

1 **Title Page**

2 **Title: The impact of Hypermobility Spectrum Disorders on musculoskeletal tissue stiffness: An exploration**  
3 **using strain elastography.**

4 **Concise title: The impact of Hypermobility Spectrum Disorders on musculoskeletal tissue stiffness.**

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1 **Abstract:**

2 **Introduction:** Hypermobility Spectrum Disorders (HSD) are conditions associated with chronic joint pain and laxity.  
3 HSD's diagnostic approach is highly subjective, its validity is not well studied, and it does not consider many of the  
4 most commonly affected joints. Strain elastography (SEL) reflects musculoskeletal elasticity with sonographic  
5 images. The study explored the impact of HSD on musculoskeletal elasticity using SEL.

6 **Method:** A cross-sectional design compared 21 participants with HSD against 22 controls. SEL was used to assess  
7 the elasticity of the deltoid, biceps brachii, brachioradialis, rectus femoris, and gastrocnemius muscles, and the patellar  
8 and Achilles tendon. SEL images were analysed using Strain Index, Strain Ratio, and colour pixels.

9 **Results:** Mean Strain Index (standard deviation) was significantly reduced in the HSD group compared to the control  
10 group in the brachioradialis muscle; 0.43 (0.10) vs. 0.59 (0.24), patellar; 0.30 (0.10) vs. 0.44 (0.11), and Achilles  
11 tendons; 0.24 (0.06) vs. 0.49 (0.13). Brachioradialis muscle and patellar tendon's Strain Ratios were significantly  
12 lower in the HSD group compared to the control group; 6.02 (2.11) vs. 8.68 (2.67) and 5.18 (1.67) and 7.62 (1.88),  
13 respectively. The percentages (%) of red colour (soft tissues) in the SEL images were significantly increased in the  
14 HSD group compared to the control group in the biceps brachii muscle; 34.72 (7.82) vs. 26.69 (3.89), and Achilles  
15 tendon; 18.14 (13.21) vs. 5.59 (8.23) ( $p \leq 0.01$ ).

16 **Conclusion:** The elasticity of the musculoskeletal system seems to be lower in people with HSD. SEL could be a  
17 supplementary tool for diagnosing and monitoring HSD.

18 **Key words:**

19 Hypermobility Spectrum Disorders, strain elastography, muscle, tendon, Joint Hypermobility Syndrome, diagnosis.

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1 **Introduction:**

2 'Hypermobility Spectrum Disorders' (HSD) refers to the conditions observed with chronic synovial joint  
3 pain and hypermobility resulting from the connective tissues deficiency [1-2]. The term HSD was introduced in 2017  
4 to highlight the wide heterogeneities within joint hypermobility-related conditions, and to replace the terms 'Joint  
5 Hypermobility Syndrome' (JHS) and 'Ehlers-Danlos Syndrome Hypermobility Type' (EDS-HT) [2]. JHS and EDS-  
6 HT greatly overlap as they are heritable connective tissue disorders associated with symptomatic multiple joint  
7 hypermobility in the absence of systemic inflammation [1-3]. EDS-HT additionally involves skin hyperextensibility  
8 and smoothness, velvety skin, and recurrent joint dislocations [1-3]. HSD diagnosis is likely to capture the majority  
9 of patients previously diagnosed as JHS/EDS-HT, although a discrete group of patients with more severe symptoms  
10 may meet the diagnostic criteria of 'hypermobile EDS' (hEDS) [3]. The present research is specific to HSD.

11 Based on meta-analysis exploring joint hypermobility-related disorders, Scheper et al. (p.12) stated:  
12 "*Clinicians should be aware that within these disorders, a large variability in phenotype exist*" [4]. HSD considered  
13 the wide variability of joint hypermobility-related conditions, therefore it involves four phenotypes: 'Localized HSD':  
14 hypermobility in one or more joints but less than five joints; 'Peripheral HSD': hypermobility in hands and feet; and  
15 'Generalized HSD': hypermobility in five or more than five joints [2]. Joint hypermobility reduces with age, therefore  
16 the 'Historical HSD' phenotype was introduced [2, 5].

17 The term HSD has been newly introduced; therefore, there is a lack of research using the new criteria.  
18 However, HSD captures the previously used terms of JHS and EDS-HT. JHS had a prevalence figure of 30% of  
19 patients attending a musculoskeletal triage clinic in London [6]. JHS etiology was related to abnormalities in the genes  
20 encoding collagen, hormonal imbalance, or environmental factors [1, 7-8]. The symptomatic manifestation of JHS  
21 varied from mild to disabling, including chronic joint pain and hypermobility, muscle weakness, proprioception  
22 impairment, recurrent injuries and dislocation, fibromyalgia, and osteoarthritis [1, 9]. JHS could be associated with  
23 cardiovascular deterioration, gastrointestinal disturbances, and psychological health decline [10]. The prevalence and  
24 disabling impact of HSD provide a remarkable rationale for optimizing strategies for its identification and diagnosis.

25 Despite the importance of the new diagnostic approaches for HSD, they are likely to encounter various  
26 limitations. Like the Brighton criteria (previously used for JHS), the HSD criteria are based on subjective questionings  
27 and clinical examinations (such as the Beighton score of joint mobility) [1, 11]. Therefore, the criteria are highly  
28 dependent on the examiner's skill and experience, and patient memory. Recent meta-analysis and a systematic review  
29 concluded that the diagnostic criteria are inadequate, where their validity has not been well studied [4, 12]. The current  
30 diagnostic approaches do not assess many of the most commonly affected joints in JHS such as the shoulder; 85.2%  
31 prevalence, neck; 59.3% prevalence, pelvis and hip; 66.7% prevalence, and ankle and foot; 77.8% prevalence [13].  
32 Using the current diagnostic approach, it is easy to reach the diagnostic cut-off point of 4/9 purely with fingers and  
33 thumb hypermobility [14]. Therefore, there is a need to identify new diagnostic approaches for people with HSD.

34 Diagnostic imaging advances could help people with HSD. Strain elastography (SEL) is an ultrasound-based  
35 system designed to examine the elasticity of human structures by providing colour-coded images [15]. Administering

1 SEL for examining HSD may be valuable, as musculoskeletal structures could show significant laxity. The mutation  
2 identified in the genes encoding collagen in hypermobility-related disorders could reduce the mechanical rigidity of  
3 the connective tissues in the musculoskeletal system, and this laxity may be identified using SEL. Other factors could  
4 lead to significant softening of the HSD musculoskeletal system, including muscular weakness, reduced muscle-  
5 tendon stiffness, the pain-muscular inhibition cycle, and reduced activity [1, 16-17]. Various studies have identified  
6 the impact of hypermobility-related disorders on tissue stiffness including skin extensibility, impaired Achilles tendon  
7 stiffness, and muscle activation and force production [16, 18-19]. Other studies found no impact of hypermobility-  
8 related disorders on skin extensibility, patellar tendon active stiffness and hamstring muscle energy absorption [20-  
9 22]. The insignificant differences could be related to type II error due to the studies' small sample sizes, which ranged  
10 from eight-nine participants per group [20, 22]. People with HSD could lose the feature of joint hypermobility due to  
11 the condition pathological course or aging factors, which would make the diagnosis and condition monitoring more  
12 difficult. SEL could aid in providing real-time images for the elasticity degree without relying on joint hypermobility  
13 feature. Identifying significant elasticity with SEL in certain muscle groups might be important for the classification  
14 of HSD patients.

15 Two studies have examined the musculoskeletal elasticity in people with hypermobility-related disorders  
16 using SEL. The Achilles tendon was explored in a 10 years old boy [23]. SEL images were analysed qualitatively  
17 showing an increase in the green and red areas, which suggested an increase in the Achilles tendon laxity [23]. SEL  
18 feasibility were studied in ten participants with JHS against ten controls [17]. Alsiri (2017) found no significant  
19 differences between the two groups in gastrocnemius medius elasticity, which could be a type II error due to the small  
20 sample size [17]. The current study introduces a novel exploration that aims to identify the impact of HSD on  
21 musculoskeletal elasticity with SEL using a larger sample size and further anatomical sites. The study clinical  
22 importance could be enhanced by exploring the relationship of musculoskeletal elasticity with joint pain. Joint pain is  
23 a dominant feature and complaint, and an essential diagnostic element for HSD [1-2]. Muscles and tendons are vital  
24 components in the joint stabilization system. Reduced stiffness might create deficiency in joint support which could  
25 lead to microtrauma and instability, ultimately leading to joint pain [24-25]. It is hypothesized that joint pain would  
26 be higher with softer musculoskeletal structures. The study objective was to examine the impact of HSD on the  
27 elasticity of the musculoskeletal system.

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## 29 **Materials and Methods:**

### 30 **Participants:**

31 A cross-sectional research design compared musculoskeletal elasticity of a HSD group against a control  
32 group. Kuwait Ministry of Health Ethical Committee approved the study (571/2017). Patients were recruited from an  
33 orthopedic hospital using convenience sampling [26]. Four physiotherapists assessed their patients against the HSD  
34 classification, including the Beighton score. HSD subdivisions are Localized, Peripheral, Generalized and Historical  
35 HSD [2]. The Beighton score is used to assess joint hypermobility including the fifth metacarpophalangeal joint

1 dorsiflexion;  $> 90^{\circ}$ , thumb opposition; if reached the forearm volar aspect, elbow hyperextension;  $> 10^{\circ}$ , and knee  
2 hyperextension;  $> 10^{\circ}$ , and spinal hypermobility [27]. A Beighton score of  $\geq 5/9$  was used to distinguish Generalised  
3 HSD from other HSD categories [2-3].

4 Patients were identified from physiotherapy department referrals. All patients referred to the four  
5 physiotherapists were screened against the HSD subcategories [2]. The chief researcher (NA) screened all patients  
6 who attended for the outpatient orthopedic clinic. Recruitment packs were provided for identified patients. Patients  
7 who were willing to participate contacted the chief researcher. The chief investigator had more than four years'  
8 experience in diagnosing JHS and was trained in HSD classification. Training in the HSD classification was provided  
9 to the four physiotherapists. Participants in the control group were recruited from among the staff at the Ministry of  
10 Health and their relatives through advertising emails and posters. A matching-pairs design was used to ensure  
11 homogeneity between the two groups in terms of gender and age. The examination lasted from June-December 2017  
12 in the Radiology department. Informed consent was obtained from participants.

13 Women and men aged  $\geq 18$  years were included in the control and HSD group. Healthy adults were excluded  
14 from the control group if they had  $\geq 4/9$  in the Beighton score with chronic multiple joint pain or met the criteria for  
15 any of the HSD subcategories. The exclusion criteria for the two groups were upper/lower limbs' injuries during the  
16 last three months; upper/lower limbs' fractures or surgery in the last year; pregnancy; one year postpartum due to  
17 ligament laxity; other connective tissues disorders including Ehlers-Danlos Syndrome; and other conditions which  
18 might compromise muscular strength. Participants were included in the HSD group if they met the criteria for HSD  
19 [2]. Chronic multiple joint pain for longer than three months was the primary symptomatic manifestation for the HSD  
20 group, and was self-reported by patients [6, 12]. The secondary symptomatic manifestations were recurrent injuries  
21 and dislocations.

## 22 **Instrumentation:**

23 Compression SEL (Voluson E8; GE Healthcare Technologies, Milwaukee, WI, USA) was used to examine  
24 musculoskeletal structures elasticity. SEL is a clinically applicable ultrasound-based system, which measures tissues  
25 perpendicular deformation in response to mild strain induced using the examination probe [15, 28]. SEL converts the  
26 compression into strain coloured-images, where each colour refers to a certain elasticity degree. SEL follows the  
27 mechanical law of hard tissues deforming less and soft tissues deforming more when subjected to the same force [15,  
28 28-29].

29 SEL reliability has been demonstrated for examining the biceps brachii muscle, plantar fascia, and the  
30 Achilles tendon [29-31]. An intra-rater reliability study was conducted for the twenty-two participants from the control  
31 group in this study, by repeating the examination twice in the same day with one-hour interval. The study showed  
32 moderate to excellent intra-rater reliability, with ICCs (type 3,1) ranging from 0.734 (95% Confidence Interval 0.279-  
33 0.903) to 0.950 (0.848-0.983), for examining the deltoid, biceps brachii, brachioradialis, rectus femoris, gastrocnemius  
34 medius muscles, and Achilles tendon. The same operator (NA), conducted the examination. NA has a doctorate in  
35 musculoskeletal physiotherapy, has received postgraduate training in musculoskeletal ultrasound and has four years'

1 experience in performing elastography and musculoskeletal ultrasound. The piloting stage was supervised by an  
2 associate professor in radiological sciences (A.A) who has 20 years' experience in ultrasonography. It was not  
3 practical to blind the operator as she needed to confirm the eligibility criteria using the HSD classification. Images  
4 were saved using the participants' ID, and the analysis was performed blind to health status being entered to the  
5 relevant participant file. SEG's visual quality indicator was used to standardise the applied compression magnitude  
6 [28].

7 Joint pain intensity was measured using Visual Analogue Scales (VAS). The VAS is a pain assessment tool,  
8 which is highly valid, reliable, and sensitive [32]. It is a 10-cm length line with two descriptors at the two anchors: no  
9 pain, and worst possible pain. Twelve VASs were used to assess the shoulder, elbow, wrist, hip, knee, and ankle joints,  
10 bilaterally. Pain assessment was limited to a one-week period to reduce recall error by asking: 'please rate the average  
11 pain over the past week in each of the following joints'

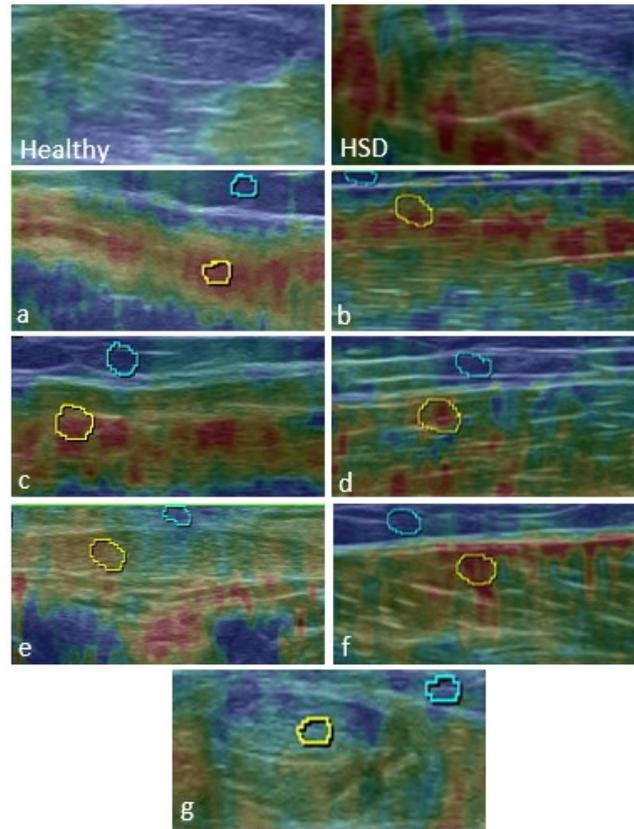
#### 12 **Sample size determination:**

13 Sample size calculation was based on Lee et al.'s study who used SEL with Strain Ratio to examine rotator  
14 cuff tendinopathy [33]. The means (standard deviation) for grade 0; 2.92 (2.13), and grade 3; 12.3 (0.00), tendinosis  
15 were used to calculate the effect size of 0.95. An appropriate sample size was estimated to be 20 at ( $\alpha$ ) 0.05, and a  
16 power (1-  $\beta$ ) of 80% [34].

#### 17 **Data collection procedures:**

18 After completing the demographic sheet and VASs, the dominant upper and lower limbs were examined. The  
19 dominant side was self-declared by the participant by reporting the hand used for writing and the leg used for kicking  
20 a ball [35-36]. SEL images were recorded by applying perpendicular force to the target area using the SEL transducer  
21 through simultaneous compressions and decompressions (Figure 1). Five muscles were examined: deltoid, biceps  
22 brachii, brachioradialis, rectus femoris and gastrocnemius medius. Additionally, the patellar and Achilles tendons  
23 were tested. The Upper and Lower Boundary of the Strain Index were measured with the SEL (Figure 1). The Higher  
24 Boundary of Strain Index refers to the elasticity of the target area, and the Lower Boundary of Strain Index refers to  
25 subcutaneous fat elasticity. The lower the Strain Index, the softer the examined structure is, and vice versa.

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2 **Fig 1** Transverse strain elastography images for the Achilles tendon for a healthy participant (Healthy), and for a  
3 participant with Hypermobility Spectrum Disorder (HSD). Red refers to soft tissues, green refers to intermittent  
4 elasticity, and blue refers to hard tissues. The size of the image box was manually adjusted to include the area of  
5 interest with adjacent subcutaneous fat as a reference. Images from (a) to (g) are for one participant with  
6 Hypermobility Spectrum Disorder, where the red colour mostly dominated SEG images. The images from (a) to (g)  
7 show the tracing approach used to attain the Higher and Lower Boundary of Strain Index following the manufacturer  
8 guidelines. (a) deltoid muscle, (b) biceps brachii muscle, (c) brachioradialis muscle, (d) rectus femoris muscle, (e)  
9 patellar tendon, (f) gastrocnemius medius muscle, and (g) Achilles tendon. Following standardized procedure, the  
10 reference area selected was always superior to the area of interest but not directly above it, following the assumption  
11 proposed by the European Federation of Societies for Ultrasound in Medicine and Biology that the stress is uniformly  
12 distributed in SEL. Initially an area encoding red pixels within the area of interest was selected to represent the Higher  
13 Boundary of Strain Index (highlighted with yellow traces). The relatively most soft area was selected in cases where  
14 red pixels were not displayed, as in image (g). Then an area encoding blue pixels was selected from the subcutaneous  
15 fat layer above the area of interest to represent the Lower Boundary of Strain Index (highlighted with blue traces)  
16 [37]. The two areas were selected to be as adjacent as possible to each other to ensure that the two areas were  
17 subjected to similar compression magnitude. Muscles and tendons are distinct structures and lack fat tissues that  
18 could serve as a reference. Using subcutaneous fat as a reference is the most applicable setup for musculoskeletal  
19 SEL, with comparable reliability to using alternative reference areas at other depth levels [37]. Representative  
20 reference tissue may be limited and sometimes difficult to obtain with comparable size and position to the area of  
21 interest, however, the size of the reference area has no significant influence on Strain Ratio [37]. The size of the two  
22 traced areas in the present investigation ranged from 0.60 – 1.00 cm<sup>2</sup> and were set to be as superficial as possible  
23 [37].

24  
25 The upper limb structures were examined then the lower limb structures. As the examined structures were  
26 superficial a linear-array high frequency transducer was used (6-15 MHz) as it penetrated 4-5 cm beneath the skin.  
27 The examinations were conducted by the operator in a sitting position, and the probe was maintained at a perpendicular

1 angle to the skin. The middle fibers of deltoid muscle were examined longitudinally in sitting, with a neutral shoulder  
2 position and supinated forearm [38]. The biceps brachii common muscle belly (5 cm above the elbow line), and  
3 brachioradialis muscles (5 cm below the elbow line laterally) were scanned longitudinally in supine, with a neutral  
4 shoulder position and elbow extension and supination [39].

5 In supine, with knee extension, the rectus femoris muscle was examined longitudinally (10 cm above the  
6 knee line), and the patellar tendon was examined transversely (midpoint between the inferior pole of the patella and  
7 the tibial tubercle). In prone, with knee extension and the feet in a relaxed position over the plinth edge, the  
8 gastrocnemius medius muscle was examined longitudinally (at 30% of its proximal length from the popliteal fossa  
9 midpoint to midpoint between the ankle malleoli). In the same position, the Achilles tendon was examined in a  
10 transverse plane (mid-position between the two malleoli) [40]. Each structure was examined 2-3 times, then one image  
11 was saved for analysis according to the compression magnitude sufficiency examined with SEL visual quality  
12 indicator. By tracing the target area and subcutaneous fat, the SEL calculates the Higher and Lower Boundary of Strain  
13 Index in real-time. The Strain Ratio was obtained from the ratio of the strain of the target area (numerator) and  
14 subcutaneous fat (denominator) [41].

#### 15 **Statistical analysis:**

16 Statistical Package for Social Sciences (SPSS 23, IBM Corp., Armonk, NY, USA) was used. SEL images  
17 were analyzed semi-quantitatively through the Strain Index, Strain Ratio and colour pixel analysis with ImageJ; a  
18 Java-based image processor (U. S. National Institute of Health, Bethesda, Maryland, USA). ImageJ counts the pixels  
19 of the red, green, and blue colour with a downloaded plugin in ImageJ, then the percentages of each colour was  
20 determined to compensate for differences in cross-sectional area between participants [17]. Data normal distribution  
21 was checked with histograms then objectively confirmed with Shapiro-Wilk tests, therefore Independent sample t-  
22 tests were used. The level of p value for statistical significance was set as  $\leq 0.01$ . The intensity of joint pain was  
23 measured and correlated with elasticity using Pearson Product Correlation Coefficient. Only pain in joints adjacent to  
24 each anatomical structure were included in this analysis.

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#### 26 **Results:**

27 Twenty-one participants in the HSD group were compared against 22 participants in the control group. The  
28 demographic characteristics are summarised in Table 1, indicating homogeneous groups in term of age, sex, height,  
29 weight, and body mass index. Regarding ethnicity, the HSD group included three Asian men, one Indian man, 16  
30 Asian women, and one white British woman. The control group included four Asian men, 15 Asian women, and three  
31 Indian women. The HSD group included 14 participants with Generalized HSD, six participants with Localized HSD,  
32 and one participant with Historical HSD [3]. Three participants in the HSD group and one participant in the control  
33 group were left side dominant. Images with artefact and tracing error were excluded; one image for the rectus femoris  
34 muscle from the HSD group, and two images for the deltoid, and two images for the biceps brachii muscles from the  
35 control group. All the results present mean values.

1           The Higher Boundary of the Strain Index for the HSD group point toward reductions in all the examined  
2 structures except for the biceps brachii muscle (Table 2). Three of the observations were statistically significant  
3 including the brachioradialis muscle, patellar tendon, and Achilles tendon ( $p \leq 0.01$ ) (Table 2). There were no  
4 significant differences between the two groups in the Lower Boundary of the Strain Index (Table 2). The Strain Ratios  
5 reveal statistically significant reduction in the elasticity of the brachioradialis muscle, and the Achilles tendon in the  
6 HSD group ( $p \leq 0.01$ ) (Table 2).

7           Colour pixel analysis shows a higher percentage of red colour in all the examined musculoskeletal structures  
8 in the HSD group, yet two of the observations were statistically significant including the biceps brachii, and Achilles  
9 tendon ( $p \leq 0.01$ ) (Table 3). The results of the HSD group demonstrate a reduction in the percentage of blue colour in  
10 all the examined structures, however, three of the seven observations were statistically significant including the biceps  
11 brachii, brachioradialis and the Achilles tendon ( $p \leq 0.01$ ) (Table 3). No significant differences were noticed between  
12 the two groups in the percentages of green colour (intermittent elasticity) ( $p \geq 0.01$ ) (Table 3).

13           Significant differences were found between the two groups in joint pain intensity;  $p \leq 0.01$  (Table 4). In the  
14 HSD group, the greatest pain was reported in the knee joint bilaterally, followed by dominant hip joint, then the  
15 shoulder joint bilaterally (Table 4). Moderate correlations were identified between the elasticity of the biceps brachii  
16 muscle and pain intensity at the shoulder, and elbow joints, and between knee joint pain and the elasticity of the  
17 patellar, and Achilles tendon;  $r \geq 0.4$  (Table 5).

	<b>HSD group n = 21</b>	<b>Control group n = 22</b>	<b>P value</b>
<b>Age (years)</b>	35.57 (14.89)	34.72 (7.00)	0.81
<b>Sex</b>	17 women, 4 men	18 women, 4 men	0.94
<b>Height</b>	161.11 (7.23)	162.63 (7.80)	0.51
<b>Weight</b>	72.66 (17.91)	74.72 (15.41)	0.68
<b>Body Mass Index</b>	27.99 (6.90)	28.06 (4.42)	0.96
<b>Beighton Score</b>	4.71 (1.82)	0.90 (1.01)	≤0.001*

*\*Indicates a statistically significant difference at  $p \leq 0.01$  with independent sample t-test.*

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Examined area	Lower Boundary of Strain Index					Higher Boundary of Strain Index					Strain Ratio				
	HSD group	Control group	P value	Mean difference	95% CI	HSD group	Control group	P value	Mean difference	95% CI	HSD group	Control group	P value	Mean difference	95% CI
<b>Deltoid muscle</b>	0.05 (0.03)	0.05 (0.01)	0.72	-0.002	-0.01, 0.01	0.44 (0.07)	0.48 (0.09)	0.24	0.03	-0.02, 0.08	11.03 (8.95)	9.48 (2.54)	0.45	1.55	-5.75, 2.65
<b>Biceps brachii muscle</b>	0.06 (0.02)	0.08 (0.02)	0.05	0.01	-0.00, 0.03	0.49 (0.09)	0.45 (0.07)	0.18	-0.03	-0.09, 0.01	10.45 (10.71)	5.79 (1.19)	0.06	4.65	-9.55, 0.24
<b>Brachioradialis muscle</b>	0.07 (0.02)	0.07 (0.02)	0.47	-0.006	-0.02, 0.01	0.43 (0.10)	0.59 (0.24)	0.01*	0.15	0.03, 0.27	6.02 (2.11)	8.68 (2.67)	≤0.001*	2.65	1.17, 4.14
<b>Rectus femoris muscle</b>	0.09 (0.03)	0.08 (0.02)	0.33	-0.009	-0.02, 0.00	0.42 (0.09)	0.45 (0.09)	0.38	0.02	-0.03, 0.08	5.10 (1.68)	6.00 (1.94)	0.11	0.89	-0.23, 2.03
<b>Patellar tendon</b>	0.06 (0.02)	0.05 (0.01)	0.72	-0.001	-0.01, 0.00	0.30 (0.10)	0.44 (0.11)	≤0.001*	0.13	0.07, 0.20	5.18 (1.67)	7.62 (1.88)	≤0.001*	2.44	1.34, 3.53
<b>Gastrocnemius medius muscle</b>	0.06 (0.03)	0.06 (0.03)	0.60	0.005	-0.01, 0.02	0.48 (0.14)	0.57 (0.19)	0.11	0.08	-0.02, 0.18	10.17 (7.64)	9.70 (4.70)	0.81	0.47	-4.43, 3.48
<b>Achilles tendon</b>	0.05 (0.03)	0.07 (0.02)	0.18	0.01	0.00, 0.02	0.24 (0.06)	0.49 (0.13)	≤0.001*	0.25	0.19, 0.31	6.24 (5.90)	7.56 (2.33)	0.34	1.32	-1.52, 4.16

*Keys: The Higher Boundary of Strain Index refers to the elasticity of the area of interest, and the Lower Boundary of Strain Index refers to the elasticity of the subcutaneous fat tissues. The lower the Strain Index, the softer the examined structure because the Strain Ratio has been calculated from the ratio of the area of interest; numerator, and adjacent subcutaneous fat; denominator. \*Indicates a statistically significant difference at  $p \leq 0.01$  with independent sample t-test. CI refers to confidence interval.*

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**Table 3: Comparison of the elasticity of the musculoskeletal structures of the Hypermobility Spectrum Disorders (HSD) group against a control group analysed with ImageJ by measuring the percentages of the colour pixels: mean (standard deviation).**

Examined area	Percentage of red colour pixel					Percentage of green colour pixel					Percentage of blue colour pixel				
	HSD group	Control group	P value	Mean difference	95% CI	HSD group	Control group	P value	Mean difference	95% CI	HSD group	Control group	P value	Mean difference	95% CI
<b>Deltoid muscle</b>	34.04 (6.70)	28.62 (7.86)	0.02	-5.42	-10.08, -0.75	41.80 (8.06)	40.28 (3.88)	0.46	-1.52	-5.64, 2.60	24.13 (11.18)	31.07 (8.44)	0.03	6.93	0.54, 13.33
<b>Biceps brachii muscle</b>	34.72 (7.82)	26.69 (3.89)	≤0.001*	-8.02	-11.96, -4.08	44.05 (3.56)	43.78 (3.50)	0.80	-0.27	-2.50, 1.96	21.20 (5.99)	29.39 (4.77)	≤0.001*	8.19	4.75, 11.62
<b>Brachioradialis muscle</b>	34.54 (10.20)	28.22 (9.88)	0.04	-6.31	-12.50, -0.12	44.69 (4.20)	42.46 (6.08)	0.17	-2.23	-5.46, 1.00	20.75 (7.43)	29.29 (11.62)	≤0.001*	8.54	2.50, 14.58
<b>Rectus femoris muscle</b>	37.73 (11.41)	29.58 (10.38)	0.02	-8.14	-15.03, -1.25	41.95 (6.09)	43.73 (5.69)	0.33	1.78	-1.94, 5.50	20.29 (7.39)	26.66 (10.80)	0.03	6.36	0.48, 12.24
<b>Patellar tendon</b>	27.69 (16.97)	20.31 (13.05)	0.11	-7.38	-16.68, 1.91	42.94 (11.31)	43.41 (8.12)	0.87	0.47	-5.56, 6.52	29.34 (18.29)	36.25 (16.63)	0.20	6.90	-3.85, 17.66
<b>Gastrocnemius medius muscle</b>	34.28 (9.17)	30.38 (8.60)	0.15	-3.89	-9.37, 1.57	45.87 (4.48)	46.50 (3.81)	0.62	0.62	-1.93, 3.18	19.82 (7.88)	23.10 (7.54)	0.17	3.27	-1.47, 8.02
<b>Achilles tendon</b>	18.14 (13.21)	5.59 (8.23)	≤0.001*	-12.54	-19.29, -5.79	43.60 (12.29)	37.56 (19.54)	0.23	-6.04	-16.15, 4.06	38.23 (17.35)	56.82 (22.81)	≤0.001*	18.58	6.06, 31.11

**Keys: The red colour refers to soft structures, the green colour refers to structures with intermittent elasticity and the blue colour refers to hard structures. \*Indicates a statistically significant difference at  $p \leq 0.01$  with an independent sample t-test. CI refers to confidence interval.**

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**Table 4: The intensity of joint pain measured using Visual Analogue Scales for the upper and lower limb joints for the Hypermobility Spectrum disorder group and the control group; mean (standard deviation)**

	<b>HSD group (n = 21)</b>	<b>Control group (n = 22)</b>	<b>P value</b>	<b>Mean difference</b>	<b>95% CI</b>
<b>Dominant shoulder pain</b>	3.25 (3.56)	0.25 (0.81)	≤0.001*	-2.99	-4.57, -1.42
<b>Non-dominant shoulder pain</b>	3.02 (3.16)	0.05 (0.27)	≤0.001*	-2.91	-4.25, -1.58
<b>Dominant elbow pain</b>	2.97 (3.08)	0.13 (0.63)	≤0.001*	-2.06	-3.44, -0.67
<b>Non-dominant elbow pain</b>	1.56 (3.06)	0.00 (0.00)	≤0.001*	-1.56	-2.88, -0.24
<b>Dominant wrist pain</b>	2.59 (3.02)	0.00 (0.00)	≤0.001*	-2.59	-3.89, -1.29
<b>Non-dominant wrist pain</b>	2.09 (2.67)	0.00 (0.00)	≤0.001*	-2.09	-3.24, -0.93
<b>Dominant hip pain</b>	3.95 (4.13)	0.27 (1.04)	≤0.001*	-3.67	-5.51, -1.84
<b>Non-dominant hip pain</b>	2.58 (3.30)	0.00 (0.00)	≤0.001*	-2.58	-4.00, -1.15
<b>Dominant knee pain</b>	4.08 (3.70)	0.15 (0.56)	≤0.001*	-3.92	-5.53, -2.31
<b>Non-dominant knee pain</b>	4.25 (3.57)	0.41 (1.33)	≤0.001*	-3.83	-5.48, -2.19
<b>Dominant ankle pain</b>	2.80 (3.45)	0.00 (0.00)	≤0.001*	-2.80	-4.28, -1.31
<b>Non-dominant ankle pain</b>	2.37 (3.52)	0.00 (0.00)	≤0.001*	-2.37	-3.88, -0.85

*\*Indicates a statistically significant difference at  $p \leq 0.01$  with an independent sample t-test.  
CI refers to confidence interval.*

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**Table 5: Correlation between the intensity of joint pain at the dominant side and the elasticity of the musculoskeletal system measured using the Higher Boundary of the Strain Index (HSI), percentage of red and blue colour pixels.**

<b>Examined structure</b>	<b>Related joint</b>	
<b>Deltoid muscle</b>	<b>Shoulder pain</b>	
HIS	-0.278	
Red pixels	0.223	
Blue pixels	-0.221	
<b>Biceps brachii muscle</b>	<b>Shoulder pain</b>	<b>Elbow pain</b>
HIS	-0.009	0.138
Red pixels	<b>0.459*</b>	<b>0.507*</b>
Blue pixels	<b>-0.537*</b>	<b>-0.499*</b>
<b>Brachioradialis muscle</b>	<b>Elbow pain</b>	<b>Wrist pain</b>
HIS	-0.105	-0.249
Red pixels	0.075	0.086
Blue pixels	-0.207	-0.212
<b>Rectus femoris muscle</b>	<b>Hip pain</b>	<b>Knee pain</b>
HIS	-0.211	-0.202
Red pixels	0.320	0.335
Blue pixels	-0.316	-0.325
<b>Patellar tendon</b>	<b>Knee pain</b>	
HIS	<b>-0.478*</b>	
Red Pixels	0.128	
Blue Pixels	-0.008	
<b>Gastrocnemius medius muscle</b>	<b>Knee pain</b>	<b>Ankle pain</b>
HIS	-0.206	-0.228
Red pixels	0.352	0.320
Blue pixels	-0.322	-0.259
<b>Achilles tendon</b>	<b>Knee pain</b>	<b>Ankle pain</b>
HIS	<b>-0.420*</b>	-0.345
Red pixels	<b>0.451*</b>	0.356
Blue pixels	<b>-0.440*</b>	-0.293

*Keys: r = 0.00-0.19 indicates very weak correlation, r = 0.2-0.39 indicates weak correlation, r = 0.40-0.59 indicates moderate correlation, r = 0.6-0.79 indicates strong correlation, and r = 0.8-1.0 indicates very strong correlation. \* refers to statistically significant correlation.*

1 **Discussion:**

2           The impact of HSD on the elasticity of the musculoskeletal system was identified. SEL images were analysed  
3 semi-quantitatively using the Strain Index, Strain Ratio, and colour pixel analysis. All analysis methods led to similar  
4 results of increased musculoskeletal elasticity in the HSD group. The results of the Higher Boundary of Strain Index  
5 point toward increased softening of the examined structures. Three of the observations were statistically significant,  
6 including the brachioradialis muscle, patellar and Achilles tendon. The deltoid, rectus femoris and gastrocnemius  
7 medius muscles showed increased elasticity in the HSD group, but the changes have not reached statistical  
8 significance. In contrast, the Higher Boundary of Strain Index for the biceps brachii muscle was higher in the HSD  
9 group, indicating a stiffer biceps brachii muscle when compared to the control group, yet this observation has not  
10 reached statistical significance. Alternatively, the biceps brachii muscle could be stiffer in people with HSD as a  
11 compensatory mechanism adopted to control elbow hypermobility. The biceps brachii muscle is essential for daily  
12 activities, which could increase the muscular tone. The Strain Ratio also demonstrated statistically significant  
13 reductions in the elasticity of the brachioradialis muscle and patellar tendon in the HSD group. Similarly, colour pixel  
14 analysis showed greater elasticity and lesser stiffness in the musculoskeletal structures of the HSD group. Three  
15 observations were statistically significant including the biceps brachii and brachioradialis muscles, and the Achilles  
16 tendon. The deltoid, rectus femoris, gastrocnemius medius muscles and the patellar tendon also showed increased  
17 elasticity and reduced stiffness in the HSD group. Colour pixel analysis for the biceps brachii, which showed increased  
18 elasticity, contradicting the Strain Index and Strain Ratio. However, the sample size was based on large effect size of  
19 0.95, which might indicate that the observed differences need to be confirmed with larger sample size study.

20           Two studies explored musculoskeletal elasticity in hypermobility-related disorders using SEL [17, 23].  
21 Kocyigit et al. (2015) examined a boy with generalised joint laxity and qualitatively analysed the SEL image of the  
22 Achilles tendon [23]. An increase in the red (soft tissues), and green (intermittent elasticity) colours were reported  
23 [23], supporting the current study, where the SEL images of the HSD group showed a statistically significant increase  
24 in the red colour and reduction in the blue colour (hard tissues) [23]. The green colour was also increased in the HSD  
25 group, but this observation was not statistically significant. A comparison with Kocyigit et al.'s (2015) study might  
26 not be appropriate as it is a case report of one boy, whereas the current study focused on adults. A feasibility study  
27 also aimed to explore the gastrocnemius medius muscle elasticity in ten adults with JHS compared to ten controls, and  
28 colour pixels were used for analysis [17]. Alsiri (2017) and the current study found no significant differences between  
29 the two groups in gastrocnemius medius elasticity [17]. This could be related to type II error, as the descriptive  
30 statistics of the current study showed 4% higher gastrocnemius muscle elasticity and 4% lower gastrocnemius medius  
31 muscle hardness in the HSD group relative to the control group. The descriptive statistics of the smaller sample size  
32 of 10 participants per group [17] identified 1.51% higher gastrocnemius muscle elasticity, and no difference in muscle  
33 hardness in the JHS group. A larger sample size study could confirm the observed differences.

34           Previous studies on non-hypermobility related conditions have found changes in the elasticity of certain  
35 musculoskeletal structures with SEL. 57% softening was identified in symptomatic Achilles tendinopathy [42].  
36 Another study found hardening in the symptomatic Achilles tendon [43]. However, with inflammatory myositis,

1 reduced elasticity was found in most of the cases in the thigh, leg, and arm muscles [44]. Future studies should compare  
2 HSD against other musculoskeletal system pathologies to examine the ability of the SEL to distinguish HSD.

3 Various reasons could explain the significant increase in musculoskeletal structures' elasticity in people with  
4 HSD. Collagen is a dominant constituent of the musculotendinous tissues, enhancing its support system and  
5 mechanical rigidity. Previous studies on hypermobility-related disorders identified mutation in the genes encoding  
6 collagen, and deficiency in the collagen modification enzymes [7]. Collagen deficiency could explain the significant  
7 reduction in musculoskeletal structures' elasticity. Hypermobility-related disorders are significantly associated with  
8 muscle weakness [17], and weak muscles might reduce the resting muscular elasticity. The current study found a  
9 moderate correlation between joint pain and musculoskeletal elasticity, indicating that joint pain is associated with  
10 softer structures. The significant increase in musculoskeletal elasticity in people with HSD indicates a degree of failure  
11 in their joint dynamic support system, leading to instability, microtrauma and pain. Alternatively, pain could lead to  
12 muscle weakness and motor inhibition [45], which could cause the significant reduction in musculoskeletal elasticity.  
13 The cause-effect relationship between pain and musculoskeletal elasticity needs to be explored in future studies.

14 The examiner was not blinded to the groups, yet expectation bias was partially controlled by conducting  
15 ImageJ analysis after acquiring the images. The sample size might not be sufficient for all the variables. Specially that  
16 the effect sizes observed were smaller than the effect size used for sample size calculation of 0.95. The observed effect  
17 sizes in the current study ranged from 0.16 – 0.77, for the rectus femoris muscle and Achilles tendon, respectively, as  
18 indicated with the Higher Boundary of Strain Index. The effect sizes ranged from 0.21 – 0.54 for the gastrocnemius  
19 medius, and biceps brachii muscles, respectively, for the red colour pixels. The Strain Ratio was obtained from the  
20 ratio of the target area and subcutaneous fat. However, the Strain Index of the subcutaneous fat varies between  
21 participants, so it might not serve as an ideal reference. Therefore, a range has also been presented in the current study;  
22 Lower and Higher Boundary of Strain Index. Some studies have used an external gel pad as a reference, which could  
23 be used for future studies. Heterogeneity in the reference area elasticity might explain the large variability noticed in  
24 some structures. Spinal hypermobility is one of the major features of HSD, however, the current study did not explore  
25 the spinal musculoskeletal structures, and this area could be explored in future research.

26 Future research should consider inclusion of further variables to allow more advanced statistical analysis to  
27 account for potential covariates and build meaningful regression models to explore the relationships between elasticity  
28 and pain. The findings clinical relevance need to be further explored. For example, determining the magnitude of the  
29 observe differences relative to data variability; diagnostic validity; sensitivity and specificity, and the relationships  
30 with functional outcomes are all areas for exploration. There are various strengths in the study, including establishing  
31 intra-rater reliability in a smaller study. A sample size calculation was used, and the methodological procedures were  
32 carefully standardized. The findings' clinical importance was increased by correlating the findings with joint pain.

33 Increased musculoskeletal system elasticity in people with HSD is evident with SEL. Using three SEL image  
34 analysis approaches, people with HSD showed significant reductions in the elasticity of muscular and tendinous  
35 structures including the biceps brachii and brachioradialis muscles, and patellar and Achilles tendons. People with  
36 HSD significantly complain of multiple joint pain which demonstrated a moderate correlation with musculoskeletal

1 elasticity. SEL could be a supplementary tool for diagnosing, monitoring and assessing the effectiveness of  
2 physiotherapy, strengthening programs and other management regimes provided for people with HSD.

3

4 **Conflict of interest:**

5           The authors declare no conflict of interest.

6

7 **Ethical standard:**

8           The study has been approved by the Ethical Committee of Kuwait Ministry of Health (571/2017), therefore,  
9 the study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki  
10 and its later amendments.

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