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New insights on contraction efficiency in patients with Duchenne muscular dystrophy

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Lacourpaille L, Hug F, Guével A, Péréon Y, Magot A, Hogrel JY, Nordez A. New insights on contraction efficiency in patients with Duchenne muscular dystrophy. *J Appl Physiol* 117: 658–662, 2014. First published August 7, 2014; doi:10.1152/japplphysiol.00544.2014.— The decrease in muscle strength in patients with Duchenne muscular dystrophy (DMD) is mainly explained by a decrease in the number of active contractile elements. Nevertheless, it is possible that other electrochemical and force transmission processes may contribute. The present study aimed to quantify the effect of DMD on the relative contribution of electrochemical and force transmission components of the electromechanical delay (i.e., time lag between the onset of muscle activation and force production) in humans using very high frame rate ultrasound. Fourteen patients with DMD and thirteen control subjects underwent two electrically evoked contractions of the biceps brachii with the ultrasound probe over the muscle belly. The electromechanical delay was significantly longer in DMD patients compared with controls (18.5 ± 3.9 vs. 12.5 ± 1.4 ms, $P < 0.0001$). More precisely, DMD patients exhibited a longer delay between the onset of muscle fascicles motion and force production (13.6 ± 3.1 vs. 7.9 ± 2.0 ms, $P < 0.0001$). This delay was correlated to the chronological age of the DMD patients ($r = 0.66$; $P = 0.01$), but not of the controls ($r = -0.45$; $P = 0.10$). No significant difference was found for the delay between the onset of muscle stimulation and the onset of muscle fascicle motion. These results highlight the role of the alteration of muscle force transmission (delay between the onset of fascicle motion and force production) in the impairments of the contraction efficiency in patients with DMD.

myopathy; force; ultrasound; electromechanical delay

DUCHENNE MUSCULAR DYSTROPHY (DMD) is one of the most severe degenerative muscle diseases characterized by a lack of dystrophin (14). Dystrophin is a protein that has a role, with other proteins, to laterally link actin filaments through the sarcolemma to the extracellular matrix (10). It is essential for the transmission of the force generated by contractile proteins (34, 35). An absence of dystrophin ultimately leads to contractile tissue wastage (27) and thus to a dramatic decrease in maximal force-generating capacity (21). This is even observed when maximal strength is normalized to muscle contractile cross-sectional area, illustrating an impairment of muscle quality (37). This alteration is mainly explained by a decrease in the

number of active contractile elements for a given muscle volume, due to fiber necrosis (1). Still, it is plausible that other electrochemical and mechanical processes might further alter the contraction efficiency.

In addition to the main muscle structural consequence of DMD, it has been shown that both muscle (12) and tendon (32) mechanical properties are altered in X-linked muscular dystrophy mice model (*mdx*). As both structural and mechanical properties may play an important role in force transmission (12, 17, 32), the efficiency of force transmission from the actomyosin cross bridges to the tendons is presumed to be altered in patients with DMD (24). The alteration of contraction efficiency in DMD patients might also be related to an alteration of electrochemical processes, as suggested by the impairment of excitation-contraction coupling reported in *mdx* mice (38). Because of the lack of experimental in vivo techniques, the effect of DMD on both muscle force transmission and excitation-contraction coupling in humans is still unknown. This information is particularly relevant for *1*) a better understanding of the physiopathology of DMD; *2*) an improvement of the methods used to follow the patients throughout the progression of their disease; and *3*) the assessment of the efficacy of new therapies.

Electromechanical delay (EMD) is the time lag between onsets of muscle activation and force production (4). The relative contribution of both electrochemical (synaptic transmission, excitation-contraction coupling) and mechanical components (force transmission) to EMD has been recently characterized in humans using very high frame rate ultrasound (15, 18, 19, 26) and mechanomyography (5, 30, 33). More precisely, the delay between muscle electrical stimulation and the onset of muscle fascicle motion 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)] (Fig. 1). This method is a unique opportunity to quantify in vivo and noninvasively the effects of DMD on both electrochemical and mechanical processes of muscle contraction and indirectly estimate the efficiency of muscle force production.

The aim of the present study was to determine the effect of DMD on the relative contribution of electrochemical (mainly synaptic transmission and excitation-contraction coupling) and mechanical (force transmission) components to the EMD in the
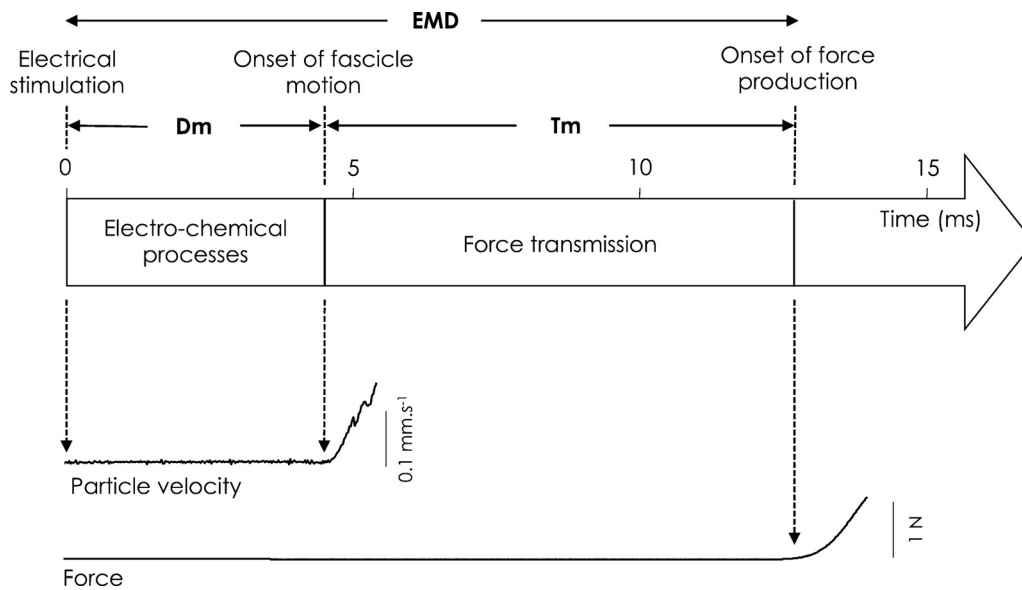
Fig. 1. Representation of the electromechanical delay (EMD) and its components. The delay between muscle electrical stimulation and the onset of muscle fascicle motion (particle velocity) is mainly attributed to electrochemical processes [referred to as time delay for muscle contraction (Dm)]. The delay 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)].

biceps brachii (BB) muscle. We hypothesized that EMD would be increased in patients with DMD compared with controls, due to an increase in the time required for both electrochemical and mechanical processes.

METHODS

Participants

Fourteen (genetically confirmed) DMD patients [age: 13.3 ± 5.9 yr (range: 5–22 yr); height: 1.41 ± 0.25 m; body mass: 40.5 ± 21.1 kg] and thirteen age-matched healthy controls [age: 12.8 ± 5.5 yr (range: 6–24 yr); height: 1.50 ± 0.20 m; body mass: 43.8 ± 17.3 kg] volunteered to participate in the present study. The medication provided to the DMD patients as part of their standard care management is listed in Table 1. Two patients were receiving corticosteroid therapy that is known to improve muscle strength in the short term (for review, see Ref. 22). Two patients were receiving muscle relaxants (thiocolchicoside), which are known to increase muscle flexibility (16, 31). Six patients were receiving cardiovascular drugs (angiotensin-converting enzyme). Finally, six and five patients were receiving calcium and vitamin D supplementation, respectively. Neither cardiovascular drugs nor calcium/vitamin D supplementation has a significant impact on neuromuscular function. All of the children and their legal guardians were informed of the purpose of the study and the potential discomfort associated with the experimental procedures before giving their written consent. The local ethics committee approved the study (CPP Nantes Ouest IV–CPP-MIP-004), and all of the procedures conformed to the Declaration of Helsinki.

Instrumentation

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, range: 0–50 lbf, insensitivity: 2 mV/V, Interface) 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).

Electrical stimulation. Percutaneous electrical stimulation was applied over the BB 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 BB. To determine the stimulation intensity required to induce the maximal elbow flexion

Table 1. *Individual data for DMD patients*

DMD Patient No.	ACE	Corticosteroids	Thiocolchicoside	Calcium	Vitamin D	EMD, ms	Dm, ms	Tm, ms
1			X	X	X	15.8	3.7	12.1
2				X	X	18.4	4.6	13.8
3	X			X	X	18.2	4.1	14.1
4						17.9	3.5	14.4
5						14.4	4.7	9.7
6	X					14.5	3.2	11.3
7			X	X	X	17.1	5.7	11.4
8	X					20.2	4.6	15.6
9			X	X	X	18.5	8.1	10.4
10	X					24.2	5.3	18.9
12	X					20.2	5.1	15.1
13		X		X		13.8	3.0	10.8
14	X					27.6	8.2	19.4

For each Duchenne muscular dystrophy (DMD) patient, both medications provided as part of their standard care management and data on electromechanical delay (and its processes) are depicted. ACE, angiontensin-converting enzyme; EMD, electromechanical delay; Dm, time delay for muscle contraction; Tm, time delay for mechanical force transmission. EMD measurements were not taken for *patient 11*.

force (Imax), the output current was increased (incremental step of 5 mA) until a maximum force output (or a maximum tolerable current output) was reached.

Ultrasonography. 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 (18, 19).

Protocol

After taking elastographic recordings to assess muscle stiffness (Lacourpaille et al., unpublished observations), two contractions of BB were electrically evoked at 70% of Imax previously determined (see above). 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 (19). During the two electrically evoked contractions, the ultrasound probe was placed over the BB muscle belly, parallel to the muscle fascicles. Participants were instructed to be fully relaxed before each stimulation.

Data Analysis

Data processing of very high frame rate ultrasound device was performed using Matlab scripts (The Mathworks, Natick, MA). The ultrasound B-mode images were used to determine the region of interest for each contraction (i.e., between the two aponeurosis of the BB muscle) (19). The displacements along the ultrasound beam axis were calculated using a one-dimensional cross correlation of the windows of consecutive radio-frequency signals (3, 9). Thus 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 region of interest, and these averaged signals were used to detect the onset of muscle motion. As previously described in Lacourpaille et al. (18, 19), the detection of the onset of both muscle fascicle motion and external force production was defined visually. We defined the EMD as the time lag between the onset of the electrical stimulation (i.e., artifact of stimulation) and the onset of force production (15, 18, 19, 26). Then delays between the onset of electrical stimulation and the onset of muscle fascicle motion (Dm) and between the onset of fascicle motion and the onset of force production (Tm) were calculated (Fig. 1). These delays have shown a good interday reproducibility in healthy participants (SE of measurement = 0.66 and 0.51 ms, and coefficient of variation = 6.8 and 12.4% for EMD and Dm, respectively; Ref. 19).

Statistical Analysis

All analyses were performed with Statistica Version 7.0 (StatSoft, Tulsa, OK). Data distributions consistently passed the Kolmogorov-Smirnov normality test, and thus all data are reported as means ± SD. The level of significance was set at $\alpha < 0.05$.

The mean maximal electrically evoked torque was compared between the two populations (DMD and control subjects) with a Student *t*-test. Because Tm was dependent on both EMD and Dm (Tm = EMD − Dm), three separate Student's *t*-tests were used to test whether EMD, Dm, and Tm were different between populations (DMD patients and control subjects). Finally, correlation analyses (Bravais-Pearson) were performed for each population to determine whether torque, EMD, Dm, or Tm were correlated to chronological age.

RESULTS

EMD measurements based on electrically evoked contractions were not taken for one DMD patient (*patient 11*). Thus this part of the protocol includes 13 DMD patients.

The mean Imax was 86.7 ± 20.0 and 83.5 ± 18.0 mA for controls and DMD patients, respectively. The results of maximal torque production, EMD, Dm, and Tm are shown in Table 1 (individual data for DMD patients) and Table 2 (averaged data). Maximal electrically evoked elbow flexion torque was significantly lower for DMD patients, representing 10.4 ± 5.9% of the torque values measured in control subjects ($P < 0.0001$). Although no significant correlation was found between the chronological age and torque for DMD patients ($r = -0.14$; $P = 0.65$), a significant positive correlation was found for healthy controls ($r = 0.90$; $P < 0.0005$).

EMD was significantly longer in DMD patients compared with healthy controls (+44.2 ± 30.6%; $P < 0.0001$; Table 2). More precisely, DMD patients exhibited longer Tm (+72.6 ± 39.3%; $P < 0.0001$), while no significant difference was found for Dm ($P = 0.28$; Table 2). EMD and Tm were strongly correlated to the chronological age of the DMD patients ($r = 0.62$, $P = 0.02$; $r = 0.66$, $P = 0.01$ for EMD and Tm, respectively), but not of the controls ($r = -0.28$, $P = 0.35$; $r = -0.45$, $P = 0.10$ for EMD and Tm, respectively) (Fig. 2). A significant positive correlation was found between Dm and chronological age of the control subjects ($r = 0.70$, $P = 0.01$), while no significant correlation was found for DMD patients ($r = 0.26$, $P = 0.4$).

DISCUSSION

This study explored the effect of DMD on EMD and its associated electrochemical/mechanical components. DMD patients exhibit longer delay between the onset of fascicle motion and the onset of force production (representative of an altered force transmission) compared with control subjects. No significant difference was found for the delay between the onset of electrical stimulation and the onset of muscle fascicle shortening (i.e., electrochemical processes). Because the increased BB stiffness reported in the same population of DMD patients (Lacourpaille et al., unpublished observations) should theoretically improve transmission of force (24), the altered force transmission we report (longer Tm) is likely explained by structural abnormalities (e.g., the absence of dystrophin, sarcomere disconnections, detached fibers from tendons) rather than changes in muscle stiffness.

Maximal electrically evoked elbow flexion torque was considerably lower for DMD patients compared with the control subjects (10.4% of the values for healthy controls). Muscle weakness is mainly explained by the loss of contractile ele-

Table 2. *Averaged data for healthy controls and DMD patients*

	n	Evoked Maximal Torque, N·m	EMD, ms	Dm, ms	Tm, ms
Controls	13	2.8 ± 1.7	12.5 ± 1.4	4.6 ± 0.7	7.9 ± 2.0
DMD	13	0.3 ± 0.2*	18.5 ± 3.9*	4.9 ± 1.7	13.6 ± 3.1*

Values are means ± SD; *n*, no. of subjects. Evoked maximal torque, EMD, Dm, and Tm values are shown for control subjects (Controls) and DMD patients (DMD). *$P < 0.05$, significant difference between the populations.
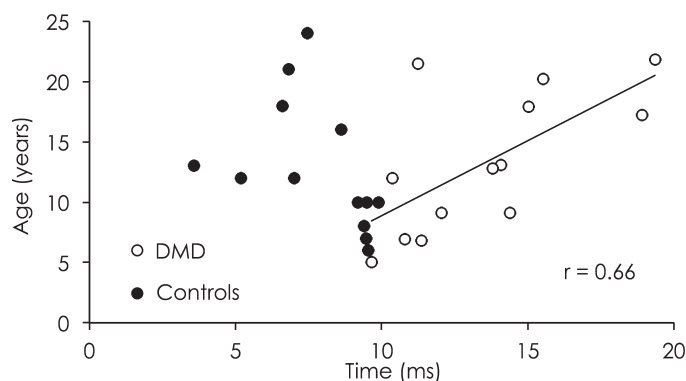
Fig. 2. Relation between force transmission (Tm) and age for both populations. Relationship is shown between the delay between onset of muscle fiber motion and onset of force production (Tm; ms) and the chronological age (years) of Duchenne muscular dystrophy (DMD) patients (○) and control subjects (●).

ments. Nevertheless, as mentioned in the Introduction, the origin of the impairments of muscle quality (i.e., maximal strength normalized by contractile cross-sectional area) is a matter of debate (37). Several factors may be responsible for this change, e.g., myofiber necrosis and changes in electrochemical (e.g., excitation-contraction coupling) and/or mechanical mechanisms (e.g., force transmission) responsible for the external force production.

DMD muscle fibers (*mdx* mice or double knockout mouse for utrophin and dystrophin) exhibit a significant impairment of excitation-contraction coupling (2, 38). More precisely, the quantity of Ca^{2+} released by the sarcoplasmic reticulum is decreased by $\approx 50\%$ in DMD muscle fibers (2, 38), which contributes to muscle weakness (11, 20). Using very high frame rate ultrasound, the present study demonstrates that the delay between electrical stimulation and onset of muscle fascicle shortening (Dm) is not different in DMD patients compared with healthy controls. As this delay is principally attributed to synaptic transmission and excitation-contraction coupling (15, 26), this result suggests that the efficiency of the excitation-contraction coupling might be affected independently of its duration. This is in line with a previous animal study showing that the time to release the maximal quantity of Ca^{2+} is not affected (≈ 4 ms; Ref. 38). It is also important to consider that this absence of change in excitation-contraction coupling duration might be explained by a compensation between multiple mechanisms [e.g., longer time to saturate the Ca^{2+}-troponin C complex (38) and shorter time for crossbridge attachment (6)].

The muscle force transmission from actin-myosin interaction to the bone may be influenced by several pathways (28), including the sarcolemma-associated cytoskeletal protein dystrophin (24). This assumption has been corroborated by ex vivo studies showing that both lateral (29) and longitudinal (6) force transmission are altered in *mdx* mice. To our knowledge, this alteration in force transmission has not been quantified in vivo. Consistent with the aforementioned studies, our results showed that DMD patients exhibit a longer delay between the onset of fascicle motion and the onset of force production (Tm) compared with healthy controls (+72.7%). Also, we showed a positive linear relationship between the age of DMD patients and Tm ($r = 0.66$), underlining the degenerative nature of this process and thus the relevance of its assessment to monitoring

disease progression. These results confirm that the absence of dystrophin in DMD patients may lead to an increase in the time to transmit the force from actin filaments to the tendon (i.e., impairment of the longitudinal and lateral force transmission pathways; Refs. 6, 29). This result may be partly explained by muscle structural abnormalities at two levels. First, in healthy skeletal muscle, dystrophin binds actin filaments to the extracellular matrix through the sarcolemma and is more densely distributed at the myotendinous junction (23). These results support the role of dystrophin for force transmission from the cytoskeleton to extracellular matrix at the level of both muscle sarcomere and the myotendinous junction (36). Second, structural abnormalities such as sarcomere disconnections (8), malformed/branched fibers (13), and detached fibers from tendons (13), may lead to an alteration of the longitudinal transmission from the force-generating structures to the myotendinous junction.

Due to the loss of regenerative capacity, DMD patient muscle fibers are gradually replaced by fibrous tissue, associated with calcium homeostasis perturbation [reviewed by Blake et al. (1)] and predominance of type I fibers (25). Together, this may contribute to the increased muscle-tendon stiffness reported in humans during quick release movements (7). Using a more direct assessment of stiffness, we found a significant higher BB muscle stiffness in DMD patients compared with healthy controls [2 times higher when the muscle was stretched (Lacourpaille et al., unpublished observations)]. As an increase in muscle-tendon stiffness should be associated with a more efficient muscle force transmission, the impairment of force transmission reported in this present study is likely explained by muscle structural abnormalities rather than altered muscle stiffness.

The noninvasive techniques used in this study offer a unique opportunity to assess muscle force transmission in patients with DMD (delay between the onset of fascicle motion and force production), contributing to a better understanding of the origin of the impairments of muscle weakness in DMD. Further investigations are necessary to determine whether this information has the potential to improve the accuracy (i.e., local measurements) and relevance (i.e., focusing on the main process altered in DMD) of patient monitoring, especially in clinical trials.

GRANTS

DISCLOSURES

AUTHOR CONTRIBUTIONS

Author contributions: L.L., F.H., A.G., Y.P., J.Y.H., and A.N. conception and design of research; L.L., A.G., Y.P., A.M., and A.N. performed experiments; L.L. analyzed data; L.L., F.H., Y.P., J.Y.H., and A.N. interpreted results of experiments; L.L. prepared figures; L.L., F.H., and A.N. drafted manuscript; L.L., F.H., A.G., Y.P., A.M., J.Y.H., and A.N. edited and revised manuscript; L.L., F.H., A.G., Y.P., A.M., J.Y.H., and A.N. approved final version of manuscript.

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