

Original Article

In-Vivo Force-Length Relationship of the Medial Gastrocnemius Muscle in Hypermobile Ehlers-Danlos Syndrome and Hypermobility Spectrum Disorders

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Abstract

Objectives: To determine the force-length relationship, muscle thickness (MT), pennation angle (PA) and echo-intensity (EI) of the medial gastrocnemius (MG) in HSD/hEDS compared to healthy controls. **Methods:** The maximal MG force-length relationship was determined throughout ankle passive ROM in 11 individuals with HSD/hEDS and 11 healthy age- and sex-matched controls. MG fascicle length (FL), MT, PA and EI were measured using ultrasonography. MG force was calculated from plantarflexion moments and MG FL. Sarcomere lengths (SL) were estimated from changes in FL and assuming an optimal SL of 2.64 μm at optimal fascicle length. **Results:** HSD/hEDS were 36% weaker across the ankle ROM ($p = 0.047$). Optimal FL was significantly shorter in HSD/hEDS (34.8 ± 8.1 mm) compared to controls (43.6 ± 6.9 mm; $p = 0.013$). A shallower descending limb of the force-length relationship was seen in HSD/hEDS ($p < 0.001$). MT, PA, and EI were similar between groups ($p > 0.05$). **Conclusion:** HSD/hEDS were weaker at all measured fascicle lengths, despite similar muscle architecture (MT, PA) and quality (EI) suggesting that reduced neuromuscular activation may contribute to muscle weakness in this population. Rehabilitation interventions should consider addressing joint-angle specific strength deficits in HSD/hEDS.

Keywords: Ehlers-Danlos Syndrome, Joint Instability, Muscle Strength, Muscle Weakness

Introduction

Joint hypermobility has been estimated to affect approximately 10% to 30% of adults depending on sex, age, and ethnicity^{1,2}. Some individuals with joint hypermobility

may be asymptomatic while others may experience a range of multisystemic symptoms including recurrent joint and tendon injury, chronic pain, fatigue, respiratory, and dermatologic complications. The latter group includes those with hypermobility spectrum disorders (HSD) and hypermobile Ehlers-Danlos syndrome (hEDS)³. HSD/hEDS share an estimated prevalence of 1 in 500^{4,5} and are often associated with reduced quality of life⁶⁻⁸. Muscular weakness in HSD/hEDS has also been documented^{7,9} and has been associated with activity limitations¹⁰, fatigue⁸, and pain⁹. Daily activities such as walking or climbing stairs are reported to be challenging and painful for some individuals living with HSD/hEDS^{11,12}. The triceps surae are highly active during the stance phase of walking and

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generate plantarflexion force during propulsion in terminal stance^{13,14}. Therefore, weakness of these muscles may contribute to pain and limited function during locomotion in this clinical population.

Muscular forces and resulting joint moments are influenced by tissue properties and neuromuscular activation¹⁵. The total muscle force consists of both active (generated by the contractile element) and passive components, the latter of which arises from various parallel and in-series connective tissue components of the muscle-tendon unit (MTU)¹⁵. As other forms of EDS result from structural issues with various forms of collagen, muscle-tendon function may be altered in HSD/hEDS and further contribute to reported symptoms and physical limitations^{9,16,17}. In addition to structural issues, altered neuromuscular function may also contribute to weakness in HSD/hEDS¹⁸. It has been shown in other EDS subtypes that reduced function within muscle tissue may be influenced by altered collagen formation in the extracellular matrix¹⁹. During contraction, muscles shorten and pull on long, compliant tendons connected in series with the muscle. For a given muscle force in HSD/hEDS, a more compliant tendon would require greater muscle shortening to stretch the tendon and affect joint rotation. This additional muscle shortening in HSD/hEDS may result in muscles operating at shorter than optimal muscle lengths for peak force production.

The relationship between muscle length and active force production is described by the sliding filament theory of muscle contraction, which posits that the amount of active force produced by a muscle is dependent on the relative overlap of actin and myosin filaments within the sarcomere^{15,20,21}. Maximal force is achieved at a length associated with maximal actomyosin overlap, and greater muscle shortening or lengthening results in a decrease in active force. This phenomenon that maximal force is achieved at some optimal length is demonstrated at multiple muscle scales, from single sarcomeres^{20,21} to whole MTUs¹⁵. To our knowledge, the force-length properties of the plantarflexor muscles have yet to be investigated in HSD/hEDS. This might offer a mechanism for muscle weakness in this population because muscles in HSD/hEDS may shorten beyond the length associated with maximal active force production.

At the whole muscle level, muscle size and architecture also have a direct influence on the force-producing capacity of the tissue, including measurements of muscle thickness (MT) and pennation angle (PA)^{22,24}. A larger muscle with more or larger fibers will allow for more contractile tissue capable of producing active force^{22,24}. Together MT and PA are used to calculate physiological cross-sectional area (PCSA), a measure of the force producing capacity of a muscle²⁴.

Higher forces are also produced when a given muscle thickness consists of muscle tissue, as opposed to inter- and intra-muscular fat. Muscle quality can be assessed using B-mode ultrasound and histogram analysis^{25,26}.

Histogram analysis of muscle tissue using ultrasonography involves utilizing the effect of differences in tissue composition and the reflection of acoustic ultrasound waves from those tissues. Tissues with varying compositions will reflect ultrasound waves at different echo intensities (EI). Higher tissue EI is typically indicative of non-contractile tissue^{22,27} and has been negatively correlated with muscular strength^{28,29}. Thus, it is plausible that muscle quality, as assessed by tissue EI, may also contribute to weakness in HSD/hEDS.

Theoretically, diminished muscular strength in HSD/hEDS could result from various structural and functional factors, including but not necessarily limited to: 1) reduced muscle quality, 2) reduced muscle quantity, 3) reduced ability to activate a similar muscle quantity, and/or 4) altered muscle properties such as differences in the muscle's force-length relationship. Determining the force-length relationship of the plantarflexor muscles and elucidating the potential mechanisms contributing to muscular weakness in HSD/hEDS may provide insight into strategies and interventions for improving pain, fatigue, and quality of life during daily activities.

The primary purpose of this study was to determine the *in-vivo* force-fascicle length relationship of the medial gastrocnemius (MG) in individuals with HSD/hEDS compared to healthy age- and sex-matched controls. A secondary purpose was to elucidate potential structural mechanisms of muscular weakness in HSD/hEDS compared to healthy controls. We hypothesized that compared to otherwise healthy individuals, those with HSD/hEDS would exhibit:

1. Reduced active force for any given length due to HSD/hEDS muscle fascicles shortening beyond optimal length and,
2. Reduced MT, smaller PA, and increased EI at rest.

Materials and Methods

Individuals with HSD/hEDS (n = 11, 10 females) were compared to 11 age- and sex-matched healthy controls. Participants with HSD/hEDS were recruited from a tertiary physiatry clinic (Connective Tissue Disorders Rehabilitation Clinic, Foothills Medical Centre, Calgary, Alberta, Canada) and included if they had a diagnosis of hEDS (n = 10) or met the criteria for generalized HSD (n = 1) based on the current diagnostic criteria^{3,6}. Age- and sex-matched healthy controls were recruited if they presented with a Beighton score $\leq 4/9$, were within three years of age with an un-matched HSD/hEDS participant, and were free of prior cardiovascular, neurological, connective tissue or musculoskeletal disorder. Participants were excluded based on the presence of any major medical conditions (except for HSD/hEDS).

Participants self-reported their assigned sex at birth and visited the lab on a single occasion. The participant laid prone with their dominant foot and ankle affixed to an isokinetic dynamometer (Biodex System 3, Biodex Medical

Systems Inc., Shirley, NY, USA) using Velcro straps. The ankle joint was aligned such that the center of rotation of the ankle was aligned with the axis of the dynamometer³⁰. The ankle was passively rotated through the participant-specific ROM at 5 deg·s⁻¹, with two full ROM increments from maximum plantarflexion to maximum dorsiflexion recorded as passive trials. Participants performed two familiarization trials of their full passive range of motion prior to recording the passive trials. Participants were then asked to perform 5-second isometric maximal voluntary contractions (MVC) of the plantarflexors at six relative percentages (0,20,40,60,80,100%) of their ankle ROM, where 0% and 100% ROM were considered maximal dorsiflexion and plantarflexion, respectively. Participants performed a 5-second isometric familiarization trial around an ankle angle of 90 degrees prior to the MVCs. The order of each MVC was randomized using a random number generator for each participant, with a 2-minute rest interval between each MVC. Plantarflexion forces during the passive and active trials were measured at 100 Hz using a commercially available instrumented insole (Loadsol, Novel.de, St Paul, MN, USA) and saved to a smart device (iPad mini-4, Apple Inc. Cupertino, CA). The reliability and validity of these insoles have been previously established³¹. Insoles were calibrated to body weight prior to affixing the insole to the dynamometer. Once the insole was secured to the dynamometer footplate using tape, the force output was centered at zero to negate any detected pressure due to securing the insole to the footplate prior to positioning the participant in the dynamometer. The isometric force-length data represents a portion of data collected as part of a larger study protocol as described previously by Sheehan et al.³². As such, plantarflexion forces were assessed using the force insole to coincide with data from Sheehan et al. who also measured plantarflexion forces using the same insole during walking³².

Plantarflexion moments during each contraction were calculated as the sum of forces measured at the center of each of the three force-sensing zones in the insole, as previously described^{31,33}. The Achilles tendon (AT) moment arm (MA_{AT}) at 90° was calculated using the tendon excursion method, accounting for passive forces according to Fletcher and MacIntosh³⁰, who demonstrated an angle-invariant MA_{AT} when accounting for passive forces during the angular rotation. The MA_{AT} determined from tendon travel was calculated as the displacement of the MG-AT myotendinous junction (MTJ) in mm from 85° to 95° divided by the ankle angular displacement (in radians). Displacement of the myotendinous junction was quantified from ultrasound imaging (MicrUS 65 mm LV8-4L65S-3 EXT-1H, Telemed, Vilnius, Lithuania) recorded at 39 Hz. The recorded ultrasound images were analyzed in *ImageJ* (v. 2.3.0, NIH, Baltimore MD USA). Two ultrasound probes with a depth of 40 mm were placed on the MG. The first probe identified the MG-AT MTJ. The second probe was placed immediately proximal to the MTJ probe approximately at the mid-muscle belly. Probe placement was aligned with the

MG-MTJ for each participant. Ultrasound image analysis was not blinded. Achilles tendon force was calculated from total plantarflexion moment divided by the measured MA_{AT} as previously described^{31,33}. Passive forces of the in-series and parallel elastic components of the triceps surae were calculated as the ratio of plantarflexion moment to MA_{AT} during the passive trials. Passive joint stiffness was calculated as the slope of the passive force vs. ankle angle between 0% and 20% ROM.

During each MVC, FL was measured at rest and at peak active force between the superficial and deep aponeurosis using *ImageJ*³⁵. In the few cases where a whole muscle fascicle could not be seen on the ultrasound image, linear extrapolation was used to measure the total FL³⁶. In total, extrapolation was used in 2/11 HSD/hEDS and 2/11 control participants, respectively, at 0% ROM (i.e. the longest fascicle lengths). Intra-rater reliability for fascicle length measurements was performed in a separate cohort of 10 individuals without HSD or hEDS (4 males, 6 females) using the procedure for ultrasound placement as described above. Fascicle lengths of the medial gastrocnemius muscle were measured at peak force from 20 separate MVCs at randomized percentages of ankle ROM. Measurements were repeated 1 week later and analyzed by the same examiner. Intra-class correlation coefficient (ICC, 3, k) was employed to assess relative reliability. Limit of agreement (LOA) and standard error of the mean (SEM) were used to assess absolute reliability. Intraclass correlation coefficient (ICC) was interpreted according to Koo et al.³⁷.

Force and ultrasound data were synchronized via an external 5 V trigger, ensuring frame-level alignment at peak torque. Active force during each MVC was calculated by subtracting the passive force (corresponding to the fascicle length at which peak force occurred) from the total plantarflexion force³⁴. Fascicle shortening during each MVC was calculated as the difference between resting FL and the FL at peak force. Briefly, peak force was calculated from the force insole, and the ultrasound frame number corresponding to the time at peak force was interpolated from the ultrasound images. Optimal FL was defined as the FL at which peak force across the entire ROM was achieved (F_{max})³⁴. Fascicle force (F_{Fas}) was calculated by dividing the active force by the cosine of the measured PA. PA was measured as the angle between the measured fascicle and the deep aponeurosis³⁸. Fascicle force was also expressed relative to maximal force (F_{Fas}/F_{max}).

From the measured force-fascicle length relationship, the relative force-sarcomere length relationship was estimated by assuming a SL of 2.64 μ m for human skeletal muscle at optimal FL³⁹. From this SL, changes in SL were assumed to be uniform within a muscle cell and therefore any change in FL represented an equal magnitude change in each SL³⁴. The SL at each ankle angle was thus calculated based on the relative FL change from optimal length³⁴. The number of sarcomeres in series (N) was calculated as the FL measured at F_{max} divided by 2.64 μ m.

MT was defined as the perpendicular distance between

Table 1. Participant characteristics.

	CON	HSD/hEDS	p-value	Effect size (Cohen's <i>d</i>)
Participants (n)	11	11		
Age (years)	32.6±14.1	33.2±14.0	0.928	0.039
Height (cm)	165.6±9.9	169.9±6.4	0.240	0.516
Weight (kg)	67.4±14.6	70.6±11.0	0.566	0.249
ankle ROM (deg)	76.0±5.0	73.6±5.3	0.292	-0.462
Beighton score (/9)	1±1	7±1	<0.001	5.013
Moment arm (mm)	37.1±6.4	44.1±7.0	0.024	1.039*
Passive stiffness (N·deg ⁻¹)	19.7±5.2	14.0±7.0	0.042	-0.926

Data are presented as mean ± standard deviation. *Significant difference between groups ($p < 0.05$). * Effect size reported as rank biserial correlation (non-parametric distribution in moment arm data across participants).

Table 2. Passive and active plantarflexion force in HSD/hEDS and Control participants.

ROM	Passive Force (N)		Active Force (N)		Difference from CON (%)
	CON	HSD/hEDS	CON	HSD/hEDS	
0%	382±129	289±140	2011±1092	1400±1107	30%
20%	84±86	83±89	2720±1566	1553±1012	43%
40%	22±36	27±60	2474±1332	1682±970	32%
60%	15±30	24±51	1794±992	1243±670	31%
80%	5±16	23±40	1181±623	697±326	41%
100%	8±21	21±43	670±263	402±288	40%

Data are presented as mean ± standard deviation. *HSD/hEDS produced significantly less active force at each % ROM compared to their age- and sex-matched pair ($p = 0.047$, $\eta^2 = 0.339$).

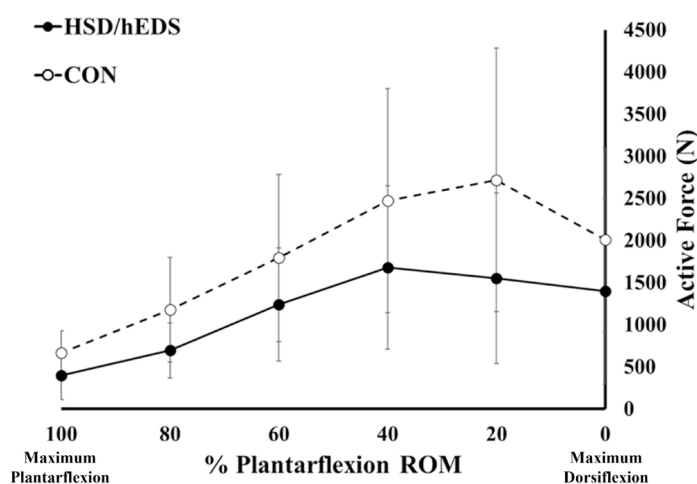


Figure 1. Active plantarflexion force vs. ankle angle at relative percentages of each participant's plantarflexion range of motion. Data are presented as mean±standard deviation. 100% represents full plantarflexion while 0% represents full dorsiflexion. Active force was significantly lower across ankle angles in hEDS ($p = 0.047$, $\eta^2 = 0.339$). Note: Inter-individual variability in the % ROM at which peak force was achieved is presented in Supplementary Table 1. Abbreviations: ROM, range of motion; HSD/hEDS, hypermobility spectrum disorder/hypermobile Ehlers-Danlos Syndrome; CON, control.

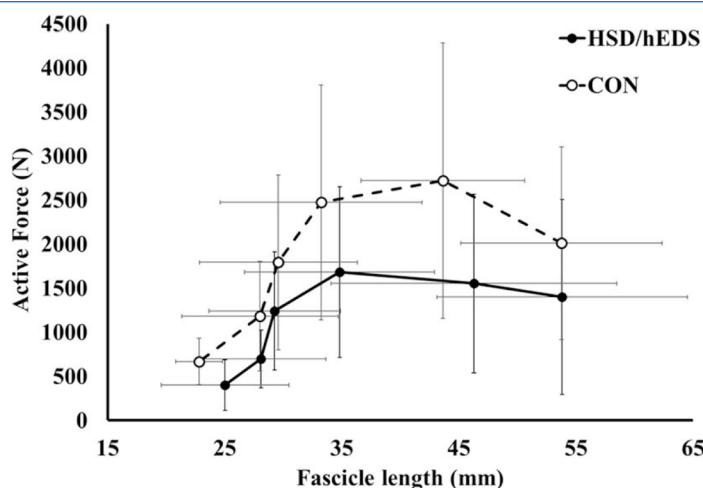


Figure 2. Active force-fascicle length relationship. Data are presented as mean \pm standard deviation. Abbreviations: HSD/hEDS, hypermobility spectrum disorder/hypermobile Ehlers-Danlos Syndrome; CON, control.

the superficial and deep aponeurosis measured at the widest point in the ultrasound field of view²². Subcutaneous thickness (SCT) was measured as the distance between the skin and superficial aponeurosis²⁵. EI was defined as the mean greyscale value between a threshold of 0 (black) and 255 (white), using histogram analysis in 8-bit resolution within *ImageJ*²⁵. A rectangular region of interest was defined to include as much of the muscle between the superficial and deep aponeurosis as possible²⁵. EI was analyzed with correction for SCT (uncorrected EI + (subcutaneous fat thickness (cm) \times 40.5278)) to reduce variability as previously described for the quadriceps muscles²⁶. As an SCT correction factor for the MG has not been validated to our knowledge, the formula for the quadriceps was applied. Analysis was performed with and without SCT correction to ensure conclusions were not significantly different. SCT-corrected EI values are reported.

Statistical Analysis

Descriptive statistics were performed. Statistical analyses were performed using *JASP* (Version 0.17.1.0) and *RStudio* (Version 2012.12.0+467). Shapiro-Wilk tests were used to check for normality and Levene's test for equality of variances. Independent samples *t*-tests assessed differences in weight, height, ROM, passive stiffness, and N between groups. Wilcoxon ranked sum test compared MA_{AT} between groups. Matched-pair analyses compared the differences between age- and sex-matched pairs of HSD/hEDS and their corresponding controls using two-way (group \times joint angle) repeated measures analyses of variance (ANOVAs) to assess active force, passive force, FL,

SL, MT, PA, EI, and SCT across the six relative joint angles. As the slope of the descending limb of the force-length relationship was not normally distributed ($p = 0.039$), the non-parametric Mann-Whitney U-test was used to evaluate differences in the slope of the descending limb of the force-length relationship between groups. Mauchly's test of sphericity was used to check for variation and Greenhouse-Geisser correction was implemented whenever sphericity was violated. Tukey's and Holm's *post-hoc* tests were used to detect significant differences between groups when a significant main effect was identified. Effect sizes for *t*-tests were reported as Cohen's *d*, with small, medium, and large effect sizes of $d = 0.20$, 0.50 , and 0.80 , respectively⁴⁰. Effect sizes for repeated measures ANOVA were reported as partial eta squared (η^2), with small, medium, and large sizes of $\eta^2 = 0.0099$, 0.059 , and 0.14 , respectively⁴¹. The *a priori* level of significance was set at $\alpha = 0.05$.

Results

Participant characteristics are reported in Table 1. All values are presented as mean \pm standard deviation. Passive and active forces across the ankle ROM are shown in Table 2 and Figure 1. Passive forces were similar between groups at each % ROM ($p = 0.74$, $\eta^2 = 0.006$). A medium, but non-significant effect in passive force at maximum dorsiflexion (0% ROM) was seen between HSD/hEDS (289 ± 140 N) and controls (382 ± 129 N; $p = 0.12$, $d = 0.69$). Individuals with HSD/hEDS produced significantly less active force at each relative ankle angle compared to their matched control ($p = 0.047$, $\eta^2 = 0.339$). On average, those with HSD/HEDS produced 36% less active force across the ankle ROM compared to controls. The % ROM at which individual

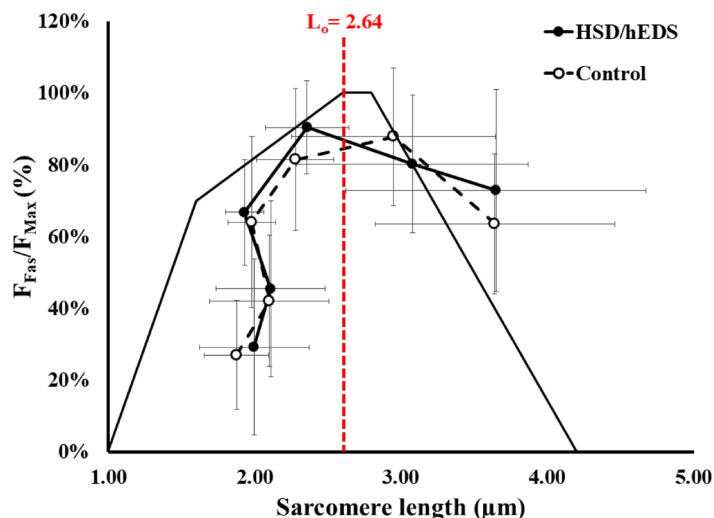


Figure 3. Sarcomere force-length relationship. Force (F_{Fas}) is expressed relative to the maximum force (F_{max}) of each pairs' age-matched control. Data are expressed as mean \pm standard deviation. The unmarked solid line illustrates the theoretical force-length relationship based on the lengths of the thick and thin filaments, as described by Gordon & Huxley²⁰. The vertical red dashed line indicates the estimated optimal sarcomere length for human muscle on the short side of the plateau of the force-sarcomere length relationship. Optimal sarcomere length was estimated from Herzog²¹. Both the solid line for the theoretical force-length relationship (Gordon and Huxley²⁰) and the red dashed line for the assumed optimal sarcomere length (Herzog & Keurs²¹) are shown for illustration purposes and were not fitted to our data. Optimal sarcomere length was calculated for each participant as the length associated with peak active force production. Changes in sarcomere length were proportional to changes in the measured fascicle length across joint angles. Note: Because not all participants achieved L_o at the same relative ankle joint angle, average F_{Fas}/F_{max} across all participants is not equivalent to 1.0 at L_o . HSD/hEDS, hypermobility spectrum disorder/hypermobile Ehlers-Danlos Syndrome; CON, control.

participants achieved peak active force production (Supplementary Table 1) varied between 0% ROM (3/11 HSD/hEDS; 1/11 CON), 20% ROM (2/11 HSD/hEDS; 7/11 CON) and 40% ROM (6/11 HSD/hEDS; 3/11 CON).

Measured FL and estimated SL are reported in Table 3. Intra-rater reliability of fascicle lengths was found to be moderate with an ICC of 0.72 (95% CI: 0.69-0.75). Absolute reliability was deemed to be acceptable with LOA = -0.6 ± 2.4 mm and SEM = 0.5 mm (95% CI: $-1.7-0.5$ mm).

The active force-FL relationship is shown in Figure 2. Optimal FL was 43.6 ± 6.9 mm for controls at 20% ROM, and 34.8 ± 8.1 mm for HSD/hEDS at 40% ROM ($p = 0.012$, Cohen's $d = -1.17$). At each relative ankle angle, both FL at rest ($p = 0.57$, $\eta^2 = 0.016$) and at peak active force ($p = 0.69$, $\eta^2 = 0.008$) were similar between groups. Together, muscle shortening during the MVC at each relative ankle angle was also similar between groups ($p = 0.67$, $\eta^2 = 0.009$). At longer than optimal FL (i.e. on the descending limb of the muscle's force-length relationship), active force declined less as a function of fascicle length in HSD/hEDS compared to controls ($p < 0.008$, Cohen's $d = 0.653$).

The relative active force (F_{Fas}/F_{max})-SL relationship

is shown in Figure 3. The longest estimated SL was similar between HSD/hEDS (3.65 ± 1.02 μm) and controls (3.64 ± 0.82 μm ; $p = 0.59$, Cohen's $d = 0.23$). The number of in-series sarcomeres (N) was also similar between groups ($N = 15,963\pm 6040$ in HSD/hEDS, $N = 15,331\pm 3486$ for CON; $p = 0.76$, Cohen's $d = -0.13$). Similar to the decline in active force on the descending limb, relative active force (F_{Fas}/F_{max}) also declined significantly less in HSD/hEDS compared to controls ($p = 0.034$, $d = 0.537$).

MT, PA, and EI at each % ROM are shown in Table 3. PA ($p = 0.99$, $\eta^2 = 2.19\times 10^{-5}$), MT ($p = 0.50$, $\eta^2 = 0.023$) and EI ($p = 0.074$, $\eta^2 = 0.28$) were similar between groups. SCT was lower in HSD/hEDS compared to controls (mean difference = 3.40 ± 1.50 mm, $p = 0.030$, $\eta^2 = 0.22$).

Discussion

To our knowledge, this is the first study to report the force-length relationship of skeletal muscle *in-vivo* in HSD/hEDS. Individuals with HSD/hEDS were relatively weaker compared to age- and sex-matched controls at all measured FL. The greatest disparity in MG muscle strength between

Table 3. Measured fascicle length and estimated sarcomere length at rest and at peak MVC force, muscle thickness, pennation angle and echo-intensity by % ROM.

ROM	Resting fascicle length (mm)		Resting sarcomere length (μm)		Fascicle length at peak MVC force (mm)		Sarcomere length at peak MVC force (μm)		Muscle thickness (mm)		Pennation angle ($^{\circ}$)		Echo-intensity (O-255)	
	CON	HSD/hEDS	CON	HSD/hEDS	CON	HSD/hEDS	CON	HSD/hEDS	CON	HSD/hEDS	CON	HSD/hEDS	CON	HSD/hEDS
0%	62.1 \pm 7.2	65.0 \pm 15.6	4.30 \pm 0.97	4.42 \pm 1.08	53.8 \pm 8.6	53.8 \pm 10.7	3.64 \pm 0.82	3.65 \pm 1.02	18.9 \pm 2.5	19.4 \pm 3.6	17.9 \pm 2.7	19.5 \pm 4.4	59.6 \pm 14.7	69.3 \pm 25.3
20%	58.4 \pm 5.4	61.1 \pm 13.4	4.06 \pm 0.93	4.17 \pm 1.04	43.6 \pm 7.0	46.3 \pm 12.2	2.95 \pm 0.70	3.08 \pm 0.79	18.2 \pm 2.6	18.9 \pm 2.9	23.1 \pm 6.3	22.7 \pm 6.1	60.5 \pm 16.7	68.7 \pm 24.9
40%	52.6 \pm 7.2	56.2 \pm 12.0	3.63 \pm 0.72	3.88 \pm 0.93	33.2 \pm 8.6	34.8 \pm 8.1	2.28 \pm 0.26	2.36 \pm 0.29	17.3 \pm 2.7	17.9 \pm 3.4	30.8 \pm 8.0	30.1 \pm 8.4	57.5 \pm 17.2	67.1 \pm 24.2
60%	49.3 \pm 5.9	51.2 \pm 12.6	3.47 \pm 0.53	3.60 \pm 0.74	29.6 \pm 6.7	29.3 \pm 5.6	1.98 \pm 0.16	1.93 \pm 0.13	16.8 \pm 2.7	17.2 \pm 3.3	34.4 \pm 7.7	35.8 \pm 8.3	54.1 \pm 18.2	64.0 \pm 24.9
80%	42.5 \pm 7.1	43.2 \pm 14.3	3.11 \pm 0.16	3.25 \pm 0.43	28.0 \pm 6.7	28.1 \pm 5.5	2.10 \pm 0.41	2.11 \pm 0.37	13.0 \pm 2.5	17.4 \pm 2.6	36.9 \pm 10.8	37.5 \pm 6.7	52.8 \pm 15.8	62.0 \pm 22.5
100%	34.0 \pm 7.8	36.5 \pm 10.5	3.21 \pm 0.32	3.32 \pm 0.48	22.8 \pm 2.0	25.0 \pm 5.5	1.88 \pm 0.22	2.00 \pm 0.38	15.9 \pm 2.4	17.0 \pm 2.6	43.8 \pm 6.5	41.8 \pm 8.5	48.8 \pm 17.6	56.5 \pm 22.0

Data are presented as mean \pm standard deviation. No significant main effect of group ($p > 0.05$) was seen for any of the measured variables.

HSD/hEDS and controls was at optimal FL for controls (43.6 \pm 7.0 mm), where those with HSD/hEDS produced 43% less active force compared to their matched control. However, at longer than optimal FL, active force was relatively maintained for large changes in fascicle lengths on the descending limb of the muscle's force-fascicle length relationship in HSD/hEDS. The mitigated decline in force beyond optimal FL (44 mm to 55 mm) in HSD/hEDS suggests an extended plateau region of the force-length relationship, which may have functional implications. As force-length properties have been shown to adapt to the requirements imposed upon the muscle⁴², it could be speculated that the MG in HSD/hEDS may be adapted to habitually acting over a greater ROM during daily activities. This extended plateau of the force-length relationship could also be attributed to the length-dependence of active force, whereby interfilament spacing is reduced at long fascicle lengths and enhancing calcium sensitivity to force production or differences in the distribution of sarcomere lengths between HSD/hEDS and their matched-controls⁴².

Alternatively, relatively higher forces at longer FL could be due to intra-cellular adaptations at the level of the sarcomere such as altered stiffness of the protein titin. Increased stiffness of titin was observed to contribute to higher forces at longer muscle lengths in classical-like EDS (a form of EDS due to tenascin X deficiency)⁴³. However, the maintenance of active force production at longer FLs in our sample is not likely due to alterations in titin, as we accounted for passive forces at the corresponding FL at which peak force occurred in our calculation of active force. Higher active force at long SLs in HSD/hEDS could also be the result of subtracting less passive force from the total force due to a more compliant AT, resulting in an active force that appears higher. However, measured passive forces were not statistically

different in the current study. Ottenheim, et al. suggested a slower detachment rate of cross bridges and prolonged force production at long SL⁴³ in classical-like EDS. This could explain the relative maintenance of force production at long lengths in our sample. This notion, however, remains speculative as our participants were not confirmed to be tenascin X deficient. While the diagnosis of HSD/hEDS is based on the exclusion of other genetically confirmed connective tissue disorders, we did not perform genotyping in our cohort and relied on previous diagnosis by a physician.

Lower active force in HSD/hEDS compared to controls at a given MG fascicle length means that walking at a given speed requires a greater relative force in HSD/hEDS. This muscle weakness may contribute to pain, fatigue, and physical limitations during daily activities as greater muscle activation would be required to produce the plantarflexion positive ankle joint power during stance. This aligns with observations from our laboratory of increased pain and fatigue in HSD/hEDS following walking¹². Greater pain and fatigue may then also reduce the ability of active contractions to stabilize the ankle joint during walking. Therefore, FL or joint angle-specific deficiencies in strength may provide targets for strength training to improve symptoms and function during challenging activities in HSD/hEDS such as while walking or navigating stairs or uneven ground.

Contrary to previous literature¹⁷, we found similar ROM between HSD/hEDS and controls. Our participants with HSD/hEDS may have limited both their plantarflexion and dorsiflexion ROMs due to pain, fear of injury, or muscular weakness, resulting in similar ROM to the control group. Additionally, MA_{AT} was greater in HSD/hEDS, implying the MTU lengthened to a greater extent for a given

change in ankle angle during passive joint rotation. This implication is supported by reduced passive joint and AT stiffness in HSD/hEDS, as previously shown by our work and the work of others^{17,32}. Therefore, for a given change in ankle angle, compliance of the tissues contributing to joint stiffness may lead to greater lengthening of the MTU in HSD/hEDS. During locomotion, this would imply a greater muscle shortening to accommodate the additional MTU length changes in HSD/hEDS. A longer MA_{AT} would also increase ankle plantarflexion moment for a given triceps surae force, which may partially compensate for a lower muscle active force.

Passive force at maximum dorsiflexion (0% ROM) was not significantly different between groups despite reduced passive joint stiffness in HSD/hEDS. Passive stiffness of the ankle may be influenced by a more compliant AT in HSD/hEDS^{17,32}, however this may not translate to differences in passive force as more structures than just the AT contribute to resisting dorsiflexion such as the joint capsule, ligaments, etc. This suggests ankle joint function may be maintained by compensation from other joint tissues, leading to similar passive forces experienced by the joint despite reduced tendon and joint stiffness.

Similar fascicle shortening during each MVC between HSD/hEDS and controls was observed. During an MVC, for a fixed MTU length, a reduced maximal muscle force would result in *lower* muscle fascicle shortening for a given AT stiffness. However, for a given force, a lower AT stiffness would result in *greater* fascicle shortening³³. Taken together, reduced maximal muscle force combined with a lower AT stiffness³² may result in similar fascicle shortening during the MVCs compared to controls.

With respect to the estimated sarcomere force-length relationship, HSD/hEDS shortened beyond optimal sarcomere length (2.64 μm) to an average estimated sarcomere length of 2.36 μm . The shift in “optimal” SL to shorter SL associated with the ascending limb in HSD/hEDS may be in response to increased tendon compliance. With reduced stiffness of the Achilles tendon, either increased muscle shortening or adjusting the joint angle to take advantage of a larger MA would be required to generate a given ankle joint moment. This shorter than optimal SL also coincides with our finding of increased MTU lengthening, as additional muscle shortening would be necessary to lengthen a more compliant tendon⁴⁴. Therefore, the shift of peak force production towards the ascending limb of the MG force-SL relationship may be in response to accommodate a more compliant in-series tendon. This increased muscle shortening would necessitate an increased energy cost, requiring more actin-myosin cross bridges and adenosine triphosphate, thereby increasing the energy cost of contraction in HSD/hEDS^{15,32}. This is indirectly supported by previous observations from our laboratory of elevated total metabolic cost during walking in HSD/hEDS³². However, while increased energy cost of muscle contraction could provide a possible explanation for muscular weakness in HSD/hEDS, muscle energy cost has yet to be measured

directly in the MG during MVC in this population.

As greater AT compliance in HSD/hEDS^{17,32} may impact the *in-vivo* force-length relationship of the MG (because the active muscle must shorten against a more compliant in-series tendon, perhaps shortening beyond optimal length), modulating tendon stiffness through exercise⁴⁵ may be a beneficial strategy for rehabilitation. Targeted exercise such as isometric MVCs to increase AT stiffness⁴⁵ may promote “optimal” function of the MG muscle, as a stronger MG and stiffer AT could shift the MG force-length relationship to longer fascicle lengths, reducing the amount of relative muscle shortening required to achieve peak force active force. This reduced shortening might help alleviate symptoms associated with muscular weakness during daily activities since any given absolute force would be a lower relative force (as a percentage of MVC), optimizing the muscle force-length relationship⁴⁶ and minimizing additional shortening that would come at a higher metabolic cost³⁵. While this remains speculative, a similar strategy using targeted exercise to modify the force-length properties of muscle has been successfully utilized in cerebral palsy⁴⁷. However, further research is needed to confirm the effectiveness of such an intervention in HSD/hEDS.

Regarding muscle size and architecture, our sample of individuals with HSD/hEDS had similar muscle morphology (MT and PA) compared to controls. Our finding of similar MG muscle thickness is supported by previous reports of similar total CSA of the plantar flexor muscles in those with and without HSD/hEDS⁷. As muscle size is used as a surrogate for muscle atrophy⁴⁸, the weakness observed in our sample is likely not due to muscle atrophy. We also observed similar EI, suggesting that muscle weakness was also not due to reduced muscle quality. Takamatsu et al.⁴⁹ have previously suggested that plantarflexion strength may not correlate well with EI of specific muscles since multiple muscles contribute to plantarflexion force production, and our EI measures were only for that of the MG, which represents a relatively small proportion (~17%) of total plantarflexor muscle volume⁵⁰. Others show a negative correlation between EI and strength²⁸, as increased EI may indicate greater amounts of intra-muscular fat or fibrotic tissue within the muscle⁵¹. Together, our results suggest the observed muscle weakness in HSD/hEDS may not be due to differences in muscle quality between groups.

Our MG fascicle forces may be overestimated as total plantarflexion force was used to calculate fascicle force because we were unable to measure PCSA for this study. Using a literature value for the PCSA of the MG would include additional assumptions as the literature is limited to mostly healthy males and PCSA is dependent on individual characteristics⁵⁰. In particular, our sample, like the HSD/hEDS population, was mostly female. Additionally, previous studies in HSD/hEDS do not provide PCSA of the MG or MG-specific CSA in HSD/hEDS to enable appropriate PCSA calculations^{17,52}. Since fascicle forces in both groups would be overestimated in this way, it is not likely to have influenced the observed relative weakness in HSD/hEDS. We were also

limited to MT as an estimate of muscle size, which may have underestimated the size of the MG as it is only an indicator of a section of the muscle. While other measurements such as muscle volume, CSA or PCSA may provide a more comprehensive assessment of total muscle size, MT may serve as a limited indicator of muscle size as it is an influential component in calculating PCSA^{23,24}. While there are limitations in comparing EI values due to differences in ultrasound device settings²⁶, we used the same device and settings in our study to allow for comparing EI between HSD/hEDS and controls. A limitation of our EI analysis was correction for SCT using methods originally derived and validated for the quadriceps muscle, due to a lack of MG-validated protocol. Analysis was performed with and without SCT to account for potential bias in the correction method. While our results were similar with and without considering SCT, validation of methods for SCT correction in the MG are still needed.

Ultrasound image analysis was not blinded, which may contribute to observer bias. Observer bias was mitigated, but not necessarily eliminated, through assessment of intrarater reliability of fascicle length measurement in a separate cohort without HSD or hEDS. As we were unable to directly measure SLs, we assumed peak force production to occur at a SL of 2.64 μm and that changes in FL were reflective of relative changes in SL, as described previously³⁴. A limitation of this approach *in vivo* is an inability to detect non-uniformity of sarcomere lengths considered on the descending limb of the sarcomere force-length relationship. This non-uniformity of sarcomere lengths may contribute to variation in estimated sarcomere lengths on the descending limb of the sarcomere force-length relationship⁵³. As such, we must consider the estimated sarcomere lengths calculated here to represent the average sarcomere lengths within the measured MG fascicles, particularly on the descending limb of the MG force-length relationship. Lastly, we also did not assess muscle activity in this study, which is likely another factor influencing muscular weakness given the lack of differences in tissue architecture or quality in our cohort.

These findings promote multiple future directions for research. Assessment of muscle activation during plantarflexion MVCs may explain weakness of the MG in HSD/hEDS, as no differences were observed in the size, architecture or quality of MG muscle tissue. Along with assessment of muscle activation, targeted neuromuscular training may be another candidate for future studies to investigate as a strategy for improving strength and physical function in HSD/hEDS. A study investigating the benefits of exercise training targeting neuromuscular adaptations compared to tendon stiffness adaptations would aid in understanding which types and effects of exercise are beneficial for those living with HSD/hEDS. Despite the limitations of this study, our estimates of SL and N provide initial findings to inform future research that can directly measure sarcomeres in HSD/hEDS. The SL at peak force production in HSD/hEDS could be confirmed by investigation

of sarcomere properties using X-ray diffraction⁴³ or *in-vivo* microendoscopy⁵⁴. Future exercise studies should consider joint angle-specific deficits in strength, as these may be beneficial targets for strength training and clinical interventions to improve function and quality of life in HSD/hEDS. Specifically, studies are called for to determine the effect of functional strength training programs to increase AT stiffness that target ankle joint angles where the greatest disparity in strength is observed (around 90° in our cohort). Such programs may improve symptoms and function during tasks such as walking or climbing stairs in people living with HSD/hEDS, but this remains to be elucidated.

Individuals living with HSD/hEDS are relatively weaker throughout the ankle joint ROM. The force-length properties of the MG muscle appear to be altered in this population at the level of the muscle fascicles and sarcomeres, with a shift towards the ascending limb for peak force production and maintenance of force production at longer muscle lengths. Muscular weakness did not coincide with alterations in muscle size, architecture, or quality in this sample, suggesting that other factors such as reduced neuromuscular activation or increased energy cost of muscle contraction may contribute to muscular weakness in HSD/hEDS. Future work includes studying the biomechanical and clinical effects of addressing neuromuscular, connective tissue stiffness, and joint-angle specific strength deficiencies through targeted exercise in individuals living with HSD/hEDS.

Ethics Approval

The experimental protocol and procedures were approved by the Mount Royal University Human Research Ethics Board (HREB ID #102279).

Consent to participate

Participants gave voluntary written informed consent to participate in the experimental protocol.

Authors' contributions

DWG: Methodology, Software, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft, review & editing. JPO: Methodology, Investigation, Data curation, Writing – Review & Editing. AJML: Investigation, Data curation, Writing – Review & Editing. KDW: Methodology, Investigation, Data curation, Funding acquisition, Writing – Review & Editing. ECB: Investigation, Writing – Review & Editing. SJS: Investigation, Writing – Review & Editing. MJA: Conceptualization, Methodology, Resources, Funding acquisition, Writing – Review & Editing. RHKM: Conceptualization, Methodology, Resources, Supervision, Funding acquisition, Writing – Review & Editing. JRF: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data curation, Visualization, Supervision, Project administration, Funding acquisition, Writing – original draft, review & editing. All authors read and approved the final version of the manuscript.

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Supplementary Table 1. Percent range of motion (ROM) corresponding to peak force production.

Participant ID	Sex	Group	Peak Force (N)	% ROM
2871	Female	HSD/hEDS	2113.3	40%
3418	Female	HSD/hEDS	3575.7	0%
5187	Female	HSD/hEDS	448.45	40%
5984	Female	HSD/hEDS	491.4	0%
7738	Female	HSD/hEDS	1988.6	20%
7744	Male	HSD/hEDS	1368.9	0%
8204	Female	HSD/hEDS	2872.6	20%
8221	Female	HSD/hEDS	1039.1	40%
8365	Female	HSD/hEDS	3274.0	40%
9292	Female	HSD/hEDS	903.3	40%
6302	Female	HSD/hEDS	2996.1	40%
1093	Female	CON	4009.2	20%
1375	Female	CON	2440.8	40%
3547	Female	CON	828.9	20%
4123	Female	CON	4015.5	20%
4292	Male	CON	2904.5	20%
5043	Female	CON	4338.4	40%
5720	Female	CON	1636.3	40%
5876	Female	CON	1160.5	20%
7598	Female	CON	2772.3	0%
9930	Female	CON	5393.5	20%
3003	Female	CON	3909.2	20%