Muscle characteristics and altered myofascial force transmission in tenascin-X-deficient mice, a mouse model of Ehlers-Danlos syndrome

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1Research Instituut MOVE, Faculteit Bewegingswetenschappen, Vrije Universiteit, Amsterdam; and 2Department of Neurology, Donders Institute for Brain, Cognition, and Behavior, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; and 3Institute for Biomedical Research into Human Movement and Health, Manchester Metropolitan University, Manchester, United Kingdom

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Huijing PA, Voermans NC, Baan GC, Busé TE, van Engelen BG, de Haan A. Muscle characteristics and altered myofascial force transmission in tenascin-X-deficient mice, a mouse model of Ehlers-Danlos syndrome. J Appl Physiol 109: 986–995, 2010. First published June 24, 2010; doi:10.1152/japplphysiol.00723.2009.—The Ehlers-Danlos syndrome is a group of inherited connective tissue disorders caused by defects in collagens or tenascin-X (TNX). Muscle involvement can be expected based on interactions between muscle and extracellular matrix molecules; however, muscle function has not yet been investigated quantitatively. This study aims to investigate effects of TNX deficiency on muscular characteristics in TNX knockout (KO) mice, a mouse model of Ehlers-Danlos syndrome. At lower muscle lengths, maximally dissected medial gastrocnemius muscle-tendon complex of TNX KO mice showed lower active force, lower maximal rate of relaxation, and longer time delay between first stimulation pulse and initial force rise, supporting the hypothesis that relatively more slack needs to be taken up, as well as more elastic length changes occurring. In addition, study of the minimally dissected lower leg muscles shows that TNX deficiency strongly affects the mechanical interaction between antagonistic, as well as synergistic, muscles, which is consistent with the concept of altered myofascial force transmission due to increased compliance of myofascial components. Altered properties of the force transmission pathways of muscle (being either part of the myotendinous or myofascial pathways) due to TNX deficiency directly affect muscle function in TNX KO mice. Such effects are likely to contribute to muscle weakness experienced by patients with Ehlers-Danlos syndrome.

quantitative muscle function; tenascin-X; connective tissue; myopathy; ECM

THE Ehlers-Danlos syndrome (EDS) is a group of inherited connective tissue disorders caused by defects in metabolism of fibrillar collagens. It presents with joint hypermobility, skin hyperextensibility, abnormal scar formation, easy bruising, and tissue fragility (1, 21). EDS is caused by mutations in the genes encoding collagen I, III, and V, and tenascin-X (TNX), molecules that are known to be abundantly expressed in the extracellular matrix (ECM) (1, 17, 21).

Primary muscle involvement in EDS can be expected based on interactions between muscle and these ECM molecules (24). In a recent case study, our laboratory indeed demonstrated reduced muscle function in two EDS patients, which could not be attributed to increased tendon compliance or disuse (23). Subsequently, our laboratory found considerable clinical muscle weakness in patients with various types of EDS accompanied by only limited histological myopathic changes (22).

This study aims to investigate effects of TNX deficiency on muscular characteristics in TNX knockout (KO) mice, a mouse model of EDS. Various intra- and intermuscular aspects of muscle force may be affected by TNX deficiency. Intramuscular aspects can be studied during isometric contractions of isolated muscles. During an isometric contraction, the muscle-tendon complex length is fixed, but the actual active length of the muscle fibers is dependent on the properties of the series elastic components, consisting of the network of endo-, peri-, and epimysium, as well as of tendon. Similarly, these properties affect the rate of length change of the fibers during the initial phase of force generation and hence influence the rate of force building up. These intramuscular aspects of muscle force may be affected by TNX deficiency via altered viscoelastic properties of the connective tissue within muscle and tendon.

Study of the intermuscular aspects of muscle force has proved to be an effective method to investigate myofascial force transmission (11). This concept is based on the ability of muscle to transmit forces between muscle fibers and connective tissue within muscle (endo- and perimysium) and between individual muscles and connective tissue between muscles (epimysium, fascia, septum, neurovascular tract). As a result, morphologically defined muscles are not independent actuators, but are capable of mechanical interaction via their connective tissue structures (13, 16, 25). As such, force exerted at the origin of a muscle within its natural context of connective tissue is not necessary equal to the force exerted at its insertion, since additional loads initiated in neighboring muscles act on the muscle. Hence, the difference in forces measured at the muscle’s origin and insertion is an unequivocal indication for net epimuscular myofascial force transmission (16). Furthermore, as epimuscular myofascial force transmission is mediated by surrounding connective tissues, the fraction of force transmitted myofascially has been found to depend on muscle length and its position relative to its surrounding structures (9).

In view of the above, we hypothesize that TNX deficiency not only affects intramuscular aspects of muscle force via altered viscoelastic properties of the connective tissues, but also reduces the stiffness of myofascial pathways, causing pathological changes in force transmitted this way. The present study, therefore, aims to investigate directly the effect of TNX deficiency on muscle characteristics in a mouse model of TNX-deficient type EDS (18). To do so, this study combines
measurement of both intramuscular and tendon aspects [i.e., force characteristics of the maximally dissected medial gastrocnemius (GM) muscle tested in isolation; series A] and intermuscular aspects [i.e., force characteristics of the triceps surae (TS) muscle and anterior crural muscles without major dissection to detect changes in mechanical interaction between these muscle groups; series B]. Contractile responses from TNX KO mice will be compared with those from wild-type (WT) mice.

MATERIAL AND METHODS

The experimental design was approved by the Ethics Committee for Animal Experimentation of the Vrije Universiteit Amsterdam. TNX KO mice were obtained, as previously reported, by inactivating murine Tnxb (7, 18). The 5′ end of the gene was targeted, thus replacing the first five coding exons with lacZ and a neomycin resistance cassette. Correct targeting of Tnxb was confirmed by Southern blotting, and, as expected, TNX KO mice lacked both TNX mRNA and protein (18). Experiments were performed on two groups of TNX KO mice that had been crossed back with six generations of C57BL/6N mice.

1) Series A: Eight female TNX KO mice (mean body mass of 39.3 g, SE = 0.6 g) and seven female WT C57BL/6 (mean body mass of 41.6 g, SE = 1.5 g) mice were tested (age 12–14 mo).

2) Series B: Six female TNX KO mice (mean body mass of 30.2 g, SE = 0.91 g) and six female WT C57BL/6 mice were used as a control (mean body mass 31.2 g, SE = 2.49 g; age 12–14 mo).

All mice were deeply anesthetized by administration (intraperitoneal 0.1 ml/10 g body mass) of a solution of fentanyl citrate (0.079 mg/ml) and flunizole (2.5 mg/ml; Hypnorm) and midazolam (1.25 g/ml; Dormicum). Additional doses were given as necessary (0.05 ml or 0.10 ml, intraperitoneally). During surgery and data collection, animals were placed on a heated water pad of ~37°C to prevent hypothermia.

Definitions

The biomechanical concepts used are summarized in Table 1.

Surgical Procedures

Dissection of sciatic nerve. The sciatic nerve was dissected free from surrounding tissues and severed as proximally as possible. Subsequently, all of its branches except the branch to the GM (series A) or the common peroneal and tibial nerves (series B) were cut. To be able to clamp the femur, small insertions were made in the musculature located anteriorly and posteriorly of the femur, and a metal clamp was inserted and tightened.

Dissection of muscles. SERIES A: Maximally Dissected GM. The GM muscle was fully dissected from the surrounding tissue, with the exception of blood supply and innervating nerve.

SERIES B: Anterior Crural and TS Muscles. For this segment of the experiments, dissection of the lower leg was minimized to free the distal tendons of target muscles. Only limited fasciotomy was performed distally to expose the distal tendons.

Table 1. Biomechanical concepts in this study

<table>
<thead>
<tr>
<th>Term Definition</th>
<th>Abbreviation (Unit)</th>
<th>Description</th>
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<tbody>
<tr>
<td>Optimum length</td>
<td>$l_o$ (mm)</td>
<td>The muscle’s length at which the length of the muscle’s sarcomeres are, on average, on the plateau of the length-force curve. This represents the length at which actin and myosin have maximal overlap.</td>
</tr>
<tr>
<td>Active slack length</td>
<td></td>
<td>Active slack length is the lowest muscle length at which active force approaches zero. At any length below that length, the active muscle is slack. Below active slack length, even a fully active muscle does not exert force on its outside world, and the distance between its proximal and distal end may not adequately reflect its true length, due to buckling of tissues.</td>
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<tr>
<td>Muscle slack (ness)</td>
<td></td>
<td>A characteristic of the series elastic components of muscle. It reflects the necessity to stretch the series elastic components minimally before they can transmit forces. This is referred to when it is mentioned that a muscle needs time to take up slackness before shortening, once contraction has started.</td>
</tr>
<tr>
<td>Passive force</td>
<td>$F_{pass}$ (mN)</td>
<td>The force required to stretch a relaxed muscle to a given length.</td>
</tr>
<tr>
<td>Active muscle force</td>
<td>$F_{act}$ (mN)</td>
<td>Total force minus passive force. This estimates the component of force that is related to the attachment of cross bridges.</td>
</tr>
<tr>
<td>Normalized active force</td>
<td>%$F_{max}$</td>
<td>Active force at a given length as a percentage of active force at optimum length ($l_o$).</td>
</tr>
<tr>
<td>Total force</td>
<td>$F_{tot}$ (mN)</td>
<td>The final force that a muscle attains following stimulation. This force includes the passive force that existed before stimulation, and the component of force that is generated in response to the stimulus.</td>
</tr>
<tr>
<td>Optimum force</td>
<td>$F_o$ (mN)</td>
<td>Maximal active muscle force at $l_o$ in a length-force curve.</td>
</tr>
<tr>
<td>Active peak force</td>
<td>peak$F_{max}$ (mN)</td>
<td>Maximal active muscle force in a force-time curve for each given length. These peak forces at a certain length are plotted in the length-force curve. The active peak force at $l_o$ equals the optimum force.</td>
</tr>
<tr>
<td>Normalized maximal rate of relaxation</td>
<td>%MRR (mN/ms)</td>
<td>Maximal rate of relaxation as a percentage of the rate of relaxation after the first contraction (in isometric fatigue protocol), after stimulation with 150 Hz (in frequency-force measurements), or after contraction at $l_o$ (in length-force measurements).</td>
</tr>
<tr>
<td>Normalized maximal rate of force rise</td>
<td>%MRFR</td>
<td>Maximal rate of force rise as a percentage of the rate of force rise after the first contraction (in isometric fatigue protocol), after stimulation with 400 Hz (in frequency-force measurements), or after contraction at $l_o$ (in length-force measurements) (mN/ms).</td>
</tr>
<tr>
<td>Maximal power production</td>
<td>(mW)</td>
<td>Highest power (muscle force $\times$ velocity) obtained from fitted power-velocity curve.</td>
</tr>
</tbody>
</table>
of the tibialis anterior (TA) muscle, extensor hallucis longus (EHL) muscle, and extensor digitorum longus (EDL) muscle, and to sever the retinaculae (i.e., the transverse crural ligament and crural cruciate ligament). Otherwise, the connective tissue at the muscle bellies and tendons was left intact. The distal tendons of EDL were tied together (Ethilon surgical suture) and severed distally of the knot. Also, the distal tendons of TA and EHL were tied (polyester yarn) and severed from their insertions. Below, this complex will be referred to as TA+EHL complex. A small piece of the epicondylus lateralis comprising the origin of the EDL muscle was cut from the femur. Similarly, a small piece of bone was cut, comprising the insertion of the TS muscle via the Achilles tendon on the calcaneus bone. All tendons described were tied to Kevlar threads (4% elongation at a break load of 800 N), that were in turn attached to rods for later connection to a force transducer. In the reference position (corresponding to a knee angle of 100° and ankle angle of 180° plantar flexion), the original position of the proximal tendon of EDL on the epicondylus lateralis of the femur was marked by placing corresponding markers on the proximal EDL tendon and lateral collateral ligament. The foot was firmly attached to a plastic foot plate.

Experimental setup, conditions, and treatment of data. See Fig. 1. SERIES A: MAXIMALLY DISSECTED GM. The distal tendon of the GM (length ~4 mm) was connected to a force transducer of an isovelocity measuring system (5). The attachment of the proximal tendon was left intact. The femur was fixed to the measuring system. Length changes of the GM tendon complex were induced with a computer-controlled servomotor connected to a lever on which the force transducer was mounted. Contractions were induced by electrical stimulation using a constant-current stimulator. Electrical pulses (width 50 μs) were applied to the sciatic nerve with a constant current (1 mA), being high enough to fully activate all muscle fibers. The muscle temperature was maintained at 34–36°C with a water-saturated airflow around the muscle, which at the same time kept the muscle moistened (5). Force and length signals were digitized (1–5 kHz) and stored on disk. At the end of the experiment, the GM muscles were excised and weighed. Thereafter, the mice were humanely killed with an overdose of anesthesia.

Muscle optimum length \( l_o \) was first estimated using a few twitch contractions (one per minute). (Tetanic) \( l_o \) was subsequently determined using only three to four tetanic contractions (stimulation frequency 150 Hz, duration 150 ms). About 10 min later, the following series of contractions started. Duration of a single pulse in all experiments was 50 μs.

1) Length-force protocol: Muscles were stimulated isometrically in random order at various lengths (steps of 0.5 mm) between \( l_o - 4 \) mm and \( l_o + 2 \) mm for 150 ms with a stimulation frequency of 150 Hz with >2 min rest intervals to prevent fatigue.

2) Stimulation frequency: Contractions were performed at \( l_o \) using the following stimulation frequencies, 25, 50, 75, 100, 250, and 400 Hz, and pulse duration of 50 μs with durations long enough to allow the muscles to reach their peak force at each particular frequency. Between the contractions, there was at least 2 min rest.

3) Force-velocity protocol: Contractions were performed during which the muscles were allowed to shorten at different constant velocities (at random; 0, 20, 30, 40, 50, 75, and 100 mm/s). Just before the start of the contraction, the muscle was (passively) stretched to 0.5 mm over \( l_o \). During the initial part of the contraction, the length of the muscle was kept constant until the (increasing) force had reached the level that was estimated to be the force that could be sustained during the shortening at the imposed velocity. In this way, the measured force was constant when the muscle passed \( l_o \) during shortening (4). Hence, the velocity of the length change of the muscle tendon complex is also the velocity of shortening of the muscle fibers, since no length change occurs in the series elastic elements at constant force. The stimulation frequency used was 400 Hz, except for contractions with shortening velocities of 0
and 20 mm/s, where 200 Hz was used. These frequencies were high enough to obtain maximal forces at all shortening velocities. After each contraction, there was at least 2 min rest.

4) Fatigability protocol: A series of 20 repeated isometric contractions was induced at $l_o$ (duration 150 ms, one contraction every 500 ms, and stimulation frequency 150 Hz).

5) Data management: From the isometric force traces, the following parameters were calculated. Active peak force [peak $F_{m_a}$ (active muscle force); mN] was taken as the highest force minus the passive force. The maximal rates for force rise ($\%$MRFR) and relaxation ($\%$MRR in mN/ms) were taken as the maxima and minima of the differentiated force signal at the beginning and end of the contraction, respectively. The above data were normalized for the data obtained at $l_o$ to study the influence of length, at a stimulation frequency of 250 Hz (for $F_{m_a}$) and 400 Hz (for MRFR and MRR) to study the influence of stimulation frequency, and to the first contraction of the series to study the fatigability, and all data are presented as percentages. The time (ms) between the first stimulation pulse and the increase of force above 2% of the maximal force was determined as time needed to take up the slack of the muscle. For the shortening contractions, the force was obtained when the muscle passed $l_o$. Power was calculated by multiplying the force by the imposed velocity (mW). For each muscle, maximal power was obtained from the fitted curve through the power-velocity data points.

SERIES B: ANTERIOR CRURAL AND TS MUSCLES. The animal was mounted in the experimental setup, at a knee angle of $\sim 110^\circ$ (measured postexperimentally in images to be equal to mean $\pm$ SE 111.8 $\pm$ 1.4° and 111.8 $\pm$ 5.1° for the TNX KO and WT group, respectively). The foot, attached to a plastic plate, was attached to a rigid frame with the ankle in extreme plantar flexion to create room for free passage of the distal tendons of EDL and TA+EHL at the ankle. The distal tendons of TA+EHL and EDL, as well as the proximal EDL tendon, were connected to force transducers (ME-Messysteme, compliance of 0.025 mm/N) mounted on single-axis micropositioners. Also, the kevlar thread attached to TS distal tendons was attached to a force transducer. The sciatic nerve was placed on a pair of silver electrodes and prevented from dehydration by covering it with paper tissue saturated with isotonic saline and a thin piece of latex.

Ambient temperature (22 $\pm$ 0.5°C) and air humidity (70 $\pm$ 2%) were kept constant by a computer-controlled air-conditioning system (Holland Heating, Waalwijk, the Netherlands). Muscle and tendon tissue was further prevented from dehydration by regular irrigation with isotonic saline. Before leg-force data were acquired, EDL was preconditioned by isometric contractions at alternating high ($l_o$) and low ($l_o - 3$) lengths, until active forces at low length were reproducible [i.e., effects of previous activity at high length (11a) are minimized]. The proximal EDL tendon was set at a position 1 mm distal of the marker position on the femur (i.e., shorter muscle). Throughout the experiment, the proximal tendon of EDL was kept at this position. The EDL distal tendon was set at 1 mm below its $l_o$ and kept at that position during the experiment. Also for TS, as well as TA+EHL, temporary estimates of optimum force (i.e., the highest active force measured as a function of length) and $l_o$ (the length of occurrence of the highest active force) were estimated. These values were used only during the execution of the experiment.

1) Length-force protocol: During measurement of TS length-force characteristics, muscle-tendon complex length of TA+EHL complex was kept relatively short (i.e., on the ascending limb of its length force curve). Initially, this length corresponded to an active force of approximately one-third of optimum force [$\frac{1}{3}$ optimum $F_{m_a}$ ($F_{mao}$)]. Similarly, for measurement of the TA+EHL length-force characteristics, TS muscle-tendon complex length was not changed and kept at a length initially corresponding to an active force of approximately one-third of optimum force. Therefore, if no myofascial muscular interaction would occur, one would expect TA+EHL and TS muscle forces, respectively, to remain constant during measurements of length-force characteristics of its antagonist muscle group.

Before excitation of the sciatic nerve, all muscles were brought passively to the desired lengths by moving the distal positioners (muscle to be manipulated; stepwise per 0.5 mm; other muscles at length corresponding to that yielding an active force of approximately of optimum force). The imposed length change was read from the micromanipulator to the nearest 0.1 mm. Postexperimentally, changes in muscle-tendon complex length are expressed as deviation from $l_o$. All muscles were activated simultaneously by supramaximal stimulation of the sciatic nerve, with a constant current (<3 mA) and a stimulation frequency of 100 Hz (pulse width 0.5 ms). Two twitches were evoked, followed by a tetanic contraction of 300 ms. For a typical example of force data collected, see Fig. 2. Timing of stimulation and analog-to-digital conversion of force data (12-bit analog-to-digital converter, sampling frequency 1,000 Hz) was controlled by a special purpose microcomputer. After each tetanic contraction, the muscles were allowed to recover near active slack length for 2 min. Passive isometric force was measured before the tetanic contraction, and total force was measured at a point during the final quarter of the tetanic force plateau.

![Fig. 2. A typical example of raw force data collected as a function of time. Force was exerted at its distal tendon by the TS complex. Force was exerted at the tied distal tendons of TA+EHL. Forces were exerted by EDL prox and at its tied EDL dist. Note differences between these two force tracings that indicate epimuscular myofascial force transmission between EDL and its surroundings. For TS and TA+EHL forces, refer to left y-axis (drawn in black), and for EDL force to the right y-axis (drawn in gray). All muscles were activated maximally with all motor units recruited. Approximate timing of stimulation is provided on the line inserted just above the x-axis. Fm, muscle force.](http://jap.physiology.org/ attachment/109_078/full/2464060811.png)
2) Treatment of data: Passive muscle force (F_{mp}), as a function of muscle-tendon complex length, was fitted with an exponential curve using a least squares criterion:

\[ y = \exp(ax + b) + C \]

where \( y \) represents F_{mp}, \( x \) represents muscle-tendon complex length, and \( a, b, \) and \( C \) are fitting constants. F_{ma} was estimated by subtracting from total force and actually measured the F_{mp} for the appropriate muscle length, which was calculated using the fitted exponential function. Active length-force data thus obtained were then least square fitted, applying a stepwise polynomial regression procedure (see Statistics below):

\[ y = b_0 + b_1 x + b_2 x^2 + \ldots b_n x^n \]

where \( y \) represents F_{ma}, \( x \) represents F_{ma} length, and \( b_0 \) through \( b_n \) are fitting constants. For TS, as well as TA+EHL, \( l_o \) of the muscle-tendon complex to be used in further analysis was defined, for each individual muscle, as the muscle length at which the fitted active force curve showed a maximum. TS and TA+EHL distal active slack lengths were estimated by selecting data at lower muscle lengths (F_{ma} < 0.3x F_{mao}) and extrapolated using the fitted curve:

\[ y = \exp(b_0 x + b_1) + b_2 \]

where \( y \) represents F_{mao}, \( x \) represents F_{mao} length, and \( b_0 \) through \( b_2 \) are fitting constants.

Similar polynomial fitting procedures were applied for forces exerted in the active and passive states by EDL at its proximal and distal tendons, as well as TS and TA+EHL distal forces in the case where they were not lengthened. For EDL, the differences in passive and active force exerted at the distal and proximal tendon were calculated by subtracting proximal from distal force, as determined from the polynomials. For all forces studied and with use of the selected polynomials, mean and SEs of F_{ma} and F_{mp} were calculated. This was done for given deviations from respective \( l_o \) values of muscles that had been changed in muscle-tendon complex length to measure its length-force characteristics.

Statistics

Series A. Possible differences in GM maximal isometric force, muscle mass, and peak power between the TNX KO and the WT group were determined using the Student t-test. A repeated-measures ANOVA was used to determine the differences in the effects of stimulation frequency, length, and fatigue between the groups. The significance level was set at 0.05.

Series B. For curve fitting of TS and TA+EHL active length-force data, the procedure starts with a first-order polynomial, and the power was increased up to the sixth order, as long as this yields a significant improvement of the statistical description of the length-active force data, as determined by one-way ANOVA (20). Bivariate ANOVA (SPSS version 14.0) were used to test for significance of main effects of muscle-tendon complex length (repeated measurements) of both TS and TA+EHL, the presence of TNX deficiency, and their interaction. This was done for 1) TS active and passive length force characteristics; 2) TA+EHL active and passive length force characteristics; 3) distally exerted EDL active and passive forces; 4) proximally exerted EDL active and passive forces; and 5) on active and passive EDL proximodistal EDL force differences. Additionally, for lengthening of TS, ANOVAs were used to determine \( \delta \) effects of TS length on active and passive force exerted distally by TA+EHL, while kept at unchanged, relatively short muscle-tendon complex length; and 7) for lengthening of TA+EHL, on active and passive force exerted distally by TS, while kept at unchanged, relatively short muscle-tendon complex length. In addition, t-tests were used to test for differences in distal active slack length of TS and TA+EHL.

RESULTS

Series A: Maximally Dissected GM

GM muscle length. Maximal forces were not different between WT (1.74 ± 0.38 N) and TNX KO mice (1.47 ± 0.36 N). Actual GM muscle belly length (excluding distal tendon length) measured at \( l_o \) was not different between the groups (12.85 ± 5.12 vs. 13.14 ± 0.69 mm for TNX KO and WT). Only at low lengths was normalized active isometric force (%F_{ma}) significantly lower in TNX KO mice compared with WT mice (at \( l_o - 4, l_o - 3.5, \) and \( l_o - 3; P = 0.030, P = 0.026, \) and \( P = 0.032, \) respectively) (Fig. 3A). At these low muscle lengths, the relaxation was slower in TNX KO mice, as indicated by a significantly lower %MRR (at \( l_o - 4, l_o - 3.5, \) and \( l_o - 3; P = 0.026, P = 0.022, \) and \( P = 0.038, \) respectively) (Fig. 3B). Note that, although these differences seem small, the relative effects are substantial. Force and relaxation rate in TNX KO mice were ~65% of WT at \( l_o - 3 \) mm and ~46% at \( l_o - 3.5 \) mm. Furthermore, the delay between the first stimulus pulse and the increase of force above 2% of the maximal active force was significantly longer in TNX KO mice at short length (\( l_o - 4 \) and \( l_o - 3.5; P = 0.025 \) and \( P = 0.026, \) respectively) (Fig. 3C), indicating that relatively more slack is needed to be taken up at shorter lengths in TNX KO mice.

GM stimulation frequency. There were no significant differences between TNX KO mice and WT mice in isometric peak forces, normalized MRFR, and normalized MRR at any stimulation frequency applied at \( l_o \) or at \( l_o - 3.5 \) (data not shown).

GM shortening velocity. No significant differences in force-velocity characteristics and maximal power production were found between TNX KO (18.2 ± 6.3 mW) and WT mice (20.9 ± 6.3 mW).

GM isometric fatigue. The fatigue protocol led to a similar reduction in force in TNX KO mice and WT mice (73.2 ± 4.0 and 70.4 ± 5.1%, respectively). There were no significantly different changes in the MRFR and MRR between TNX KO mice and WT mice during the series of repeated isometric contractions.

Series B: Anterior Crural and TS Muscle Forces

Effects of TS length change on forces exerted by antagonistic muscles at constant length. EFFECTS ON TA+EHL. ANOVA showed significant effects of TS length on active forces exerted by antagonistic muscles, while the length of this complex (TA+EHL) was kept unchanged and relatively short. Normalized active force decreased substantially (by maximum 80% of initial force) as a function of increasing TS length (Fig. 4A). ANOVA also indicated significant effects of TNX deficiency, but no significant interaction could be shown. The effect of
TNX deficiency is to limit this TS length-dependent decrease in active force to levels not exceeding 33% of initial force.

**EFFECTS ON EDL.**

1) Proximal force: ANOVA showed significant main effects (i.e., TS length, TNX deficiency) on EDL proximal active force, as well as significant interaction. Note that proximal active force increases (maximum increase $0.1 \text{ N}$) as a function of increasing TS length. These effects are still present, but less pronounced in TNX KO mice (Fig. 4B).

2) Distal force: ANOVA showed significant effect of TS length on EDL distal active force, as well as significant interaction of effects of TS length and TNX deficiency. Note that distal active force decreases as a function of increasing TS length (Fig. 4C).

3) Proximodistal EDL total force differences: This difference in force exerted in the proximal and distal tendons of EDL is indicative of net epimuscular myofascial force transmission between EDL and other muscular or nonmuscular tissues. A positive difference (distal force exceeding proximal force) indicates that a net load is exerted on EDL in the proximal direction. The force corresponding to this load is integrated into the force exerted in the distal tendon (see schematic insets in Fig. 5). For negative proximodistal force differences, the
reverse is the case. ANOVA showed significant effects of TS length on the EDL total proximodistal force difference, as well as significant interaction of effects of TS length and TNX deficiency. Therefore, it is concluded that both TS length and TNX deficiency affect myofascial force transmission within the mouse lower leg (Fig. 5). At very low lengths (i.e., for TS \( l_o \) less than \( l_o \approx 3.5 \)), a proximal load is exerted on EDL in both WT and TNX KO mice. The pattern of change of this difference varies substantially between WT and TNX KO mice. The pattern of change of this difference varies substantially between WT and TNX KO mice: with increasing TS length in WT mice, the EDL force difference decreases rapidly to zero to change sign (i.e., loading direction), while for TNX KO mice, this proximodistal EDL force difference only decreases gradually (to a value of \( \Delta F = 0 \) at highest TS lengths, indicating no net myofascial force transmission). Note that this means that, for TNX KO mice, myofascial loading direction of EDL is not changed at all over the whole length range studied. The insets illustrate schematically the direction of the net myofascial loads on EDL, where P and D indicate proximal and distal directions, respectively. \( F_{\text{mt}} \), total muscle force.

but also with regard to the direction of loading (Fig. 5). Comparison with Fig. 4, B and C, shows that this effect of the TNX deficiency is mediated predominantly by preventing a high increase in proximal EDL force at higher TS lengths.

Fig. 5. Changes in proximodistal EDL force differences with changes in length of antagonistic or synergistic muscles. A: effect of changing TS muscle-tendon complex length. B: effects of TA+EHL length. The EDL proximodistal total force difference is plotted. Note that any such difference is indicative of net myofascial force transmission between EDL and surrounding muscular and/or nonmuscular tissues. For WT mice, note that, with increasing TS length, as well as TA+EHL length, this EDL proximodistal force difference decreases rapidly to zero to change sign (i.e., loading direction), between \( l_o \approx 3.5 < l_o \leq 4 \). At higher lengths, this distally directed load increases substantially with further increasing lengths for WT mice. In contrast, for TNX KO mice, this proximodistal EDL force difference only decreases gradually (to a value of \( \Delta F = 0 \) at highest TS lengths, indicating no net myofascial force transmission). Note that this means that, for TNX KO mice, myofascial loading direction of EDL is not changed at all over the whole length range studied. The insets illustrate schematically the direction of the net myofascial loads on EDL, where P and D indicate proximal and distal directions, respectively. \( F_{\text{mt}} \), total muscle force.

Effects of TA+EHL length change on forces exerted by antagonistic muscles at constant length. EFFECTS ON TS. ANOVA showed significant main effects (TA+EHL length, TNX deficiency) on active forces exerted by antagonistic TS, while the length of this muscle was kept unchanged and relatively short, as well as significant interaction between effects of length and TNX deficiency. Normalized active force decreased substantially (by maximum 40% of initial force) as a function of increasing TA+EHL length (Fig. 6A). The effect of TNX deficiency is to limit the length-dependent decrease in TS active force to levels not exceeding 7.4% of initial force.

EFFECTS ON EDL. 1) Proximal force: ANOVA showed significant main effects (of TS length and TNX deficiency) on EDL proximal force (Fig. 6B). Proximal \( F_{\text{ma}} \) increases as a function of increasing TA+EHL length at low lengths, but remains at similar values for higher TA+EHL lengths. This effect is still present in TNX KO mice, but occurs at lower force levels: the curve is shifted downward. C: distal force exerted by EDL with changing lengths of TA+EHL. Distal \( F_{\text{ma}} \) decreases as a function of increasing TA+EHL length in both WT mice and TNX KO mice. However, such decrease in \( F_{\text{ma}} \) is much smaller in TNX KO mice.

Fig. 6. Effects of changes in TA+EHL length on forces exerted by antagonistic muscles kept at constant length. A: force exerted by TS with changing lengths of TA+EHL. Normalized \( F_{\text{ma}} \) decreased substantially (by maximum 40% of initial force) as a function of increasing TA+EHL length. The effect of TNX deficiency is to limit this length-dependent decrease in TS \( F_{\text{ma}} \) to levels not exceeding 7.4% of initial force. B: proximal force exerted by EDL with changing lengths of TA+EHL. Proximal \( F_{\text{ma}} \) increases as a function of increasing TA+EHL length at low lengths, but remains at similar values for higher TA+EHL lengths. This effect is still present in TNX KO mice, but occurs at lower force levels: the curve is shifted downward. C: distal force exerted by EDL with changing lengths of TA+EHL. Distal \( F_{\text{ma}} \) decreases as a function of increasing TA+EHL length in both WT mice and TNX KO mice. However, such decrease in \( F_{\text{ma}} \) is much smaller in TNX KO mice.
proximal active force, but no interaction. Note that, at low lengths, proximal active force increases (maximum increase >0.1 N) as a function of increasing TA+EHL length, but remains at similar values for higher TA+EHL lengths. This effect is still present in TNX KO mice, but occurs at lower force levels (Fig. 6B): the curve is shifted downward.

2) Distal force: ANOVA showed significant effects of TA+EHL length on EDL distal active force, as well as significant interaction of effects of TS length and TNX deficiency. Note that distal active force decreases as a function of increasing TA+EHL length (Fig. 6C) in both WT mice and TNX KO mice. However, such decrease in active force is much smaller in TNX KO mice.

3) Proximodistal EDL total force differences: ANOVA showed significant main effects on the EDL total proximodistal force difference, as well as significant interaction of effects of TS length and TNX deficiency. Therefore, it is concluded that TA+EHL length and TNX deficiency affect myofascial force transmission within the mouse lower leg. Similarly as for TS length change, the pattern of effects of TA+EHL length changes show the following. At low lengths, the force difference is always positive for both WT and TNX KO mice, indicating that a net load is exerted on EDL from the proximal direction. In both cases, this load decreases to very low values with increasing TA+EHL lengths. However, for WT mice (Fig. 5B), this occurs after minor TA+EHL length increase (≈0.6 mm), after which the direction of loading is reversed and increases progressively with further length increases. For TNX KO mice, the proximally directed net myofascial load decreases much more gradually to levels approaching zero at high TA+EHL lengths, i.e., the reversal of loading direction is absent.

TS and TA+EHL length-force characteristics. ANOVA showed a significant main effect (i.e., for factor length) for both TS (Fig. 7A) and TA+EHL (Fig. 7B) active, as well as passive, length-force curves. However, despite non-overlapping curves, any possible effects of TNX deficiency, as well as its interaction with length, could not be shown to be significant (ANOVA) for either muscle due to a relatively high individual variation of force values.

**Discussion**

The results of this study show that altered properties of any series elastic component of muscle due to TNX deficiency directly affect muscular characteristics. More specifically, study of the intramuscular aspects (series A) points to changes in the series elastic component within the (maximally dissected) muscle-tendon complex, and study of muscle within its connective tissue context showing altered mechanical interaction between muscles (series B) points at altered compliance of connective tissues located outside the individual muscles. Changes in connective tissue compliance both within and between muscle influence myofascial force transmission.

Mechanical interaction between antagonistic and synergistic muscles due to myofascial force transmission has been described in the last few years (3, 10, 12, 23). Altered intra- and epimuscular myofascial force transmission may drastically affect muscular coordination required for physiological movements. Main findings are summarized below.

**Series A. Intramuscular Changes: Increased Muscle Compliance**

At l0, several properties of dissected GM are unchanged in TNX KO mice compared with WT mice: 1) maximal isometric force and maximal power production; 2) stimulation frequency-force relationship; and 3) fatigability during a series of repeated isometric contractions. In contrast, at low lengths (l0 - 4, l0 - 3.5, and l0 - 3), some GM properties were affected significantly in TNX KO mice: 4) active force exerted at lower lengths was lower; 5) the MRR was lower; and 6) the time delay between first stimulation pulse and the time of attainment of 2% of maximal active force was longer in TNX KO mice. This last finding is not directly related to our hypothesis, but indicates that relatively more slack must be taken up at lower lengths in TNX KO mice. The other findings are related to the higher series elastic compliance present in the disease, which causes more shortening to be imposed on the muscle fibers in TNX KO mice at the onset of contraction at low lengths. Vice versa, more lengthening is imposed on the muscle fiber at the onset of relaxation at low lengths. These findings are in accordance with the results of our laboratory’s previous pilot study in two TNX-deficient EDS patients, who were only tested at long length (23).

**Series B. Intermuscular Effects: Reduced Myofascial Interaction Between Synergists and Antagonistic Muscles**

TNX deficiency strongly affects the mechanical interaction between muscles, which reflects the reduction of epimuscular interaction.
myofascial force transmission (i.e., transmission directly between muscle belly and its surrounding tissues). The decrease in normalized distal active force in their agonistic muscle (TA+EHL and TS, respectively) with increasing length of the antagonistic muscle (TS and TA+EHL, respectively) in normal mice results from myofascial force transmission between these muscle groups. The effect of TNX deficiency is to limit this antagonist length-dependent decrease in active force, which is compatible with the hypothesis of increased compliance of tissues surrounding the individual muscles. Second, the difference in force exerted at proximal and distal tendons of EDL is indicative of net epimuscular myofascial force transmission between EDL and other muscular or nonmuscular tissues. This difference also proved to be a function of the antagonistic or synergistic muscle-tendon complex length (TS and TA+EHL, respectively) and was affected by TNX deficiency. The deficiency significantly affects net epimuscular myofascial force transmission, not only in the magnitude of the net myofascial load on EDL, but also with regard to the direction of loading. Whereas, in WT mice, the direction changes rapidly from proximal to distal to distal loading of EDL with increasing TS length, such change of direction does not occur in TNX KO mice. In other words, in comparable experimental conditions, TNX KO muscles act more independently than healthy muscles. It seems inevitable that such altered function will require altered patterns of muscular coordination to allow effective movement. The structure that is most likely responsible for a proximal load is the neurovascular tract (i.e., the connective tissues reinforcing blood vessels and nerves outside of the muscle) (10). This load and loading direction is thought to be present permanently [unless the muscle is lengthened proximally (12), note that this is only possible in polyarticular muscles]. The similarity of proximal loads on EDL (see Fig. 4 at low lengths for TA+EHL and TS) is hypothesized to indicate that the compliance of the neurovascular tract to this muscle is not affected in a major way by the TNX deficiency. This would agree with the clinical observation that EDS patients with TNX deficiency, in contrast to other types of EDS, do not suffer from major damage to blood vessels and nerves. In healthy animals, as the synergistic or antagonistic muscles are lengthened, a distally directed myofascial load on EDL is enhanced and compensates for proximally directed load at slightly higher lengths (change of net loading direction) and becomes dominant (net distally directed load) at even higher lengths. In contrast, for TNX KO mice, enhanced compliance of collagenous tissues connecting EDL to synergistic or antagonistic muscles necessitates much higher length changes of these muscles to even attain equilibrium between the opposing myofascial loads on EDL at very high lengths, let alone attaining a change in direction of loading.

Taken together, these findings indicate that the series elastic components of the muscle-tendon complex located within and between muscles is changed in TNX KO mice. As such, these findings support the hypothesis formulated previously that TNX deficiency reduces the stiffness of myofascial pathways and thus causes a pathological reduction of the force transmitted this way (23). This study and previous animal experiments have shown that myofascial force transmission occurs between antagonistic muscles, which points to the high interdependence of muscles and their role in higher levels of motor organization (10, 12). Whether and to what extent reduced myofascial force transmission changes the muscular coordination and interferes with mechanical interaction between antagonists muscles in TNX-deficient EDS patients needs to be studied in detail.

In addition, the results of the present study constitute a new type of evidence supporting the concept of myofascial force transmission: altered ECM elastic properties affect quantity and quality of myofascial force transmission. So far, evidence of myofascial force transmission was based on use of physiological animal models, or on experiments in human patients suffering from spastic paresis (10).

Altered myofascial force transmission in TNX KO mice is most likely related to TNX deficiency. TNX is abundantly expressed in various tissues during embryonic development, among which are tendons and perimysium of skeletal muscle (2, 19). In adulthood, TNX is predominantly expressed in connective tissue of skeletal and cardiac muscle (19). TNX is involved in collagen deposition and maturation (6, 21), and several studies suggest that TNX acts as a bridge between collagen fibrils and, as such, may be important for the compliance of connective tissues (14). First, TNX is located between collagen fibrils organized in bundles (15), and the interfibrillar distance is increased in the skin of TNX-deficient patients. Furthermore, TNX was found to assemble into disulfide-linked oligomers, of which trimers are the predominant form. This disulfide-linked trimer structure of TNX is a property that is probably important for bridging (14). TNX interacts with types I, III, and V fibrillar collagen molecules and with decorin and binds to the fibril-associated types XII and XIV collagens (8, 14). Finally, the FNIII domains of TNX may be important for elastic properties of the molecule (14).

In short, altered muscular function in TNX KO mice is partially explained by changes of series elastic components of the muscle-tendon complex, which results in altered intra- and epimuscular myofascial force transmission. We hypothesize that this direct effect of altered ECM composition contributes to muscle weakness in EDS patients, in addition to mild myopathic effects of the disease on muscular histology.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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