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Effects of Duchenne muscular dystrophy on muscle stiffness and response to electrically-induced muscle contraction: A 12-month follow-up

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Abstract

The present study aimed to assess the ability of muscle stiffness (shear modulus) and response to electrically-induced muscle contraction to detect changes in muscle properties over a 12-month period in children with Duchenne muscular dystrophy (DMD). Ten children with DMD and nine age-matched healthy male controls participated in two experimental sessions (T_0 and $T_{+12months}$) separated by 12.4 ± 0.9 months. Two contractions of the *biceps brachii* were electrically-induced during which an ultrasound probe was placed over the muscle. The resting shear modulus was measured using elastography from six muscles. Evoked maximal torque was increased at $T_{+12months}$ in controls (+11.2 ± 7.6%, $P < 0.001$) but was not modified in children with DMD ($P = 0.222$). Electromechanical delay (+12.9 ± 11.3%, $P < 0.001$) and its force transmission component (+10.1 ± 21.6%, $P = 0.003$) were significantly longer at $T_{+12months}$ than T_0 for children with DMD. The results revealed an increase in muscle stiffness at $T_{+12months}$ in children with DMD for *tibialis anterior* (+75.1 ± 93.5%, $P = 0.043$), *gastrocnemius medialis* (+144.8 ± 180.6%, $P = 0.050$) and *triceps brachii* (+35.5 ± 32.2%, $P = 0.005$). This 12-month follow-up study demonstrates that electromechanical delay and elastography may help detect subtle muscle impairments in patients with DMD. These sensitive outcomes may improve the follow-up of innovative therapeutic interventions within the field of DMD.

Keywords: Elastography; Ultrafast ultrasound; Evoked muscle force; Electromechanical delay; Myopathy

1. Introduction

Promising therapeutic options are being developed for children with Duchenne muscular dystrophy (DMD) [1,2]. Because some therapies aim to target a specific muscle or limb region [3,4], the development of non-invasive and sensitive methods to locally assess both muscle function and muscle biomechanics is needed. Recent studies have revealed the potential of two approaches that have the advantage of obviating voluntary and maximal contraction by the patients [5,6].

The first approach is the electromechanical delay (EMD), defined as the time lag between the onset of muscle activation and onset of force production [7]. EMD can be assessed during electrically-induced contractions using very high frame rate ultrasound (up to 5 kHz) [8–10]. The first part of the EMD is the delay between muscle electrical stimulation and the onset of muscle fascicle motion, which is mainly attributed to electrochemical processes such as the excitation–contraction coupling. Its second part is the delay between the onset of fascicle motion and the onset of force production, which is attributed to the force transmission processes. By determining the time required for the electrochemical and mechanical processes this technique provides information on both the function and the biomechanics of a targeted muscle. Applying this method to children with DMD, Lacourpaille et al. [5] reported a longer EMD in patients with DMD compared to healthy age-matched participants. This was mainly explained by an increased time required for the force to be transmitted to the skeleton (+75%). In addition, the electrically-evoked torque was lower in children with DMD.

Second, resting muscle stiffness can be reliably estimated in DMD using ultrasound shear wave elastography [6,11]. Although an increased stiffness was found in these patients for

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five muscles *(tibialis anterior*, *gastrocnemius medialis*, *vastus lateralis*, *biceps brachii* and *triceps brachii),* there was no change for the distal *abductor digiti minimi* muscle (hand muscle) [6]. This latter result is in line with previous works showing that DMD progresses according to a proximal-distal pattern [12].

The assessment of both muscle stiffness and responses to electrically-induced contractions (EMD and torque amplitude) can provide important information related to muscle function and structure with potential relevance to clinical and therapeutic assessments. In order to bring new arguments for the use of these innovative biomechanical approaches, the present study aimed to assess their ability to detect changes in muscle properties over a 12-month period in children with DMD. We hypothesized that both muscle stiffness and the electromechanical delay would be increased after a 12-month period in patients with DMD.

2. Materials and methods

2.1. Participants

Ten DMD patients (genetically confirmed) and nine age-matched healthy controls volunteered to participate (Table 1). The experimental procedures were approved by the local ethics committee (Nantes Ouest IV – CPP-MIP-004) and all of the procedures conformed to the declaration of Helsinki. All the control subjects and patients with DMD have been previously reported elsewhere [5,6]. These prior articles dealt with the effects of DMD on muscle stiffness and response to electrically-induced muscle contraction whereas in this manuscript we report their ability to quantify the degenerative effects of DMD over a 12-month period.

2.2. Measurements

Elbow flexion force. Participants were seated with their right shoulder abducted (90°), elbow flexed at 90° with their wrist in a neutral position. To measure the force produced during elbow flexion, a force transducer (SML-50, Interface, USA; range: 0–50 lbf, sensitivity: 2 mV/V) was incorporated in a homemade ergometer and connected with Velcro straps to the wrist to ensure constant contact. The force signal was digitized at a sampling rate of 5 kHz (MP36, Biopac, Goleta, USA).

Electrical stimulation. Percutaneous electrical stimulation was applied over the *biceps brachii* to elicit its contraction. A constant-current stimulator (Digitimer DS7A, Digitimer, Letchworth Garden City, UK) delivered a single electrical pulse (pulse duration = 200 μs, 400 V) through two electrodes (2 × 1.5 cm, Compex, Annecy-le-Vieux, France) placed on the main motor point (previously determined as the location inducing the strongest twitch with the lowest electrical stimulation) and on the distal portion of the *biceps brachii* muscle. To determine the stimulation intensity required to induce the maximal elbow flexion force (Imax), the output current was increased by steps of 5 mA until a maximum force output or a maximum tolerable current output was reached.

Ultrasound. A very high frame rate ultrasound scanner (Aixplorer, version 7, Supersonic Imagine, Aix-en-Provence, France), coupled with a linear transducer array (4–15 MHz, SuperLinear 15–4, Vermon, Tours, France), was used in "research" mode to acquire raw radio-frequency signals at 4 kHz. Force and ultrasound data were synchronized using transistor–transistor logic pulses, as previously described [10,13].

Muscle stiffness. An Aixplorer ultrasound scanner (Aixplorer, version 7, Supersonic Imagine, France) coupled with a linear transducer array (4–15 MHz) was used in shear wave elastography mode [11]. This technique provides a 2-dimensional map of shear modulus (*i.e.*, index of muscle stiffness) of a localized area in real-time at one sample/s. Good reliability of this technique has been demonstrated [14]. For each muscle and each position, ten successive shear elastic measurements were averaged to obtain a representative value [14].

2.3. Protocol

DMD patients and healthy participants participated in two experimental sessions (T_0 and $T_{+12months}$) separated by 12.4 ± 0.9 months.

Electrically-induced contractions. Electrically-induced contractions were used to assess the amplitude of the elbow flexion torque and the electromechanical delay. To determine the minimal stimulation intensity required to induce the maximal elbow flexion force (Imax), the output current was incrementally increased (incremental step of 5 mA) until a maximum force output was reached [13]. The maximal elbow flexion force produced at Imax was used to calculate the evoked maximal torque. Two selective contractions of the *biceps brachii* were elicited by means of percutaneous electrical stimulation at 70% of Imax, with 15-s rest in between. This submaximal intensity was chosen to limit the discomfort associated with the stimulation and because we previously demonstrated that the EMD was not affected by an increase in stimulus intensity above 70% of Imax [13]. During these two electrically-evoked contractions, the ultrasound probe was placed over the thickest part of the *biceps brachii* muscle belly, parallel to the muscle fascicles [5]. Participants were instructed to be fully relaxed prior and during the stimulations.

Table 1
Individual data for DMD patients.

DMD patients (#)	Age	ACE	Corticosteroids	Thiocolchicoside	Calcium	Vitamin D
1	10			X	X	X
2	12				X	X
3	8	X			X	X
4	10					
5	7					
6	23	X				
7	8		X		X	X
8	22	X				
9	22	X				
10	14		X		X	

For each DMD patient, both age and medications provided as part of their standard care management are depicted. ACE, angiotensin-converting enzyme.

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Muscle stiffness. The resting shear modulus was measured at two muscle lengths (shortened and stretched) from six muscles on the right side of the body: *gastrocnemius medialis*, *tibialis anterior*, *vastus lateralis*, *biceps brachii*, *triceps brachii*, and *abductor digiti minimi*. Calcium sensitivity being higher at long muscle length [15,16], increase in muscle shear modulus subsequent to structural alterations is larger when the muscle is stretched [17]. Therefore, we hypothesized that change in shear modulus would be larger in patients with DMD at long muscle length compared to short muscle length. However, as the stretched positions can be difficult to reach for patients with high degree of joint contractures (50% of the DMD patients over 9 years of age [18]; – not observed in our population) we also tested the short position that might be easier to use in clinical settings. To assess the *gastrocnemius medialis* muscle the knee was flexed at 90° (shortened) or fully extended (stretched) with the ankle in neutral position. For the *tibialis anterior* muscle, the knee was extended fully with the ankle in neutral position (shortened) or plantarflexed at 20° (stretched). For the *vastus lateralis* muscle, the knee was extended fully (shortened) or flexed at 90° (stretched). For the *biceps brachii* muscle, the elbow was flexed at 90° (shortened) or overextended along the body (stretched), with the hand in neutral position. For the *triceps brachii* (long head) muscle, the arm was extended along the body (shortened) or abducted and flexed at 90° (stretched). For the *abductor digiti minimi* muscle, the hand was placed in pronation, and the examiner manually maintained the fifth finger in maximal abduction (shortened) or in alignment with the fifth metacarpal (stretched). For each elastographic measurement, the probe was placed on the thickest part of the muscle belly [10]. Participants were lying on a plinth and were asked to relax during each recording that lasted approximately 10 s for each muscle.

2.4. Data analysis

Data processing of very high frame rate ultrasound device was performed using Matlab scripts (The Mathworks, Natick, MA).

Electrically-induced contractions. The ultrasound B-mode images were used to determine the region of interest (ROI) as the muscle region between the two aponeuroses of the *biceps brachii* muscle [6,10]. The displacements along the ultrasound beam axis were calculated using a one-dimensional cross correlation of the windows of consecutive radio-frequency signals [19,20] and therefore the tissue motion between the two consecutive images (*i.e.*, particle velocity) was measured with micrometric precision. Displacements were then averaged over the previously determined ROI, and these averaged signals were used to detect the onset of muscle fascicle motion. As previously described in Lacourpaille [10], the detection of both muscle fascicle motion and external force production onsets was defined visually and blinded (the experimenter was not aware of the condition when doing the detection). The EMD was defined as the time lag between the onset of the electrical stimulation (*i.e.*, artifact of stimulation) and the onset of force production (Fig. 1) [8,9,21]. Then, delays between the onset of electrical stimulation and the onset of muscle fascicle motion

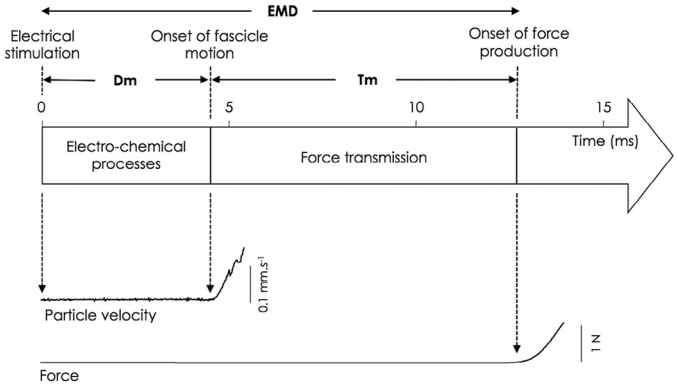

Fig. 1. Representation of the electromechanical delay (EMD) and its components. The delay between muscle electrical stimulation and the onset of muscle fascicle motion (particle velocity) is mainly attributed to electrochemical processes [referred to as time delay for muscle contraction (Dm)]. The delay between the onset of fascicle motion and the onset of force production is attributed to the force transmission [referred to as time delay for force transmission (Tm)].

[referred to as electrochemical processes (Dm)] and between the onset of fascicle motion and the onset of force production [referred to as force transmission processes (Tm)] were calculated (Fig. 1). A good inter-day reproducibility of these delays has been shown in healthy participants (standard error of measurement = 0.66 and 0.51 ms, and coefficient of variation = 6.8% and 12.4% for EMD and Dm, respectively) [13].

Muscle stiffness. SSI recordings for muscle stiffness were exported from software (Version 7.0, Supersonic Imagine, Aix en Provence, France) in “mp4” format and sequenced in “jpeg” with custom-written scripts designed using Matlab (version 10.0, The Mathworks, Inc., Natick, Massachusetts). Image processing converted the colored map into shear elastic modulus values. For each image, the average value of shear elastic modulus was calculated over a ROI corresponding to the largest muscular region by avoiding fascia.

2.5. Statistics

Due to the inability to properly maintain the ultrasound probe over the *vastus lateralis* belly in patients in wheelchairs and to identify clearly the *vastus lateralis* for some patients with DMD, this muscle was not included in the statistical analysis.

Analyses of variance (ANOVAs) with repeated measures were applied to test the effect of time on each variable. To assess the effect of DMD on mechanical response to electrically-evoked muscle contractions, two-way ANOVAs (within-subject factor: Time; between-subject factor: Group) were performed separately for each parameter (Imax, torque, EMD, Dm and Tm). Changes in stiffness were assessed for each muscle using three-way ANOVAs (within subject factors: Time and Position; Between-subject factor: Group). As baseline results have been previously published [5,6], the analysis focused on the time × group interactions. *Post-hoc* analyses were performed using the Newman–Keuls method. Statistical significance was

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set at $P < 0.05$. In addition, Cohen's d values were calculated to compare the magnitude of differences between groups (polled standard deviation as the standardizer) as measures of effect size with 0.2, 0.5, and 0.8, as small, moderate, and large effects, respectively [22].

3. Results

No significant time × group interaction was found for Imax ($P = 0.163$). The mean Imax was 83.4 ± 20.5 mA. When considering the electrically-induced contractions, no significant time × group interaction was observed for Dm ($P = 0.455$) but a significant time × group interaction was found for EMD ($P = 0.008$), Tm ($P = 0.039$) and evoked maximal torque ($P < 0.001$). *Post hoc* analyses revealed that the evoked maximal torque increased at $T_{+12months}$ in controls (+11.2 ± 7.6%, $d = 2.1$, $P < 0.001$) but Tm ($P = 0.382$) and EMD ($P = 0.999$) did not change. In contrast, when considering children with DMD, no change in the evoked maximal torque was found ($P = 0.222$) (Fig. 2a) but both EMD (+12.9 ± 11.3%, $d = 2.5$, $P < 0.001$) and Tm (+10.1 ± 21.6%, $d = 1.27$, $P = 0.003$) were significantly longer at $T_{+12months}$ than T_0 (Fig. 2b, c). Together, these results show a progressive lengthening of the time required for the muscle force to be transmitted to the skeleton in children with DMD while electrochemical processes and maximally evoked torque were unchanged.

Fig. 3 depicts representative examples of the shear modulus (index of stiffness) maps obtained for *gastrocnemius medialis* [stretched position; (a)] and *tibialis anterior* [shortened position; (b)] for controls and DMD patients. A time × group interaction was found for *tibialis anterior* ($P = 0.043$) and *triceps brachii* ($P = 0.005$). More precisely, *tibialis anterior* (+75.1 ± 93.5%, $d = 1.04$, $P = 0.009$) and *triceps brachii* shear modulus (+35.5 ± 32.2%, $d = 0.29$, $P < 0.001$) were significantly higher at $T_{+12months}$ than T_0 in patients with DMD, regardless of the muscle length (no significant interaction involving the factor length, all P values > 0.133) (Table 2) but there was no change for controls (all P values > 0.369). A significant time × length × group interaction was found for *gastrocnemius medialis* shear modulus ($P = 0.050$) showing an increase in *gastrocnemius medialis* shear modulus measured in the lengthened position at $T_{+12months}$ (+123.6 ± 180.2%, $d = 1.05$; $P < 0.001$) compared to T_0 in patients with DMD (Table 2). No significant changes were found for healthy subjects ($P > 0.715$). Neither significant main time effect, time × group interaction nor time × length × group interaction was found for *biceps brachii* and *abductor digiti minimi* (P values ranging from 0.053 to 0.653). Together, these results indicate an increased stiffness of some but not all muscles in patients with DMD over the 1-year follow-up while stiffness was stable in controls.

4. Discussion

This 12 month follow-up study demonstrated the ability of two non-invasive approaches to detect changes in both muscle function and muscle biomechanics in children with DMD. Interestingly, the present data showed a progressive lengthening of the time required for the muscle force to be transmitted to the skeleton in patients with DMD while maximally evoked torque was unchanged. It therefore suggests that, during childhood, growth and maturation may partly compensate for strength decrease but not for force transmission deterioration, which in turn is more inherent to the disease process. For adult patients ($n = 3$), it is likely that the degenerative processes occurring over 12 months are not sufficient to reduce significantly a maximal evoked torque already very low (10% of the healthy participants). The results also revealed an increased localized muscle stiffness in patients with DMD for *tibialis anterior*, *gastrocnemius medialis* and *triceps brachii* (up to +145% for *gastrocnemius medialis*/stretched).

The present study reports no significant changes in evoked maximal torque over a 12-month period in patients with DMD. This is in line with previous results showing that maximal voluntary elbow flexion torque was not significantly changed over 6 years in patients with DMD (from 6 to 12 years old), while maximal knee extension torque was highly reduced [18].

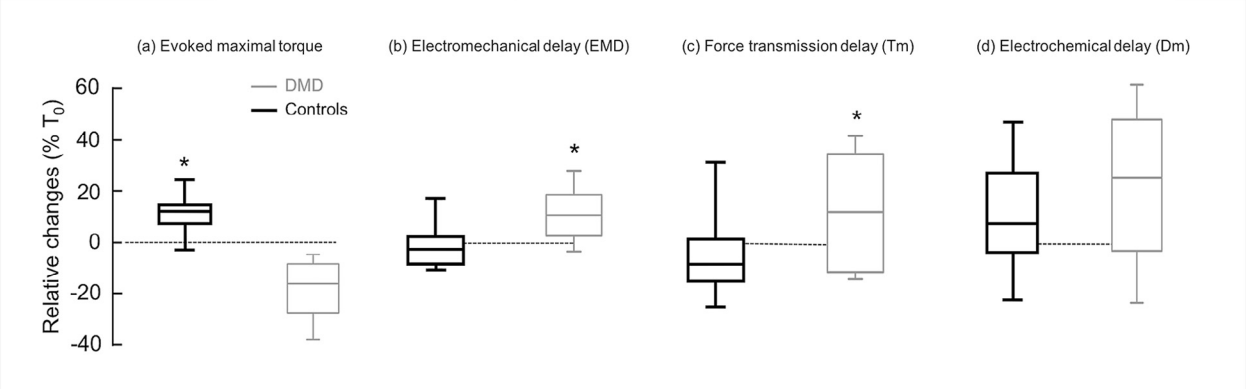

Fig. 2. Changes in force-generating capacities over 12 months in control subjects and patients with DMD. Change in maximal evoked torque (a), electromechanical delay (b), time delay between the onset of fascicle motion and the onset of force production (force transmission delay, Tm) (c) and time delay between the onset of electrical stimulation and the onset of fascicle motion (electrochemical process, Dm) (d) expressed in percentage of baseline values (T_0) for both control subjects (Controls, black box) and patients with DMD (DMD, grey box). Error bars denote the 95% confidence interval; box denotes the 25th–75th percentiles with the median. The asterisk symbol indicates that the change from baseline is significant.

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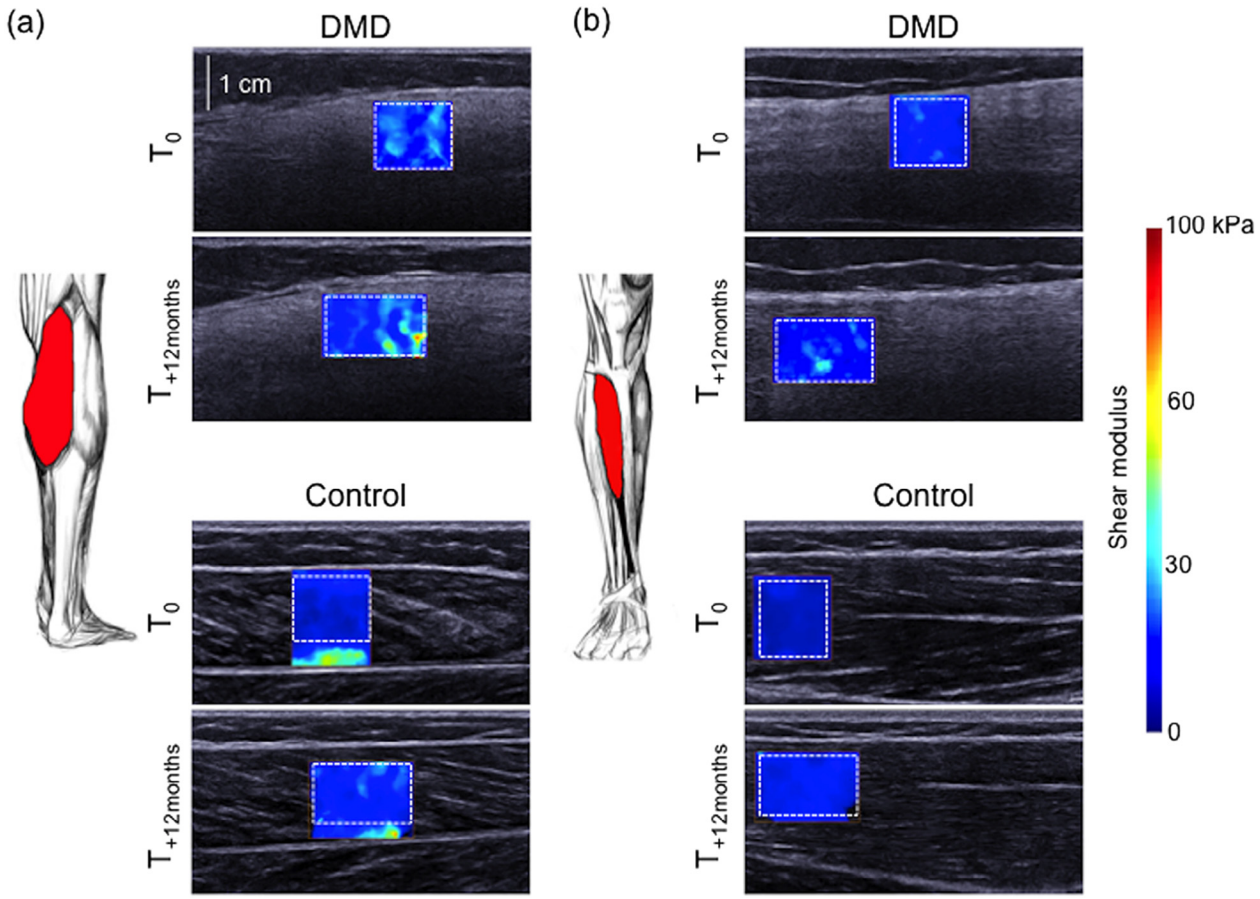

Fig. 3. Individual example of shear modulus maps of the *gastrocnemius medialis* and *tibialis anterior*. The map of shear modulus (*i.e.*, index of stiffness) is superposed onto the B-mode image, with the color scale depicting the graduation of shear modulus (scale in kPa, blue being soft and red being stiff). Typical example of shear elastic modulus maps of the *gastrocnemius medialis* (a) and *tibialis anterior* (b) obtained for a DMD patient (on the top) and a control subject (on the bottom) at T_0 and $T_{+12months}$. The shear modulus (in kPa) was averaged over the region of interest indicated by the white dashed rectangle. Note the stability of the shear modulus map in the controls, and the slight increase of stiffness in the muscles of DMD patients.

Therefore, even if muscle weakness is a striking feature of DMD patients that is commonly assessed throughout the course of the disease, its sensitivity to detect degenerative effects of DMD differs among muscles. This may be due in part to the proximo-distal progression associated with the natural history of the disease [12]. In parallel, there are several biomechanical factors affecting muscle force that can be differentially affected by DMD, *e.g.*, muscle size, architecture, typology, force transmission efficiency [23–25]. In the case of degenerative effects of DMD for children, compensations for the changes in muscle force should occur involving growing-up and maturation processes. In the present study, the localized assessment of EMD using ultrafast ultrasound is sensitive to the degenerative effects of DMD (*i.e.*, large effects size). We found that the time between the onset of *biceps brachii* fascicle shortening and the onset of force production (Tm) was significantly lengthened over 12 months in patients with DMD (+1.55 ms; Fig. 2c). In contrast, the time delay between the electrical stimulation and the onset of fascicle motion (Dm) was not significantly changed over 12 months in both healthy

Table 2
Shear modulus of the 5 muscles measured in control subjects and patients with DMD over 12 months.

		TA		GM		BB		TB		ADM	
		Short	Long	Short	Long	Short	Long	Short	Long	Short	Long
Controls (kPa)	T_0	7.2 ± 2.2	11.5 ± 1.9	4.6 ± 0.5	15.6 ± 3.4	4.1 ± 0.4	18.6 ± 4.1	4.6 ± 1.0	8.1 ± 1.1	8.0 ± 2.2	10.7 ± 3.0
	T_{+12m}	6.9 ± 2.0	12.8 ± 3.1	4.8 ± 0.7	14.6 ± 3.2	3.8 ± 0.5	19.0 ± 7.4	4.8 ± 1.0	7.4 ± 1.6	7.4 ± 2.1	11.2 ± 4.2
DMD (kPa)	T_0	8.2 ± 1.8	21.3 ± 14.9	7.1 ± 4.0	23.0 ± 11.3	6.0 ± 4.4	37.3 ± 27.9	6.3 ± 1.8	9.1 ± 1.8	8.4 ± 3.3	13.6 ± 5.4
	T_{+12m}	**14.6 ± 10.8**	**31.5 ± 19.7**	6.8 ± 2.8	**41.3 ± 23.2**	6.0 ± 2.6	36.2 ± 26.0	**8.3 ± 2.4**	**12.9 ± 2.3**	8.6 ± 1.9	16.8 ± 6.7

Shear modulus values (kPa) are presented for both muscle lengths (see Methods for descriptions of the short and long tested positions). DMD, Duchenne muscular dystrophy; TA, *tibialis anterior*; GM, *gastrocnemius medialis*; BB, *biceps brachii*; TB, *triceps brachii*; ADM, *abductor digiti minimi*. Significant differences between T_0 and T_{+12m} ($P < 0.05$) are identified in bold.

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participants and patients with DMD ($P = 0.455$). Together, these findings suggest that EMD and its force transmission component can be considered as a more sensitive measure of muscle alteration in DMD patients than maximally evoked torque. As EMD is a simple addition of Dm and Tm, it could be an easy to use and useful outcome to study muscle degeneration *in vivo*. In addition, the longer delay between muscle fascicle shortening and force production reported in the present study provides a better understanding of DMD muscle biomechanics. It provides evidence of the progressive impairment of muscle force transmission efficiency in DMD, as previously demonstrated in animal cross-sectional studies [26,27]. Thus, the present study reinforces our previously mentioned hypothesis [5] suggesting that structural abnormalities such as sarcomere disconnections [28], malformed/branched fibers, and detached fibers from tendons [29] lead to an alteration of the longitudinal transmission from the force-generating structures to the bone. This phenomenon may compensate the increase in muscle shear modulus which should increase the force transmission efficiency. The technique proposed in the present study enables to non-invasively assess these effects *in vivo*.

Although joint contractures are commonly observed in patients with DMD [18], data on changes in muscle mechanical properties are scarce [6,30–32]. Cross-sectional studies showed an increased stiffness of some but not all muscles in patients with DMD compared to controls [6], however no study quantified these changes over time. In the present study, we found an increase in *tibialis anterior*, *gastrocnemius medialis* and *triceps brachii* stiffness over 12 months in patients with DMD (up to 145% for *gastrocnemius medialis*/stretched). This result is likely due to the progressive loss of muscular tissue and its replacement by fibrous connective tissue, which represents approximately 50% of the *gastrocnemius medialis* muscle content for a 14-year-old DMD patient [33]. Note that other structural changes such as the proportion of fast- and slow-twitch muscle fibres, muscle damage and necrosis can also contribute to the observed increase in muscle stiffness [34,35]. Interestingly no significant changes were observed for *biceps brachii* and *abductor digiti minimi* muscles that are known to be affected at a later stage [12,18].

In degenerative muscle disease, continuous muscle fascicles are less visible and visualizing their main orientation is therefore challenging (see Fig. 3). Consequently, we chose to measure the shear modulus in the muscle shortening direction. Note that this direction is the most important one for the measurement of muscle stiffness and corresponds to the direction measured on isolated muscles using traditional material testing. Therefore, we consider that our measurement represents the "physiological" stiffness. However, it is important to note that this measurement does not represent the stiffness of fascicles and is influenced by the relative angle of the transducer to the muscle fascicles [36,37].

5. Conclusions

Overall, the non-invasive and localized evaluation methods used in this study offer a unique opportunity to non-invasively assess muscle stiffness, force transmission and force production in patients with DMD. The development of such assessments could contribute to a better understanding of the origin of progressive impairments in two key-domains: muscle contracture and muscle weakness. The results suggest that, during childhood, growth and maturation may partly compensate for strength decrease but not for force transmission deterioration, which in turn is more inherent to the disease process. These new sensitive outcomes may improve the accuracy of the patient monitoring during therapeutic interventions in the field of DMD, especially during localized therapy.

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