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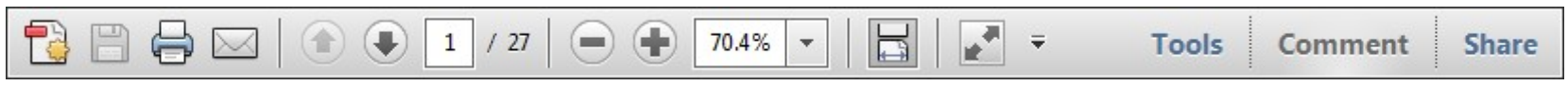

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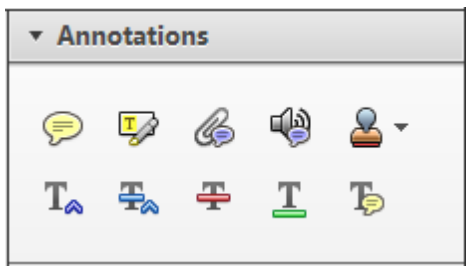

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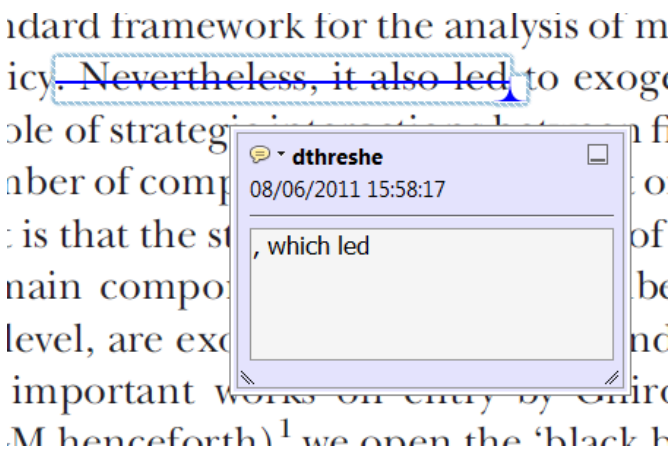

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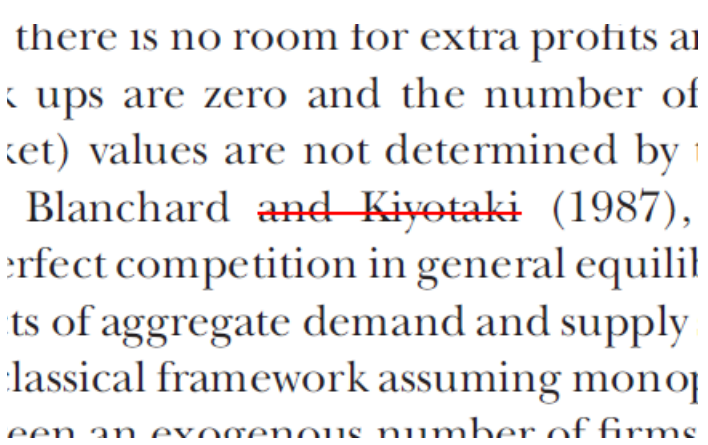

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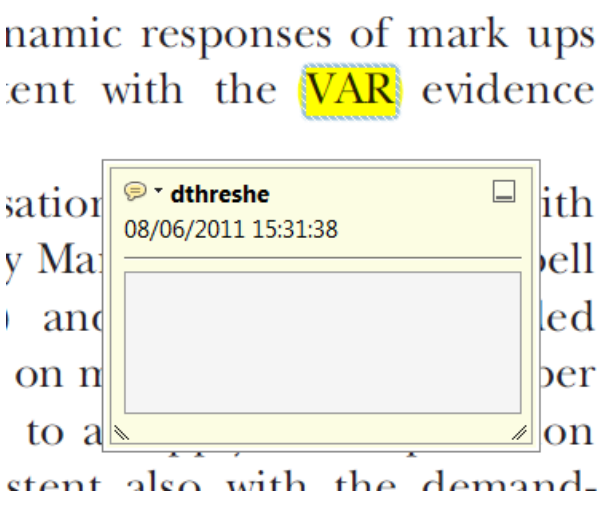

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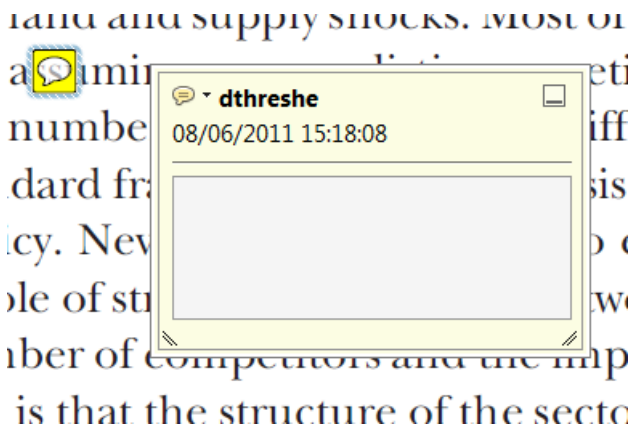

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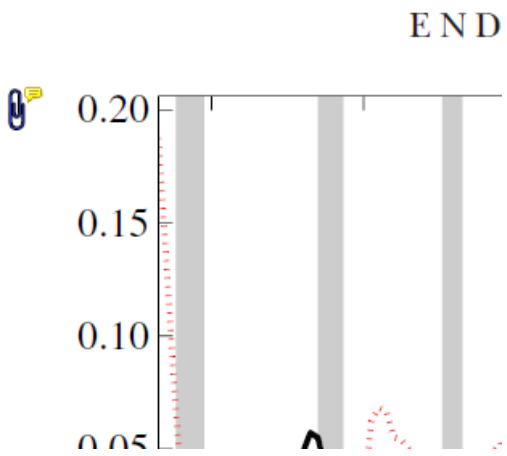

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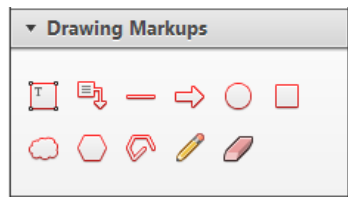

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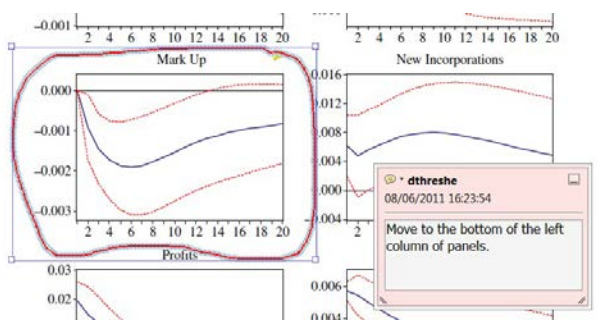

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SHORT REPORTS

NON-INVASIVE ASSESSMENT OF MUSCLE STIFFNESS IN PATIENTS WITH DUCHENNE MUSCULAR DYSTROPHY

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ABSTRACT: *Introduction:* Assessment of muscle mechanical properties may provide clinically valuable information for follow-up of patients with Duchenne muscular dystrophy (DMD) through the course of their disease. In this study we aimed to assess the effect of DMD on stiffness of relaxed muscles using elastography (supersonic shear imaging). *Methods:* Fourteen DMD patients and 13 control subjects were studied. Six muscles were measured at 2 muscle lengths (shortened and stretched): gastrocnemius medialis (GM); tibialis anterior (TA); vastus lateralis (VL); biceps brachii (BB); triceps brachii (TB); and abductor digiti minimi (ADM). *Results:* Stiffness was significantly higher in DMD patients compared with controls for all the muscles (main effect for population, $P < 0.033$ in all cases), except for ADM. The effect size was small ($d = 0.33$ for ADM at both muscle lengths) to large ($d = 0.86$ for BB/stretched). *Conclusions:* Supersonic shear imaging is a sensitive non-invasive technique to assess the increase in muscle stiffness associated with DMD.

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DMD is associated with alteration of muscle mechanical properties.[1–4] Increased stiffness is correlated with the stage of disease[4] and contributes to the patient's progressive loss of autonomy.[2,5] Consequently, assessment of muscle mechanical properties may provide clinically important information for follow-up of patients through the course of their disease and for assessment of the efficacy of potential therapies.[2]

Muscle mechanical properties have been assessed to date using sinusoidal perturbations or quick release techniques,[3,4] which have several limitations. First, they require specific dynamometers designed for a given articulation, which makes it difficult to assess mechanical properties of different joints. Second, they cannot isolate the behavior of an individual muscle, but rather they represent the global behavior of several muscles acting around a given joint. This is particularly problematic, as muscles can be affected differently, even within the same limb.[6–8] Finally, these techniques require patients to perform voluntary muscle contractions, which can be painful, difficult to perform, and deleterious.[9]

In this study we aimed to assess the effect of DMD on stiffness of relaxed muscles using elastography (supersonic shear imaging, SSI).

METHODS

Participants. Fourteen DMD patients [13.3 ± 5.9 years (range 5–22 years)] and 13 age-matched, healthy controls [12.8 ± 5.5 years (range 6–24 years)] volunteered to participate in this investigation. The local ethics committee approved the study, and all procedures conformed to the Declaration of Helsinki. Data from the biceps brachii have been published elsewhere.[10]

Shear Elastic Modulus. An ultrasound scanner (Aixplorer; Supersonic Imagine, France) coupled with a linear transducer array (4–15 MHz) was used in shear wave elastography mode. This technique quantifies the shear elastic modulus (stiffness) of a localized area of tissue and generates 2-dimensional maps of elasticity in real-time (1 sample/s; see other studies[11,12] for further information). Good reliability of this SSI technique has been demonstrated previously.[12] For each muscle and each position, 10 shear elastic measurements were averaged to obtain a representative value.[12]

Protocol. The shear elastic modulus was measured at 2 muscle lengths (shortened and stretched) from 6 muscles (right side): gastrocnemius medialis (GM); tibialis anterior (TA); vastus lateralis

Abbreviations: ADM, abductor digiti minimi; ANOVA, analysis of variance; BB, biceps brachii; DMD, Duchenne muscular dystrophy; GM, gastrocnemius medialis; SSI, supersonic shear imaging; TA, tibialis anterior; TB, triceps brachii; VL, vastus lateralis

This study was supported by grants from the European Regional Development Fund (No. 37400), the French Muscular Dystrophy Association (Contract No. 14084), and the Region des Pays de la Loire.

Key words: elastography; evaluation; myopathy; shear elastic modulus; supersonic shear imaging

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Table 1. Shear elastic modulus of the 6 muscles measured in control subjects and patients with DMD.

	TA		GM		VL		BB		TB		ADM	
	Short	Long	Short	Long	Short	Long	Short	Long	Short	Long	Short	Long
Controls Mean (kPa)	7.0	12.5	4.9	14.5	5.4	9.6	3.9	18.9	5.0	7.3	7.5	11.9
SD (kPa)	1.9	3.1	0.9	3.5	1.7	2.2	0.4	6.4	0.9	1.4	2.2	5.0
n	13	13	13	13	13	13	13	13	13	13	13	13
DMD Mean (kPa)	7.9	23.1	8.2	21.9	12.9	21.5	5.8	34.9	6.2	8.7	8.7	14.1
SD (kPa)	1.8	14.7	5.1	12.7	11.3	18.8	4.2	23.3	1.7	2.1	4.6	7.8
n	13	13	13	13	12	9	13	11	12	12	13	13
Statistics Population	**0.014**		**0.026**		**0.004**		**0.033**		**0.019**		0.394	
Length	**0.000**		**0.000**		**0.001**		**0.000**		**0.000**		**0.000**	
Interaction	**0.026**		0.201		0.069		**0.048**		0.681		0.446	
Post hoc	0.991	**0.004**	—		—		0.999	**0.017**	—		—	
Cohen *d*	0.48	0.99	0.91	0.79	0.89	0.89	0.63	0.86	0.84	0.74	0.33	0.33

Data are presented for both muscle lengths (see Methods for descriptions of the short and long tested positions). Statistics data are from results of analyses of variance (between-subject factor: population; within-subject factor: length; and population × length interaction). Significant P-values are shown in bold. DMD, Duchenne muscular dystrophy; n, number of participants and patients included in the data analysis; TA, tibialis anterior; GM, gastrocnemius medialis; VL, vastus lateralis; BB, biceps brachii; TB, triceps brachii; ADM, abductor digiti minimi.

(VL); biceps brachii (BB); triceps brachii (TB); and abductor digiti minimi (ADM). Briefly, for GM the knee was flexed at 90° (shortened) or fully extended (stretched) with the ankle in neutral position. For TA, the knee was extended fully with the ankle angle in neutral position (shortened) or plantarflexed at 20° (stretched). For VL, the knee was extended fully (shortened) or flexed at 90° (stretched). For BB, the elbow was flexed at 90° (shortened) or overextended along the body (stretched), with the hand in neutral position. For TB, the arm was extended along the body (shortened) or abducted and flexed at 90° (stretched). For ADM, 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). Participants were lying on a plinth and were asked to relax during each recording that lasted approximately 10 seconds for each muscle.

Statistics. Measurements were not performed in 1 DMD patient who was unable to remain relaxed. Data are thus reported for 13 patients. VL was not measured in 1 DMD patient in shortened position, and in 4 patients in stretched position. BB was not measured in 2 DMD patients in stretched position. TB was not measured in 2 DMD patients in both positions. These missing data are explained by the inability to reach the desired position due to contractures in 1 patient or by experimental constraints (i.e., inability to properly maintain the ultrasound probe over the VL belly in patients in wheelchairs and the quality of the B-mode image in 1 patient that made it impossible to localize the muscle). Given the exploratory nature of this study, and because of missing data, an analysis of variance (ANOVA) was performed separately for each muscle (within-subject factor: muscle length; between-subject factor: population). *Post-hoc* analyses were performed using the Tukey method. Cohen *d* values are reported as measures of effect size, with 0.2, 0.5, and 0.8 as small, moderate, and large effects, respectively.[13] A separate correlation analysis was performed for each population and each muscle to determine whether the shear elastic modulus correlated with the age of participants/patients. Significance was set at $P < 0.05$.

RESULTS

A significant main effect of population was found on the shear elastic modulus for TA ($P = 0.014$), GM ($P = 0.026$), VL ($P = 0.004$), BB ($P = 0.033$), and TB ($P = 0.019$), but not for ADM ($P = 0.394$) (Table 1). A significant population × muscle length interaction was found for TA ($P = 0.026$) and BB ($P = 0.048$). At long muscle length, the TA shear elastic modulus ($P = 0.005$) and BB ($P = 0.017$) were significantly higher in DMD than in healthy participants, but no difference between the populations was found at the short length ($P = 0.991$ and 0.999 for TA and BB, respectively). The effect size (Cohen *d*) was moderate to large for all the muscles except ADM (small effect at both muscle lengths; Table 1). T1

Only the shear elastic modulus of GM at both muscle lengths correlated with age in the DMD patients ($r = 0.74$, $P = 0.005$; $r = 0.55$, $P = 0.050$, for long and short muscle length, respectively). No significant correlation was found for healthy participants ($r < 0.43$ in all cases).

DISCUSSION

Taking advantage of a non-invasive technique, we showed that muscle stiffness is increased significantly in patients with DMD (up to +136% for VL/shortened). Although a small effect size was reported ($d = 0.33$), no significant difference was found for ADM. This is in line with previous studies showing that disease severity evolves mainly in a proximal–distal manner.[14,15] The increase in stiffness was similar between the shortened and stretched position for 3 of 6 muscles (GM, VL, and TB). This is particularly relevant for patients with a high degree of joint contractures (50% of the DMD patients over age 9 years[16]) who may be unable to reach the stretched positions. Finally, the large correlation between GM stiffness and age of the DMD patients suggests that the increase in muscle stiffness is possibly related to the stage of the disease.

Overall, the results show that SSI is a sensitive non-invasive technique to quantify the increase in muscle stiffness in DMD patients. Quantification of muscle stiffness is particularly relevant in clinical settings, as increased stiffness contributes to progressive loss of autonomy.[2,5] Longitudinal studies are now necessary to determine the sensitivity of the SSI technique to detect changes in muscle stiffness throughout the course of the disease. Ultimately, this direct assessment may help clinicians to detect change in stiffness of targeted muscles early in order to adapt physiotherapy treatments and thus to prevent joint deformities and prolong autonomy. Finally, the first step of clinical trials classically consists of intramuscular injection into a specific muscle,[17–19] making it necessary to target the evaluation to the specific muscle. Therefore, the ability of SSI to quantify stiffness of a localized area of muscle tissue makes it a potentially useful tool for monitoring in clinical trials. Further study is needed to test these assumptions.

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