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ORIGINAL ARTICLE

Muscle–Tendon Tissue Properties in the Hypermobility Type of Ehlers-Danlos Syndrome

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Objective. **To investigate the passive properties of the plantar flexors muscle–tendon tissue in patients with the hypermobility type of Ehlers-Danlos syndrome (EDS-HT).**

Methods. **Twenty-five women with EDS-HT and 25 sex- and age-matched healthy control subjects participated in the study. Passive resistive torque (PRT) of the plantar flexors was measured with an isokinetic dynamometer during 2 standardized stretch protocols to obtain the passive muscle tension. Protocol 1 consisted of 4 continuous cycles to a predetermined angle of 10° dorsiflexion. Protocol 2 consisted of a slow stretch to the onset of pain. Torque, angle, and electromyography were simultaneously recorded during the tests. To take muscle thickness into account, muscle cross-sectional area (MCSA) was obtained with peripheral quantitative computed tomography. Stiffness of the Achilles tendon was assessed using a dynamometer in combination with ultrasonography.**

Results. **The results demonstrate a significantly larger maximal joint angle in the EDS-HT patients accompanied by a similar PRT compared to the control subjects (protocol 2), indicating a lower passive muscle tension in the patient group. PRT for the predetermined angle (protocol 1) was the same for both groups and there was no difference in MSCA. Furthermore, a significantly lower Achilles tendon stiffness was seen in the patient group than in the control group.**

Conclusion. **This study is the first to provide evidence for altered passive properties of the muscle–tendon unit in EDS-HT patients. These changes are thought to be associated with structural modifications in connective tissue.**

INTRODUCTION

The Ehlers-Danlos syndrome (EDS) comprises a clinically and genetically heterogeneous group of heritable connective tissue disorders characterized by defects in the biosynthesis or secretion of fibrillar collagens (1–3). The 3 prominent features of this disorder are skin laxity, joint hypermobility, and tissue fragility (4).

The current EDS classification proposes 6 types based on clinical, biochemical, and molecular grounds. However, ~90% of all cases represent the hypermobility type of EDS (EDS-HT) in which generalized severe joint hypermobility, recurrent joint dislocations, and debilitating chronic joint pain are unequivocally the most striking features (4).

Excessive musculoskeletal mobility contributes to joint instability, which consequently gives cause to joint dislocations (5). Joint instability has been reported in almost every joint of patients with EDS-HT (6,7). Stability of the joint is based on both active and passive mechanisms. Active mechanisms include muscular strength as well as neuromuscular factors. Passive mechanisms include joint morphology and viscoelastic properties of soft tissue structures surrounding the joint, i.e., ligaments, joint capsules, and muscle–tendon tissue (8,9). Although never confirmed, joint hypermobility in patients with inherited connective tissue disorders is traditionally believed to be related to modified extensibility of the connective tissue in the soft tissues mentioned just above (10,11). As genetic aberrations affecting the fibrillar collagens distort their biomechanical structure, they may impair their tensile properties, resulting in reduced tension and stiffness.

Since the middle of the 1990s, isokinetic dynamometers have been utilized to measure the passive muscle tension. Passive muscle tension, or the resistance to elongation, is determined by the passive resistive torque (PRT) during a stretch maneuver (12–14). In addition, dynamometer measurement combined with ultrasonography is the most commonly used technique to investigate the stiffness of

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Significance & Innovations

- This is the first study to confirm modified extensibility of muscle–tendon tissue in patients with the hypermobility type of Ehlers-Danlos syndrome (EDS-HT).
- The observed reduction in passive plantar flexor muscle tension and Achilles tendon stiffness provides evidence for modified passive properties of muscle tendon in women with EDS-HT.
- These changes are thought to be associated with structural modifications in connective tissue.

tendon structures (15,16). Stiffness can be defined as the change in tension per unit change in length. These techniques allow the investigation of the passive muscle–tendon tissue properties in a noninvasive manner.

Until now, only 1 study investigated the passive muscle–tendon properties in hypermobile subjects. Magnusson et al (12) could not demonstrate altered passive properties of the hamstring muscle group in patients with benign joint hypermobility syndrome (BJHS), which closely resembles EDS-HT. They suggested a greater subjective tolerance to stretch loading in the patient group compared to the healthy control group. Tendon stiffness was not measured. However, whether the muscle–tendon tissue properties are modified in patients diagnosed with EDS-HT remains unclear.

Therefore, the purpose of the present study was to investigate the muscle–tendon tissue properties of the plantar flexor muscle–tendon unit, i.e., passive muscle tension of the plantar flexor muscles and stiffness of the Achilles tendon, in patients with EDS-HT compared to age- and sex-matched control subjects.

SUBJECTS AND METHODS

Subjects. The study protocol was reviewed and approved by the Ethical Committee of the Ghent University Hospital and written informed consent was obtained from all participants. As more than 90% of the EDS-HT patients are women (4), the current study included only women. Twenty-five women diagnosed with EDS-HT with a mean ± SD age of 41 ± 10 years participated. Patient selection was performed in the Centre for Medical Genetics at the Ghent University Hospital on the basis of the revised Villefranche criteria, including the presence of generalized joint hypermobility (Beighton score of ≥5/9) (Table 1) and/or skin hyperextensibility/fragility, in combination with recurring joint dislocations, and/or chronic musculoskeletal pain, and/or a positive family history. Also, 25 healthy volunteers, individually matched for sex and age (mean ± SD 41 ± 9.9 years), were included in the study. Control subjects were excluded if they had a history of lower leg injuries, a generalized disease affecting joints or ligaments, a Beighton score of >4/9, or if they participated in recreational sports with a frequency of >2 times per week or in competitive sports. In addition, surgery at the dominant lower leg and pregnancy or delivery in the last year were exclusion criteria for all subjects. The anthropometric characteristics of the subjects are presented in Table 2.

Table 2. Anthropometric details*

Variable	EDS-HT group	Control group	*P*
Age, years	41 ± 10.0	41 ± 9.9	0.833
Height, cm	168 ± 6.2	167 ± 6.5	0.609
Weight, kg	73.6 ± 14.29	68.4 ± 8.36	0.091
BMI, kg/m^2	26 ± 4.8	24 ± 3.2	0.136
MCSA, cm^2	61.2 ± 11.42	64.3 ± 8.64	0.390

* Values are the mean ± SD unless indicated otherwise. EDS-HT = Ehlers-Danlos syndrome hypermobility type; BMI = body mass index; MCSA = muscle cross-sectional area at 66% tibia.

Questionnaire. Before the measurements, all participants completed a questionnaire to assess their frequency of ankle distortion, their use of supportive ankle/foot devices, and their experience with calf muscle stretching. Pain severity was scored as current lower leg or foot pain at rest on the dominant side, measured with a visual analog scale. A score of 0 indicates no pain and a score of 10

Table 1. Clinical characteristics*

Variable	EDS-HT group (n = 25)	Control group (n = 25)	*P*†
Joint hypermobility, mean ± SD Beighton score	6 ± 1.3	1 ± 0.8	< 0.001‡
Lower leg and foot pain, mean ± SD VAS score	2 ± 2.4	0 ± 0.0	< 0.001‡
Frequency of ankle distortion, no. L/R			< 0.001‡
Never	2/3	21/19	
Seldom	1/2	2/4	
Sometimes	9/7	2/2	
Often	13/13	0/0	
Use of supportive ankle/foot devices, no.	18	2	< 0.001‡
Regularly stretching the plantar flexors, no.	4	7	0.306

* EDS-HT = Ehlers-Danlos syndrome hypermobility type; VAS = visual analog scale; L/R = left side/right side.
† By chi-square test.
‡ $P < 0.05$.

indicates unbearable pain. Leg dominance was determined as the leg the subject would use to kick a ball.

Measurement of the passive muscle tension of the plantar flexors. *Instrumentation.* To determine the PRT during a stretch maneuver, a Biodex Medical System 4 isokinetic dynamometer was used. The subject was placed in supine position with the knee maximally extended. The foot was securely strapped to a footplate connected to the lever arm of the dynamometer. The standard Biodex ankle-unit attachment with the provided straps was used. The attachment of the foot was constructed so that the movement of the ankle joint was not impeded, in order to avoid an overestimation of the PRT. The measurements took place in a quiet room and subjects were blindfolded in order to relax maximally. During the test, electromyographic (EMG) activity from the plantar and dorsiflexor muscles was recorded (Muscle Tester ME3000, Mega Electronics). Surface Ag-AgCL electrodes with an electrical surface contact of 1 cm^2 (BlueSensor, Medicotest, Ambu) were placed on the soleus, the tibialis anterior, and the medial head of the gastrocnemius muscle according to the guidelines of Basmajian (17), with an interelectrode distance of 10 mm. The raw EMG signals were amplified and filtered, and the analog signals were converted to digital data at a sampling rate of 1,000 Hz.

Because the maximal angle during stretch is individually determined, comparisons of PRT between the groups were made based on a predetermined joint angle common to all subjects (see protocol 1) and the subject's maximal joint angle (see protocol 2). Both protocols were administered to the dominant side.

Protocol 1. This protocol was administered to examine PRT during a predetermined ankle range of motion (ROM) used in many functional activities. The dynamometer moved the ankle passively through 4 continuous cycles of motion from 20° plantar flexion to 10° dorsiflexion at 5°/second (18), with neutral being the line of the tibia perpendicular to the footplate. A slow joint angular velocity was used to ensure that the stretch did not elicit a reflexive muscle activity, which is believed to be achieved using 5°/second (19). Throughout the test, subjects were requested to relax completely and not offer any voluntary resistance. The EMG tracings were monitored to ensure that muscle activity was <0.05 mV above baseline during the passive stretch cycles (19). The test was repeated if the subject was not relaxed sufficiently, that is, if the muscle activity was >0.05 mV above baseline.

The peak PRT (Nm), recorded from the dynamometer during the 4 cycles of motion, was used in the statistical analysis. A pilot study demonstrated that the reproducibility was high (intraclass correlation coefficient [ICC] 0.92–0.94, $P < 0.001$).

Protocol 2. This protocol was administered to determine the PRT during maximal ROM. The dynamometer moved the ankle passively at 2°/second from the starting point of 20° plantar flexion, with neutral being the line of the tibia perpendicular to the footplate, toward dorsiflexion to the onset of pain (stretch tolerance) (20). At this point, the subjects were instructed to press a switch that instantaneously stopped the lever arm. The ankle was immediately released from the attachment. Subjects were thoroughly instructed in this maneuver. Throughout the stretch maneuver, subjects were requested to relax completely and not offer any voluntary resistance. EMG activity was recorded to ensure baseline muscle relaxation. The maximal joint angle (degrees) and corresponding maximal PRT (Nm) were obtained and used in the statistical analysis.

Measurement of muscle cross-sectional area (MCSA). Passive resistance to stretch will depend on both the quality and quantity of tissue in parallel. A positive relationship between MCSA and resistance to stretch has been shown in which a thicker muscle offers more resistance (21). Therefore, calf MCSA (cm^2) of the dominant leg was determined with peripheral quantitative computed tomography (XCT2000 scanner, Stratec) as an indicator of muscle mass. Slices with a voxel size of 0.8 mm were obtained at 66% of the tibia length, the position with the greatest diameter of the plantar flexors. MCSA was estimated using a threshold below water equivalent linear attenuation set at 0.22/cm. This threshold eliminated skin and fat mass with lower linear attenuation in the cross-sectional slice. Bone area was subtracted from the remaining area, revealing the calf muscle at its maximum cross-sectional area. This procedure has been validated against measurements with magnetic resonance imaging (22), which was not possible due to the fact that many EDS-HT patients can not lie still in the supine position on a hard surface for at least 10 minutes.

Measurement of the stiffness of the Achilles tendon. The relationship between the supplied muscle force (F_m) during isometric plantar flexion and the elongation of the Achilles tendon is a measure of the stiffness of the Achilles tendon.

Measurement of torque. The dynamometer (Biodex System 4) was used to determine torque output during isometric plantar flexion. The subject lay prone on a bench. The dominant ankle was placed in a 90° position (anatomic position) with the knee joint at full extension and the foot securely strapped to a footplate connected to the lever arm of the dynamometer. Before the test, the subjects performed 3 to 5 submaximal contractions to become accustomed to the test procedure. After warming up, the subjects were instructed to develop an isometric maximal voluntary contraction for 5 seconds. The measurement was repeated 3 times per subject, with 30 seconds of rest between the trials. Visual examination was undertaken to ensure that the subject's ankle joint did not move during this muscle work. When motion was observed, the trial was discarded. Each subject was verbally encouraged to exert maximal voluntary effort by contracting as hard as possible. The maximal isometric strength was defined as the peak torque (TQ) recorded. The supplied F_m was estimated from the plantar flexion TQ, the physiologic cross-sectional area ratio of the medial gastrocnemius (MG) to all the plantar flexors, and the moment arm (see formula below).

Measurement of tendon elongation. At the same time, measurement of the elongation of the tendon structures

Table 3. Muscle–tendon tissue properties of the plantar flexors*

Variable	EDS-HT group	Control group	*P*
PRT with predetermined ROM, Nm	23.9 ± 4.89	24.0 ± 3.23	0.952
Maximal ROM, degrees	35.6 ± 9.19	26.4 ± 6.53	< 0.001†
PRT with maximal ROM, Nm	45.7 ± 15.72	45.8 ± 8.41	0.977
STIFFN, N/mm	12.3 ± 4.84	15.6 ± 4.43	0.033†

* Values are the mean ± SD unless indicated otherwise. EDS-HT = Ehlers-Danlos syndrome hypermobility type; PRT = passive resistive torque of the plantar flexors; ROM = range of motion; STIFFN = stiffness of the Achilles tendon.
† $P < 0.05$.

was performed, according to the method of Fukashiro et al (23). In the present study, a real-time ultrasonic apparatus (GE Vingmed Vivid7 Dimension) was used to obtain a longitudinal ultrasonic image of the MG muscle at 30% of the lower leg's length (± one-third of the distance between the popliteal crease and the center of the lateral malleolus) (24). An electronic linear array probe of 13 MHz wave frequency was enclosed with a thermoplastic case and secured with straps on the skin. The ultrasonic images were recorded. One tester (LR) identified the echoes from the aponeurosis and the MG fascicles. The point (*x*) at which 1 fascicle was attached to the aponeurosis was visualized on the ultrasonic image. This point (*x*) moved proximally during isometric torque output. The distance traveled by *x* (delta *x*) was measured and is considered to indicate the lengthening of the aponeurosis and, therefore, of the tendon (25,26). The mean value of the 3 measurements was used as a representative value for the elongation of the tendon (ELONG).

Calculation of Achilles tendon stiffness. The ratio of the calculated F_m and the elongation of the Achilles tendon (ELONG) provided a measure of the stiffness of the Achilles tendon. With respect to the F_m, the measured TQ (Nm) during maximal isometric plantar flexion was first converted to the F_m (N) using the following equation: $F_m = kTQ \times MA^{-1}$, where *k* is the relative contribution of the physiologic cross-sectional area of the MG within the plantar flexor muscles (18%) (23) and MA is the moment arm length of triceps surae muscle at 90° of the ankle joint flexion (neutral position) (50 mm) (27). Therefore, F_m = 18/100 x TQ/0.05. Second, the ratio of F_m and ELONG provided the stiffness of the tendon ($N \times mm^{-1}$). In this study, the calculations were based on those of Kubo et al (15). The test–retest reliability of measuring the stiffness of the Achilles tendon using ultrasonography has been shown to be good (ICC 0.80–0.82) (16).

Analyses and statistics. Data analysis was performed using PASW Statistics 18. Descriptive statistics are shown as the mean ± SD for continuous data and as absolute frequencies for categorical data. To compare the anthropometric data, the subjects' characteristics, and the plantar flexor muscle–tendon tissue properties between the EDS-HT group and the control group, independent *t*-tests for means and chi-square tests for frequencies were used. *P* values less than 0.05 were considered statistically significant.

RESULTS

Anthropometric characteristics of all subjects are illustrated in Table 2. Both the EDS-HT and control groups were homogenous with respect to age, sex, height, weight, and body mass index. Also, MCSA of the lower leg was similar for EDS-HT patients and control subjects ($P = 0.390$).

Table 1 shows that the Beighton score was greater for EDS-HT patients than for control subjects ($P < 0.001$). Also, ankle distortions were significantly more frequent in the EDS-HT group ($P < 0.001$) than in the control group (frequency distribution shown in Table 1), and 72% of the patients regularly used supportive devices such as elastic bandages, braces, orthopedic shoes, or therapeutic inlays. Furthermore, 14 patients (56%) reported mild pain on the lower leg or foot tested with a mean ± SD pain severity score of 2 ± 2.4, in contrast to the pain-free control subjects ($P < 0.001$). The number of subjects who regularly stretched their plantar flexors was small and comparable in both groups ($P = 0.306$).

Table 3 demonstrates that for joint-ankle motion between 20° plantar flexion and 10° dorsiflexion (protocol 1) there was no difference in PRT of the plantar flexors between both groups ($P = 0.952$). During the stretch maneuver until the onset of pain (protocol 2), the maximal joint angle was greater for EDS-HT patients than for control subjects ($P < 0.001$), whereas the corresponding PRT was not significantly different between both groups ($P = 0.977$). Furthermore, the stiffness of the Achilles tendon in the EDS-HT group was significantly lower compared to the control group ($P = 0.033$).

DISCUSSION

The main findings of the study were: 1) the maximal joint angle for stretch toward dorsiflexion was significantly larger in the patient group than the control group, whereas the corresponding PRT was not significantly different. In other words, a greater joint angle was achieved in the EDS-HT patients with a same load compared to the healthy subjects, indicating a lower passive muscle tension of the plantar flexors in the patient group; 2) the lower leg MSCA was similar in both groups and consequently unlikely to affect the muscle tension results; and 3) the stiffness of the Achilles tendon was significantly lower in the EDS-HT group compared to the control group. Therefore, structural

changes in the plantar flexor muscle–tendon tissue of EDS-HT patients could be suggested from the present results.

Our results of reduced passive muscle tension are not in agreement with those of Magnusson et al (12). They demonstrated a greater maximal joint angle as well as a greater corresponding PRT during knee extension in patients with BJHS compared to healthy matched control subjects. As a change in the tissue properties may only be concluded if a decrease in PRT was observed at the same joint angle, or if a greater joint angle was achieved with the same load (20), Magnusson et al could not conclude altered passive mechanical properties of the connective tissue in the muscle–tendon unit of hypermobile patients with BJHS, which is in contrast with our findings. Magnusson et al suggested that BJHS subjects have a greater subjective tolerance to passive stretch loading, which may explain the greater flexibility. The structures and mechanism for an altered stretch tolerance are presently unknown, but it is possible that nociceptive nerve endings in the joint and muscle play a role (28). As such, it is conceivable that a change in stretch perception could also be present in EDS-HT patients, but this cannot be derived from the present results. Even so, it is unknown whether enhanced subjective tolerance to passive stretch plays a role in the development of musculoskeletal complaints.

There are some factors that can be responsible for the different result in passive muscle tension. First, although EDS-HT and BJHS represent the same phenotypic group of patients (3,29), it stays controversial if both conditions are one and the same pathologic entity as long as the genetic background of EDS-HT and BJHS remains elusive (30). Second, it should be noted that the sample size of the study of Magnusson et al (12) was rather small (n = 9 women with BJHS).

The abnormal passive muscle tension shown in the EDS-HT patients indicates that the connective tissue of the muscle–tendon unit is affected. It has been shown that passive muscle tension is mainly influenced by the extensibility of connective tissue elements, i.e., the endomysium surrounding each muscle fiber, the perimysium surrounding groups of muscle fibers, and the epimysium surrounding the muscle as a whole (31). Although all 3 components contribute, the quantitatively most abundant perimysium has been considered as the major contributor to extracellular passive resistance to stretch (32).

Skeletal muscle belly connective tissue predominantly consists of collagen types I, III, V, and VI (33). In fact, most EDS subtypes are caused by mutations in genes encoding fibrillar collagens type I, III, or V, as well as genes encoding enzymes involved in the posttranslational modification of these collagens (1,2). In addition, mutations in the 3 genes coding for the alpha chains of collagen VI result in Ullrich and Bethlem congenital muscular dystrophy, which shows clinical and ultrastructural overlap with EDS (34). In this way, even though the genetic background of EDS-HT is presently unknown (35), it seems reasonable that fibrillar collagens in the muscle of EDS-HT patients may also lose their tensile properties, resulting in reduced passive muscle tension. In fact, Voermans et al recently showed a reduction of the density of collagen fibrils and mild structural abnormalities, including increased variation in fiber diameter and sporadic isolated atrophic fibers, on muscle biopsies of the quadriceps muscle in some EDS-HT patients (36).

In contrast to the protocol with maximal joint angle (protocol 2), our study could not demonstrate a difference between both groups in PRT of the plantar flexors for a predetermined ROM (protocol 1). This result may be explained by the fact that the predetermined dorsiflexion angle of 10° did not induce enough stretch on the plantar flexors muscle–tendon structures to provoke a difference in passive muscle tension.

A second aim of our study was to investigate whether patients with EDS-HT show altered stiffness of the Achilles tendon. Our results indicate that the tendon stiffness was significantly lower in the EDS-HT group compared to the control group. Unfortunately, there are no previous studies in hypermobile subjects with which to compare our results. Still, the present findings support the common explanation that enhanced tendon compliance due to increased extensibility of tendons also contributes to the frequently occurring muscle weakness in EDS-HT (36, Rombaut L, et al: unpublished observations) by modified myotendinous force transmission. In contrast, Voermans et al (37) lend no support for this interpretation as they could not find a delay between stimulation and initial muscle contraction nor a change in relaxation rate in the quadriceps muscle of 2 EDS patients with the rare tenascin-X deficient type. However, muscle function was studied at a relatively long muscle length, which may have masked the effects of increased tendon compliance.

Unfortunately, no biochemical or biomechanical studies of surgical or postmortem tendons in EDS subjects have been performed yet to confirm our results, thereby warranting further investigation. However, we can assume, in line with the structural changes in muscle connective tissue discussed above, that also the tendon can be affected, as tendon tissue consists predominantly of fibrillar collagen molecules, which is for the most part represented by type I collagen (38).

Recent published studies have shown that female EDS-HT patients have impaired proprioception (39) and muscle strength (36, Rombaut L, et al: unpublished observations), and both affect their joint stability. In addition, according to the concept of functional joint stability (9,40), the muscle–tendon complex also contributes to joint stability by their mechanical properties providing a certain passive restraint. In line with this, it could be suggested that the observations of reduced muscle tension and tendon stiffness in the present study may possibly contribute to the joint instability and hypermobility in EDS-HT. On average, the EDS-HT patients reached a maximal ankle joint angle that was ~26% greater than that of control subjects. In addition, a high frequency of ankle distortions was observed in our patient group, which is in agreement with previous results of our research group (7) and Berglund et al (41). For example, in the latter study, ankle problems such as sprains, unstable ankles, and weakness were reported by 73% of the EDS patients (41). This explains why a large number of EDS patients (72% of pa-

tients in the present study) appeal to supportive shoes or technical foot/ankle aids.

The findings of this study have important clinical implications. The results may contribute to a better understanding of how the complaints present and affect the EDS-HT patient, which will help the clinician to better manage the condition. Clinicians need to endeavor to rehabilitate EDS-HT patients with careful handling, bearing in mind the fragility of the connective tissues in both passive and dynamic soft tissues, which makes the patient vulnerable to trauma and overuse problems. In addition, attention should be paid to the associated neurophysiologic deficits in EDS-HT (39) to avoid excessive joint movement, which could lead to tears and ruptures of the soft tissues surrounding the joint.

Our results must be viewed within the limitations of the study. First, although subject sensation is the most frequently used end point in human muscle stretching research (like protocol 2), there is little consensus regarding which sensation (onset of pain, stiffness, discomfort, stretch) is most clinically relevant. End-range joint angles can vary among subjects, depending on the sensation (42). However, all subjects were thoroughly instructed to report the onset of pain during this maneuver. Second, the study only included female patients. As some studies showed differences in the viscoelastic properties of tendon structures between sexes (43), we should be cautious with generalization. However, 90% of patients with EDS-HT are women (4).

In conclusion, this is the first study to investigate the mechanical properties of the muscle–tendon unit in patients with EDS. The results demonstrate a larger maximal plantar flexor stretch angle in the EDS-HT patients accompanied by a similar corresponding PRT compared to the control subjects, indicating a lower passive muscle tension in the patient group. Also, a lower Achilles tendon stiffness was seen in the patient group. Therefore, these findings suggest altered passive properties of the plantar flexor muscle–tendon unit in women with EDS-HT. These changes are thought to be associated with structural modifications in connective tissue.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Rombaut had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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