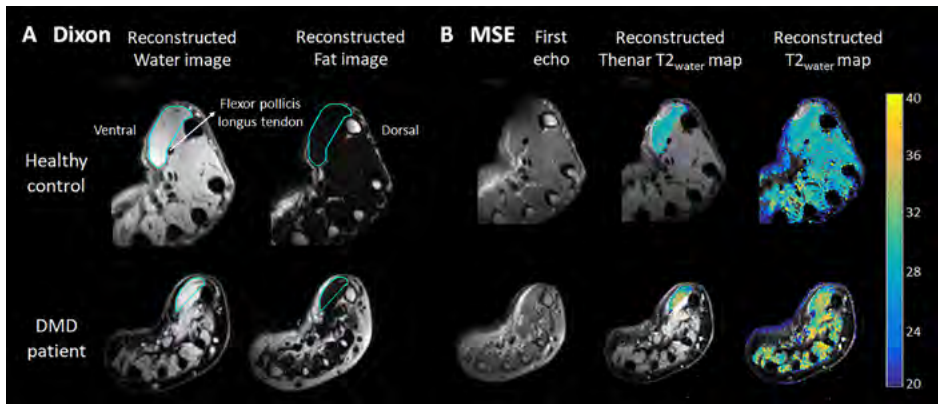


Figure 1. Dixon and multi-echo spin-echo (MSE) MRI acquisitions and analyses

(A) Example of a Dixon reconstructed water and fat image of a healthy control (HC; 16 years old) and a Duchenne muscular dystrophy (DMD) patient (13 years old). ROIs of the thenar muscles were drawn (light blue line) with the dorsal boundary defined as a line on the palmar side of the tendon of the flexor pollicis longus muscle and first metacarpal bone. (B) Example of the first echo from the MSE scan of the same HC and DMD patient and the following reconstructed images: T_{2_water} map for voxels within the thenar ROI and T_{2_water} map of all hand muscles. MSE voxels that fitted on the physiological boundaries of the dictionary, were excluded.

pinch strength in kg per cm^3 of CV. Performance of the Upper Limb (PUL) 2.0 was performed for the right arm, and only in DMD patients because a maximum score was assumed in HCs. The PUL 2.0 consists of 22 items and yields a maximum total score of 42 points.²⁵ Items are divided over three dimensions with a maximum score of 12 for the shoulder dimension, 17 for the elbow dimension and 13 for the distal wrist/hand dimension. The adapted DMD Upper Limb Patient Reported Outcome Measure (PROM) questionnaire was used to investigate patient reported upper limb function. The questionnaire contains 32 daily-life activity items which are scored on a three-level scale ('cannot do'; 'with difficulty'; 'easy') with a maximum possible score of 64 points.²⁶ Furthermore, DMD patients were asked to play a video game (Rocket League®, Psyonix LLC, California, USA) for 10 minutes using a game controller (GC-100XF Wired Gaming Controller, NACON™, Bigben group, France). In this game, patients used their right thumb to steer a vehicle on the screen. Afterwards patients rated tiredness and difficulty on two 10-point numeric rating scales (NRS) ranging from 'not at all tiring/difficult' (score 0) to 'very tiring/difficult' (score 10) with matching facial cartoons based on the Wong-Baker Faces Rating Scale.²⁷

Statistical analysis

Interrater variability of qMRI results in DMD patients was assessed using Bland-Altman analyses to determine bias and limits of agreement. Differences in baseline characteristics, qMRI values, specific strength, and pinch strength between DMD patients and HCs were assessed with the Mann-Whitney U test. Differences in clinical assessments over 12 months were assessed using Wilcoxon signed-rank test. Statistical significance was set at $p < 0.05$.

Bonferroni-Holm correction was used to correct for multiple comparisons of qMRI results between patients and HCs, and of clinical assessments between baseline and follow-up. Spearman's rank correlation coefficient was used to correlate qMRI values to pinch strength and grip strength in DMD patients and HCs, and to PUL total and distal, PROM total and NRS scores in patients. The correlation was considered very strong (0.9-1.0), strong (0.7-0.9) or moderate (0.5-0.7).²⁸

Data availability

Anonymized data and analysis software can be made available to qualified investigators on request.

Results

Characteristics of participants and qMRI data inclusion

Twenty-two DMD patients and 14 HCs participated. Five patients and one HC could not undergo MRI. Characteristics of the remaining 17 patients and 13 HCs are presented in Table 1. Due to unforeseen restrictions during the COVID-19 pandemic, the 12 months follow-up visit could only take place for 13 patients. All patients used corticosteroids at baseline, except for two patients who had temporarily ceased treatment for six weeks prior to the visit due to weight gain and non-compliance.

All HCs and 10 DMD patients could maintain the standard position, while three patients had their forearm positioned halfway between pronation and supination. The MRI scans were acquired in supine position in four DMD patients due to discomfort lying on the side, one with the forearm in maximum pronation and three with their forearm positioned halfway between pronation and supination. Scans in a separate group of HCs showed no or minimal differences in qMRI results between two forearm positions (supporting information and Figure S2). Interrater variability (Figure S3) showed a mean bias in FF of 0.4%, and 95% limits of agreement -1.3 to 2.0%, for TV this was 0.4 cm³ (-0.8 to 1.7 cm³), for CV 0.3 cm³ (-0.8 to 1.5 cm³), and for T2_{water} 0.1 ms (-0.8 to 0.9 ms).

Four Dixon scans and five MSE scans of DMD patients and four MSE scans of HCs were excluded due to insufficient signal or movement artefacts. Thirteen Dixon scans from patients and HCs, and 12 MSE scans from patients and nine from HCs were included in the analyses (see flowchart in Figure S4).

Thenar FF, muscle size and T2_{water}

Thenar FF was only slightly elevated in patients compared to HCs (median 9.7 versus 7.7%, $p=0.043$; Figure 2A). By contrast, TV and CV were significantly lower in patients (median 6.9 and 6.3 versus 13.0 and 11.9 cm³, both $p=0.010$; Figure 2B and C), whereas thenar T2_{water} was higher (median 31.5 versus 28.1 ms, $p<0.001$; Figure 2D). The higher thenar T2_{water} in DMD patients could be visually confirmed for all intrinsic hand muscles (Figure S5). In

patients, whole thenar TV, CV and specific strength correlated very strongly ($\rho=0.96-0.97$) to these same values determined on only the slice with the largest cross-sectional area (CSA), FF correlated moderately ($\rho=0.67$; Figure S6).

Table 1. Baseline characteristics

	Healthy controls (n=13)	DMD patients (n=17)	p-value
Age, years	15.7 (11.1-20.7)	13.4 (12.5-16.7)	0.536
Calculated height, m	1.73 (1.47-1.75)	1.54 (1.47-1.66)	0.053
BMI, kg/m ²	19.5 (16.4-22.3)	27.5 (23.4-31.6)	0.001
Righthanded	12 (92.3%)	13 (76.5%)	
Prednisone intermittent	NA	8 (47.1%)	
Deflazacort intermittent	NA	8 (47.1%)	
Deflazacort daily	NA	1 (5.9%)	
Steroids on-day at baseline	NA	7 (41.1%)	
Steroids off-day at baseline	NA	10 (58.8%)	
Age at start steroid use, years	NA	5.7 (4.6-8.0)	
Age at loss of ambulation, years	NA	11.6 (10.1-12.8) range 8.6-18.9	
Time since loss of ambulation, years	NA	2.6 (1.4-4.0)	

Values are median (first-third quartiles) or number of patients (%). Abbreviations: DMD = Duchenne muscular dystrophy, BMI = body mass index. Statistical significance was set at $p<0.05$.

Clinical assessments

Clinical assessments at baseline and follow-up are presented in Table 2. At baseline, pinch strength in patients was lower compared to HCs (Figure 2E), and it decreased significantly over 12 months from median 2.857 to 2.243 kg (Figure 3A). PUL total also significantly decreased from a median of 29 to 23 points. Grip strength declined from median 8.47 to 6.39 kg and PROM total scores from 49 to 44 points, but both were not significant after correction. PUL distal and NRS tiredness and difficulty scores did not show a decline over 12 months. At baseline, all patients scored NRS tiredness score ≤ 4 (i.e. 'not really tiring'). Similarly, NRS difficulty score was ≤ 4 (i.e. 'not really difficult') for all patients except two, who scored 5 and 6 (i.e. 'a little difficult'). Fifteen out of 17 patients were able to play the video game for 10 minutes at baseline, including the patient with the lowest pinch strength (0.723 kg), and 10 out of 13 patients were able at 12 months follow-up. Two patients at baseline and one at follow-up did not want to continue playing after five minutes, but were able to play for 10 minutes at a later study visit. The other two patients at follow-up refused or quit after five minutes, but claimed to easily play for 10 minutes.

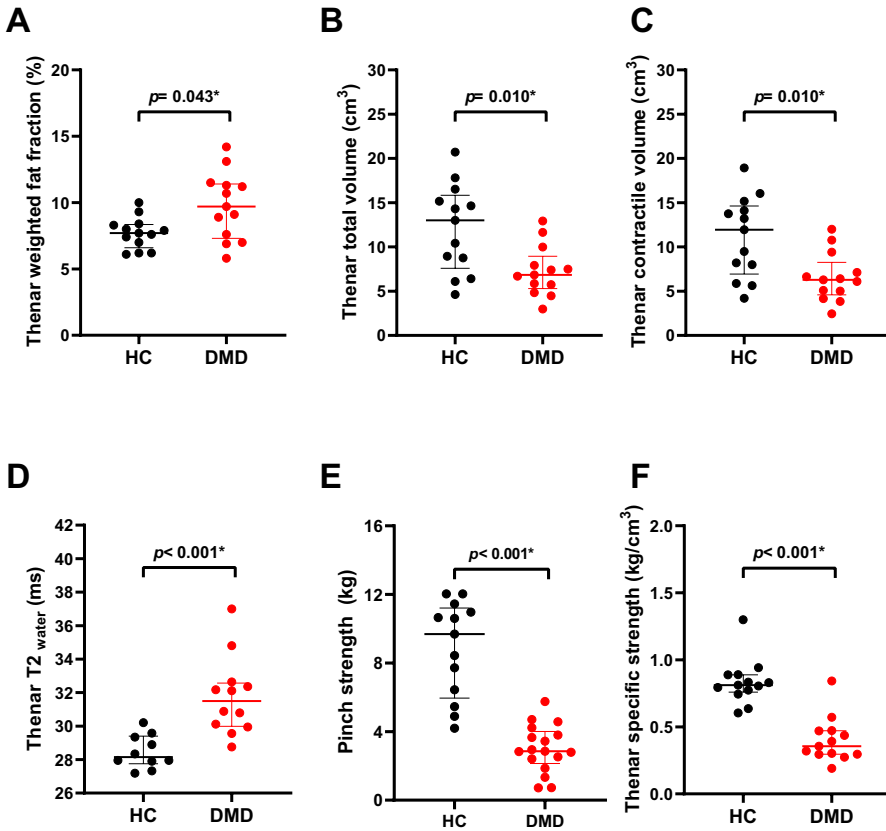


Figure 2. Dixon and pinch strength results of healthy controls (HC) compared to Duchenne muscular dystrophy (DMD) patients

Thenar weighted fat fraction (FF) (A), total volume (TV) (B), contractile volume (CV) (C), T₂_{water} (D), pinch strength (E), and specific strength (F), are presented for healthy controls (HCs; black) and DMD patients (red). TV, CV, specific strength and pinch strength were lower in DMD patients. T₂_{water} was higher compared to HCs, while FF was only slightly elevated. Statistical significance was set at $p < 0.05$. Uncorrected p -values are reported and statistical significance after Bonferroni-Holm correction is shown by *

Relations between qMRI and clinical assessments

Table 3 shows correlations between qMRI parameters and clinical assessments. Apart from the smaller TV and CV, specific strength (kg/cm³), was also significantly lower in patients (median 0.36 versus 0.81 kg/cm³; Figure 2F). In both groups, FF did not correlate with pinch or grip strength (Figure 3B). In patients, pinch strength correlated strongly with TV (Figure 3C) and moderately with CV. There was a moderate correlation between grip strength and TV and CV, between PUL total and TV (Figure 3D), CV and T₂_{water}, between PUL distal and TV and CV, between PROM total and TV and CV, and between NRS tiredness and TV. In HCs, correlations of pinch and grip strength with TV and CV were also strong or very strong.

Table 2. Clinical assessments in DMD patients at baseline and 12 months follow-up

DMD patients	Baseline (n=17)	12 months follow-up (n=13)	p-value
Pinch strength, kg	2.857 (2.137-4.010)	2.243 (1.930-3.339)	<0.001*
Grip strength, kg	8.47 (5.25-11.08)	6.39 (4.92-9.93)	0.016
PUL 2.0 total score, points (max 42)	29 (20-36)	23 (17-30)	<0.001*
PUL 2.0 shoulder score, points (max 12)	4 (0-8)	1 (0-6)	0.109
PUL 2.0 elbow score, points (max 17)	13 (10-16)	11 (7-14)	0.001 *
PUL 2.0 distal score, points (max 13)	11 (10-12)	11 (10-12)	0.227
PROM total score, points (max 64)	49 (36-57)	44 (30-54)	0.041
NRS tiring, points (max 10)	0 (0-1) ⁿ⁼¹⁵	1 (0-4) ⁿ⁼⁹	0.250
NRS difficult, points (max 10)	0 (0-2) ⁿ⁼¹⁵	0 (0-1) ⁿ⁼⁹	1.000

Values are median (first-third quartiles). Abbreviations: DMD = Duchenne muscular dystrophy, PUL = Performance of the Upper Limb, PROM = Patient Reported Outcome Measure, NRS = Numeric Rating Scale. Statistical significance was set at $p < 0.05$. Uncorrected p -values are reported and statistical significance after Bonferroni-Holm correction is shown by *. If a certain value was not available for all patient, the number of patients for whom the data was available was presented after the result with $n =$ number.

DISCUSSION

The thenar muscles were assessed for their value to monitor treatment effects in DMD using qMRI, strength and functional assessments. TV, CV, specific strength and pinch strength were lower and $T2_{\text{water}}$ was higher in DMD patients compared to HCs, while FF was only slightly elevated. Pinch strength and PUL total decreased significantly over 12 months in DMD patients, and there were moderate to strong correlations of qMRI muscle size with pinch strength and PUL total and distal. Despite this decrease in strength, operating a game controller was still possible for all our participants.

In DMD, hand function is preserved years after loss of ambulation and the ability to raise the arms². Previous qMRI studies support this pattern of decline on tissue level by showing a proximal to distal involvement, where thigh, shoulder and upper arm muscles are, on average, more affected than lower leg and forearm muscles.^{29, 30} Hand function and corresponding activities are vital for independence, e.g. operating a wheelchair and using a smart phone or computer, and for entertainment. Compared to ambulant patients, non-ambulant patients spend significantly more time (in total 2-4 hours/day) on playing (online) video games, which in recent years has become an important tool for social interaction.⁷

Drugs that preserve muscle and make use of dystrophin products are assumed to have the greatest effect on progression at an early disease stage, because they rely on the presence of sufficient muscle tissue.^{4, 5} In addition, measurable disease progression is of vital importance to detect this potential preserving effect of a drug. All aspects of the thenar

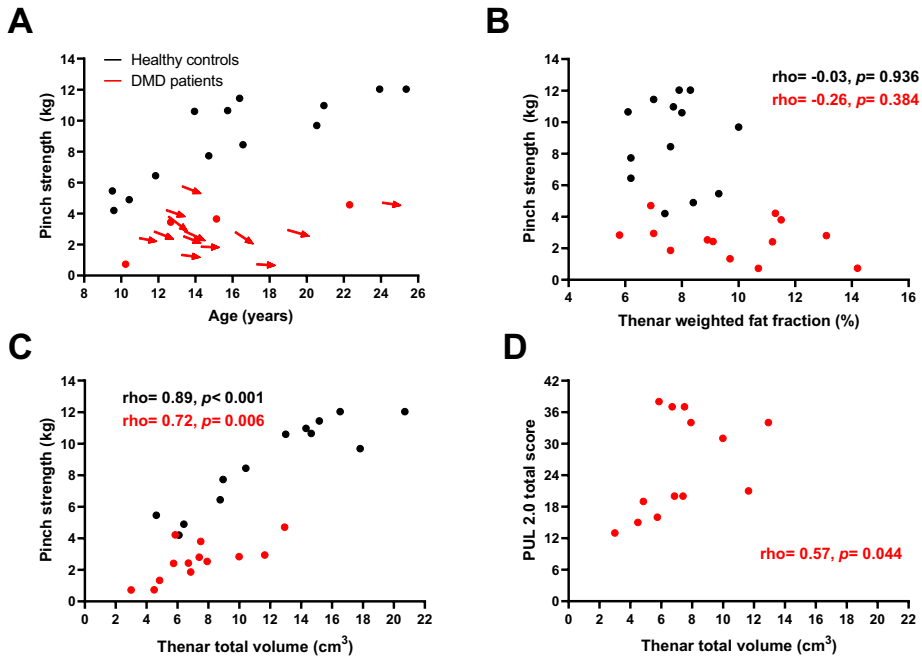


Figure 3. Pinch strength declines over time and correlates with quantitative MRI muscle size in Duchenne muscular dystrophy (DMD)

(A) Pinch strength is plotted versus age for both healthy controls (HC; black) and DMD patients (red). In all patients with 12 months follow-up data, pinch strength decreased over that time-period. (B) No clear relation between the plotted thenar fat fraction and pinch strength can be observed. Thenar total volume (TV) is plotted against pinch strength in (C) and Performance of the Upper Limb (PUL) 2.0 total score in (D). A strong correlation between pinch strength and TV in HCs and DMD patients can be observed, as well as a moderate correlation between PUL 2.0 total score and TV in DMD patients.

muscles that were assessed in this study pointed towards a relative preservation of the thenar muscles, and some also showed measurable disease progression: tissue characteristics as measured by qMRI, strength via pinch strength, function by assessing PUL and PROM, and activities in daily life via playing a video game. On tissue level, we found a slightly increased FF and an elevated $T2_{\text{water}}$ in the thenar muscles of DMD patients. $T2_{\text{water}}$ reflects active pathological processes, such as inflammation, myocyte swelling, edema and necrosis,¹⁶ and previous qMRI studies of lower extremity muscles showed that young DMD patients have limited fat replacement and elevated $T2_{\text{water}}$.^{15,31} Other qMRI techniques might show early signs of muscle pathology and treatment effects in DMD, such as ionic dysregulation by sodium MRI,³² or pH or phosphodiester alterations by ³¹P or ¹H magnetic resonance spectroscopy.^{33,34} Thenar muscle volume was clearly reduced compared to HCs, which could be inherent to the dystrophic process or related to long-term corticosteroid use.^{1,35}

Table 3. Correlations between qMRI results and clinical assessments

	Fat fraction	Total volume	Contractile volume	T2 _{water}
Healthy controls				
Pinch strength	rho= -0.03 p= 0.936	rho= 0.89 p< 0.001	rho= 0.89 p< 0.001	rho= -0.39 p= 0.266
Grip strength	rho= 0.03 p= 0.936	rho= 0.91 p< 0.001	rho= 0.91 p< 0.001	rho= -0.44 p= 0.200
DMD patients				
Pinch strength	rho= -0.26 p= 0.384	rho= 0.72 p= 0.006	rho= 0.68 p= 0.010	rho= 0.16 p= 0.618
Grip strength	rho= -0.17 p= 0.578	rho= 0.69 p= 0.009	rho= 0.67 p= 0.013	rho= -0.15 p= 0.633
PUL 2.0 total score	rho= -0.22 p= 0.480	rho= 0.57 p= 0.044	rho= 0.51 p= 0.075	rho= -0.59 p= 0.043
PUL 2.0 distal score	rho= -0.35 p= 0.249	rho= 0.58 p= 0.039	rho= 0.53 p= 0.065	rho= 0.10 p= 0.765
DMD Upper Limb PROM	rho= -0.32 p= 0.292	rho= 0.55 p= 0.051	rho= 0.52 p= 0.070	rho= -0.03 p= 0.931
NRS tiredness score	rho= 0.26 p= 0.415	rho= -0.54 p= 0.068	rho= -0.50 p= 0.101	rho= 0.12 p= 0.721
NRS difficulty score	rho= -0.18 p= 0.569	rho= -0.28 p= 0.384	rho= -0.24 p= 0.446	rho= 0.02 p= 0.951

Abbreviations: DMD = Duchenne muscular dystrophy, PUL = Performance of the Upper Limb, PROM = Patient Reported Outcome Measure, NRS = Numeric Rating Scale.

For the second aspect, muscle strength, we observed a decline in pinch strength over 12 months in our non-ambulant cohort, which is in agreement with the slightly older and weaker cohort described by Seferian et al.³⁶ As onset of weakness in pinch strength can already be quantified in preschool infants with DMD,³⁷ this process is apparently slow. The higher baseline pinch strength in our cohort compared to literature could be explained by the younger age at inclusion in the present study, and higher age at and shorter duration since loss of ambulation, and more consistent corticosteroid use.³⁶ The lower specific strength we observed, was also reported in other muscles of DMD patients, as well as in other muscular dystrophies.^{30, 38, 39} The strong correlation between muscle size and strength suggests that therapies that manage to increase muscle volume have the potential to also increase muscle strength.⁴⁰

The PUL 2.0 total has been accepted as a primary endpoint in non-ambulant DMD (e.g. NCT03406780 and NCT04371666). We showed quantifiable disease progression on function level in the upper extremity, reflected by a decline in the PUL 2.0 total within the same range of the non-ambulant cohort of Pane et al.⁴¹ A decline in PUL distal was reported in a much

larger cohort over 24 months, but this was small (0.8 points), not reported as significant and therefore arguably not clinically relevant.⁴¹ We did not observe a decline in the distal domain, supporting the preservation of hand function. The decline in PUL total was not reflected in a significant decline in PROM total score in our data, which could have been caused by our small cohort. Ultimately, it is important that therapies are shown to have an effect on activities in daily life, especially from a regulatory and patient's perspective.⁴ Therefore, we studied the ability to play video games for 10 minutes using a game controller as clinical endpoint. None of the patients had reached this endpoint during our study, although some patients were not motivated to complete the 10 minutes or preferred smaller game controllers than used in the current setup. Therefore, the search for a direct connection between results on tissue characteristics, strength and function level with this or another robust clinical endpoint will be continued.

Obtaining qMRI data of the thenar muscles in non-ambulant DMD patients led to some challenges and technical considerations. Contractures of knee flexion, shoulder extension and internal rotation, forearm pronation and wrist flexion caused difficulties in maintaining the desired standardized position or any comfortable position in the MRI scanner. To address the effects of this on our outcome parameters, we studied the effect of two forearm positions on qMRI results separately, and found that thenar FF and $T2_{\text{water}}$ were comparable between both positions (supporting information). Even though TV and CV differed significantly between positions, average differences were small (0.55-0.63 cm³). We also found that TV and CV correlated strongly with these same values determined on only the slice with the largest CSA (supporting information), indicating that (contractile) CSA can be used as a proxy for whole muscle values. Thenar FFs, however, should always be determined via whole muscle analysis, as correlations between single slice and whole muscle were moderate for thenar FF. This could be explained by the known proximal-distal differences in fat replacement in DMD.^{42,43} Furthermore, both the small size of the hand and positioning away from the center of the MRI scanner led to reductions in image quality. The best scan quality was observed when patients were positioned on the right side with the arm placed beside the body with either the elbow in 90° flexion and the forearm halfway between pronation and supination, or the elbow in maximum extension and the forearm in any comfortable position. However, the more severely affected patients in our study were often unable to lie on their right side, which makes the use of qMRI of the thenar muscles as biomarker challenging when using a conventional MR scanner. Drawing ROIs consistently may be challenging because of the small size of the thenar muscles, but the low interrater variability showed that this did not influence our qMRI results. The positioning away from the center of the MRI scanner resulted in both DMD patients and HCs in difficulties with obtaining sufficient B1 for the MSE scans. However, as the $T2_{\text{water}}$ values in all intrinsic hand muscles in DMD were also elevated, and values in our HCs are comparable to those of our previous study in upper arm and lower extremity muscles,²² we are confident that the analysis was sound.

In conclusion, the minimal fat replacement within the thenar muscles and increased $T2_{\text{water}}$ indicate that the thenar muscles are in an early stage of muscle pathology in this cohort of non-ambulant patients. The simultaneous decrease in pinch strength and PUL total over one year shows that there is measurable disease progression within the possible duration of a clinical trial. Together with the correlation between muscle size and function, these results indicate that the thenar muscles are a valuable and quantifiable target for systemic or local therapy in later stages of the disease.

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Supplementary material

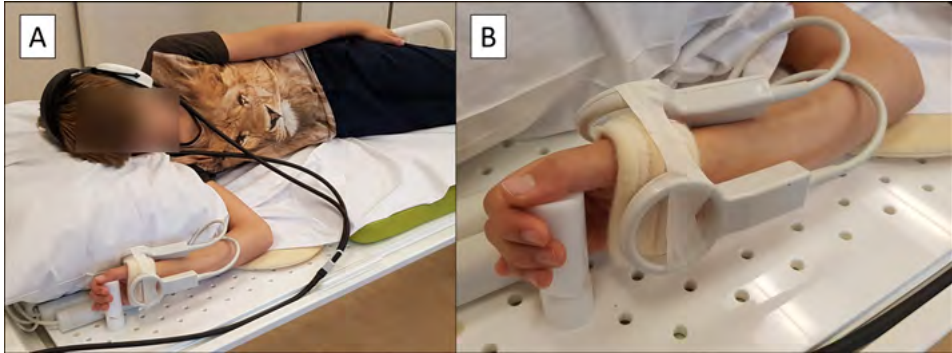


Figure S1. Example of a participant and forearm positioned in maximum pronation
 In (A) the standard position is shown, where participants lay on the right side with the right shoulder in 90° flexion, with the right elbow in 90° flexion and the forearm in maximum pronation, which was supported by holding a handle. During the actual scanning procedure a sheet would be placed around the arm to prevent direct contact between the skin and the coils and cables. A detail of the 47 mm surface coils placed on the ventral and dorsal side of the thenar muscles is shown in (B).

5

Effect of different positioning

Healthy controls (HC) ≥ 18 years old without MRI contra-indications were included in a separate study to assess the effects of different positioning in the MRI scanner on thenar quantitative MRI (qMRI) results. Medical ethical approval for this protocol was waived, and written informed consents from participants were obtained.

Seven HCs were included. MRI scans of the right hand were acquired, while the participant was positioned on his right side with the right shoulder in 90° flexion, the elbow in 90° flexion, and the forearm in maximum pronation or halfway between pronation and supination. The Dixon scan was acquired in these two positions in all HCs, and the multi-echo spin-echo (MSE) scan was acquired in both positions in three of the HCs. Thenar fat fraction (FF), total volume (TV), contractile volume (CV) and T2 relaxation time of the muscle compartment ($T2_{\text{water}}$) were determined as described previously. We assessed differences between qMRI results in the different positions using Wilcoxon signed-rank test.

Thenar FF and $T2_{\text{water}}$ results were comparable in both positions (figure S2A and D). Both TV and CV differed significantly between the two positions, although the average difference was small with 0.55 cm³ for TV and 0.63 cm³ for CV (Supporting information figure S2B and C).

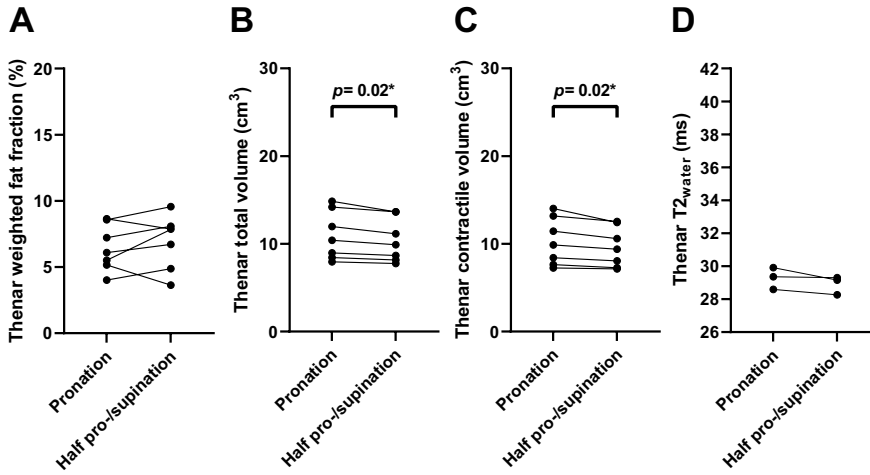


Figure S2. Thenar qMRI results in different forearm positions
 Dixon and multi-echo spin-echo whole muscle thenar results are shown for seven HC. Participants are positioned with a 90° elbow angle, and the forearm in maximum pronation or halfway between pronation and supination. Both positions are shown in (A) for FF, (B) for TV, (C) for CV and (D) for T_{2water}. Thenar FF and T_{2water} results are comparable in both positions. Total volume and contractile volume differ significantly between both positions, but the resulting difference is small.

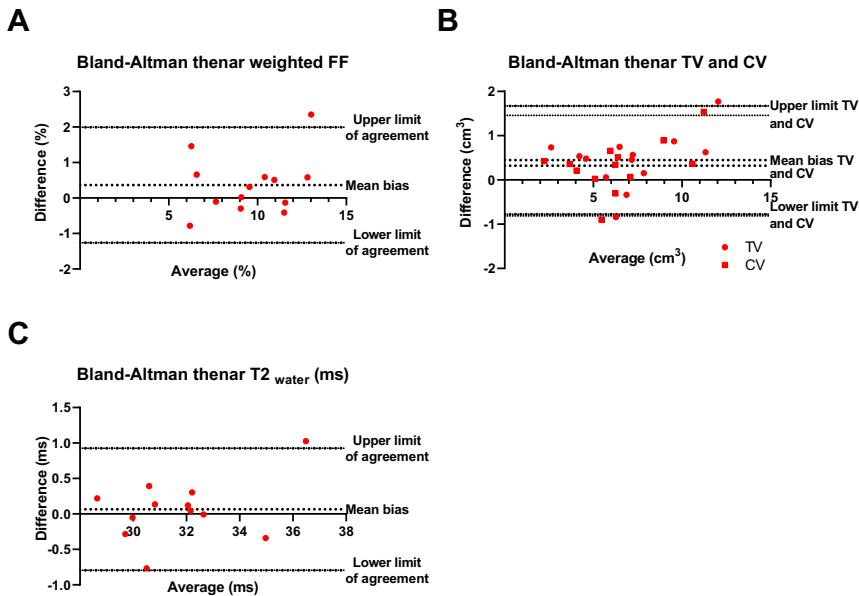


Figure S3 Interrater variability for thenar qMRI results
 The thenar ROIs of DMD patients were drawn by two raters: K.J.N. and A.J.P. The Bland-Altman plots for the interrater variability in DMD patients is shown for FF in (A), TV and CV in (B) and T_{2water} in (C). The mean bias and 95% limits of agreement for FF were 0.4% (-1.3 to 2.0%), for TV 0.4 cm³ (-0.8 to 1.7 cm³), for CV 0.3 cm³ (-0.8 to 1.5 cm³), and for T_{2water} 0.1 ms (-0.8 to 0.9 ms).

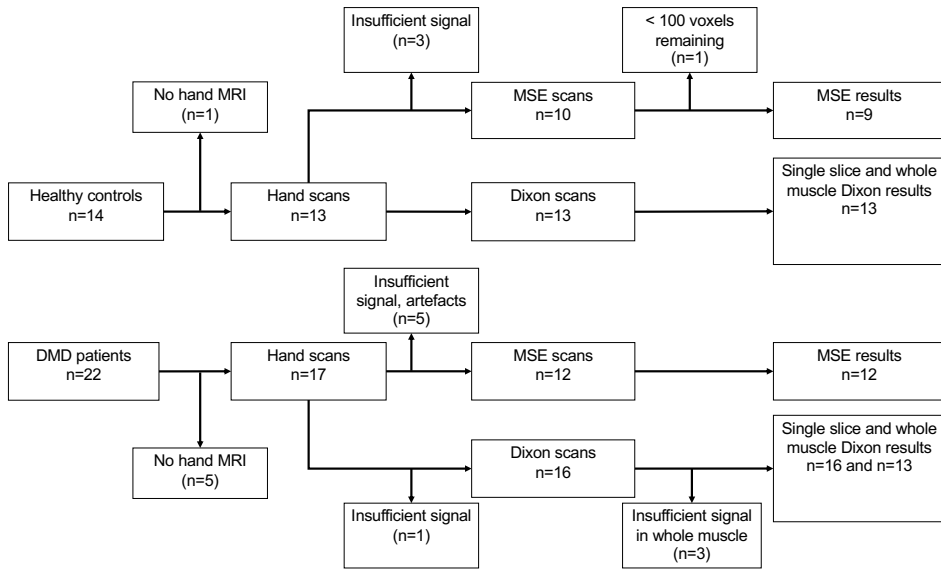


Figure S4 Flowchart of MRI data inclusion

Twenty-two DMD patients and 14 HC participated in the study. Five DMD patients could not undergo the MRI scans of the hand muscles: two patients decided not to undergo the MRI at the baseline visit, two patients were unable to complete the scans due to discomfort, and a technical failure of the MRI led to absence of the scans for the last patient. In one HC no MRI scans were acquired due to the relative contra-indication of dental braces. Four Dixon scans and five MSE scans of DMD patients were excluded due to insufficient signal or movement artefacts, and four MSE scans of HCs were excluded due to insufficient signal or because less than 100 thenar voxels with a sufficient B1 remained. In total 13 Dixon scans from HCs and 13 from DMD patients were included in the analysis of the whole thenar muscles. Similarly, MSE scans from nine HCs and 12 DMD patients were included in the analysis.

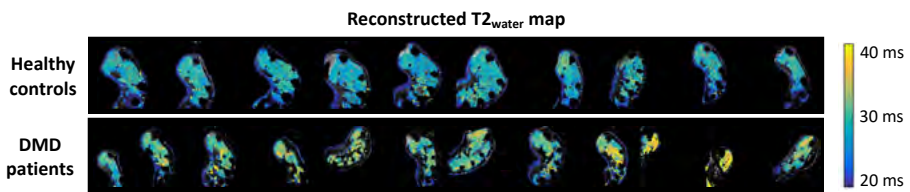


Figure S5 $T_{2\text{-water}}$ of all hand muscles for HCs and DMD patients

The reconstructed $T_{2\text{-water}}$ map for one slice per HC and DMD patient is shown in all participants to illustrate the higher $T_{2\text{-water}}$ values in hand muscles in DMD patients versus HCs.

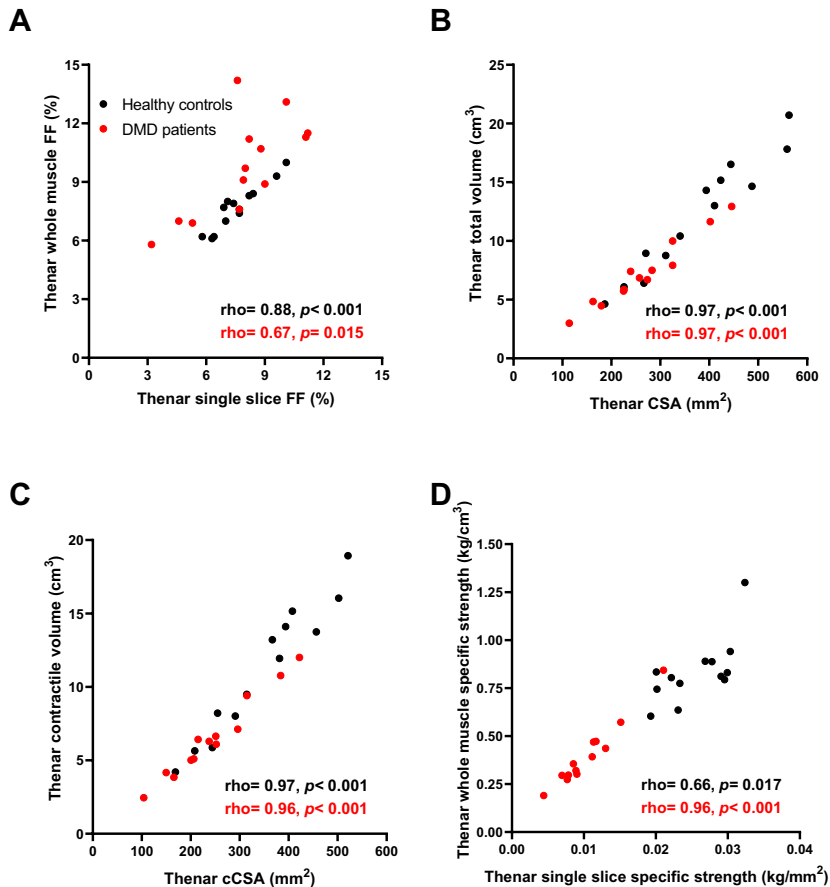


Figure S6 Single slice versus whole muscle Dixon thenar results
Dixon thenar whole muscle results are plotted versus the single slice results, for HCs (black; n=13) and DMD patients (red; n=13). The comparison is presented for FF in (A), CSA and TV in (B), cCSA and CV in (C), and specific strength in (D). All correlations between single slice and whole muscle values were strong, except FF in DMD patients and specific strength in HCs, which were moderate.

Single slice versus whole muscle results

If patient positioning would influence thenar qMRI results, this effect is expected to be larger if results would be determined on one slice instead of the whole muscle. In addition, to reduce scan time in the future, we assessed if qMRI results could be determined only on a single slice. To study this in all DMD patients and HCs, we also assessed all Dixon results for the slice with the largest cross-sectional area (CSA). This yielded a single slice thenar FF, CSA, contractile CSA (cCSA), and specific strength (pinch strength/cCSA). Spearman correlation coefficient was used to assess the correlation of the single slice and whole muscle Dixon results for HCs and DMD patients separately.

All correlations between single slice and whole muscle Dixon results were strong, except for FF in DMD patients and specific strength in HCs, which were moderate (figure S6A until D).

