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1 **Sonographic Evaluation of the Effect of Long-Term Exercise on Achilles Tendon Stiffness using** 2 **Shear Wave Elastography**

3

4 **Abstract**

5 *Objectives:* This study aimed to assess the feasibility of using quantitative Shear Wave Elastography
6 (SWE) to assess the stiffness (Young's modulus) of Achilles tendon and to investigate the effect of
7 long-term weight-bearing exercise on the stiffness of human Achilles tendon by comparing the
8 frequent and infrequent exercise groups.

9 *Design:* Case-control study.

10 *Methods:* A total of 36 healthy subjects aged 19-25 were recruited. Subjects were categorized into
11 frequent and infrequent exercise groups dependent on their level of lower limb weight bearing
12 exercise. B-mode and shear-wave ultrasound examination of Achilles tendon were performed.
13 Measurements of the Achilles tendon stiffness were conducted with the foot position standardized by
14 an ankle fixer. To evaluate inter and intra-operator reliability of Young's modulus measurements, each
15 subject was scanned by three operators and each operator scanned the subject three times.

16 *Results:* The intra-operator reliability of Young's modulus measurements ranged between 0.803 and
17 0.845. The inter-operator measurement reliability was 0.585. Result showed that Achilles tendon on
18 non-dominant ankle in frequent-exercisers (median: 320.1kPa) was significantly stiffer than that in
19 infrequent-exercisers (median: 296kPa) ($p < 0.05$), whereas there was no significant difference in
20 Achilles tendon stiffness on dominant ankle between the two groups ($p > 0.05$).

21 *Conclusions:* Shear Wave Elastography is feasible for assessing the Achilles tendon's stiffness in vivo.
22 The stiffness of Achilles tendon of frequent-exercisers was significantly higher than that of
23 infrequent-exercisers on non-dominant ankle but not on dominant ankle. With the use of an ankle fixer,
24 there was high repeatability and moderate reproducibility in SWE measurement of Achilles tendon
25 stiffness.

26

27 *Keywords:* physical activity, exercise physiology, ultrasonography, elasticity imaging techniques,
28 elastic modulus

29 **Introduction**

30 Achilles tendon is the strongest and largest tendon in the human body, originating from the
31 aponeurosis of Soleus and gastrocnemius muscles and inserts at the mid-portion of the posterior
32 calcaneal tuberosity. Owing to increasing recreational athletics and greater physical demand on sport,
33 the number of Achilles tendon injuries has risen drastically ^{1,2}. People who perform frequent exercise,
34 especially athletes, have a higher chance of Achilles tendinopathy than those who infrequently
35 perform exercise ³. The current practice for diagnosis of Achilles tendon diseases is mainly based on
36 the clinical history and imaging examinations.

37 Measuring the elasticity is one of the methods to assess Achilles tendon, and is useful for
38 assessing Achilles tendinopathy and tendon degeneration ⁴. It has been reported that Achilles tendons
39 with tendinopathy are softer than normal Achilles tendons ⁵. Measuring the stiffness of Achilles
40 tendon could be a potential diagnostic method for assessing tendinopathy. Since different factors
41 affect the mechanical properties of Achilles tendon including weight-bearing exercise (mechanical
42 loading) and dominance of leg ^{6,7}, it is necessary to understand the normal variation of the stiffness of
43 Achilles tendon so that accurate diagnosis of abnormalities can be made.

44 Ultrasonography is a common imaging tool in the assessment of musculoskeletal structures
45 including Achilles tendon ^{8,9}. Sonoelastography is an ultrasound technique which assesses the
46 elasticity of soft tissue by evaluating its Young's modulus or calculating the strain ratio of the target
47 tissue relative to its adjacent soft tissue. Moreover, sonoelastography is fast, inexpensive, and easily
48 available which makes it an ideal imaging technique to assess the stiffness of musculoskeletal
49 structures ¹⁰. Among different sonoelastography techniques, strain sonoelastography (strain EUS) and
50 shear wave elastography (SWE) are common real-time sonoelastography techniques applied in
51 detecting and characterizing a range of lesions in different tissues and organs, including the Achilles
52 tendon ¹¹.

53 Shear wave elastography is a new development in soft tissues elasticity imaging. With the use of
54 focused ultrasound beam, acoustic radiation force impulses are produced and transmitted to the soft
55 tissues resulting the generation of shear waves within the tissues. Using the ultrafast ultrasound
56 tracking techniques, the velocity of shear wave propagation is measured and the Young's modulus (i.e.

57 stiffness) of the soft tissue is obtained. SWE can quantify soft tissue stiffness, and provide absolute
58 value of the stiffness of the soft tissue. Therefore, the results of SWE are objective and the
59 quantitative data can be used for clinical references. Although SWE assessment of Achilles tendon
60 elastic property has been reported ¹²⁻¹⁶, literature has scant information about the changes of normal
61 Achilles tendon stiffness after prolonged physical activities. Therefore, this study was undertaken to
62 investigate the effect of long-term weight bearing exercise on Achilles tendon stiffness of human
63 subjects by comparing frequent and infrequent exercisers.

64 **Methods**

65 Healthy Chinese subjects were recruited from different local universities for the study. Subjects
66 with history of hormone therapy, previous contraceptive pills intake or contraceptive injections were
67 excluded because different levels of estrogen would exert an effect on collagen synthesis which alters
68 the Achilles tendon mechanism¹⁷. Subjects with history of tendon injury, hypothyroidism, treatment
69 with corticosteroids, metabolic and inflammatory diseases such as gout, ankylosing spondylitis, and
70 rheumatoid arthritis were also excluded because these may affect the normal size, thickness and
71 stiffness of Achilles tendon⁹. This study was approved by the Human Subject Ethics Subcommittee
72 of the Hong Kong Polytechnic University. An information sheet about the study's aims, examination
73 procedures, ultrasound's safety and the volunteers' rights was given to each subject. Informed written
74 consent was obtained from each subject before the examination.

75 Each subject was requested to fill in the Victorian Institute of Sports Assessment – Achilles
76 questionnaire (VISA-A). It evaluates the effect of tendinopathy on function and quantifies the
77 symptoms and dysfunction of Achilles tendon¹⁸. According to a previous literature, a recreational
78 person with Achilles tendinopathy will not score higher than 70 on the VISA-A. Moreover, a patient
79 who has VISA-A score reached 70 indicated that the patient was cured¹⁸. Therefore, in the present
80 study if the subject's score in the questionnaire was below 70, the subject would be excluded from the
81 study. Also, the age, weight (kg), height (m), the exercise's duration, frequency and types of exercise
82 of each subject were recorded.

83 For convenient sampling, university students were recruited in the campus and thus subjects
84 aged between 18 and 30 years old were recruited. Subjects were recruited and classified into two
85 groups based on the following criteria according to Ying et al.⁹:

- 86 1. frequent exercise group (having 6 or more hours of weight-bearing exercise per week)
- 87 2. infrequent exercise group (having less than 6 hours of weight-bearing exercise per week)

88 The weight-bearing exercise is defined as the force-generating activities which generates loading to
89 the skeletal regions with intensity stronger than daily activities¹⁹. Subjects in the frequent exercise
90 group were recruited from the athletics teams of local universities, whilst subjects in the infrequent
91 exercise group were recruited from acquaintance.

92 It has been reported that long-term exercise causes different extents of effect on dominant and
93 non-dominant legs ⁹. The dominant leg of each subject was recorded. To identify the dominant leg of
94 subject, subjects were asked to electively use either the left or right foot for kicking a ball. The elected
95 side for ball kicking was defined as the dominant side while the other side was the non-dominant side.
96 In the study, basketball, football and high jump were the most common exercises that subjects in the
97 frequent-exercise group performed (Table 1).

98 All ultrasound examinations were performed with the Aixplorer ultrasound unit (Supersonic
99 Imaging, Aix-en-Provence, France) in conjunction with the Supersonic Super Linear™ SL15-4
100 transducer (4 – 15 MHz). Before the ultrasound examination, each subject was asked lying prone for 5
101 minutes to ensure the subject was at resting state during the ultrasound measurement ¹⁰. In the
102 ultrasound examination, each subject lay prone with the feet hanging over the edge of the examination
103 couch. To standardize the position of subject's foot for ultrasound scanning, the foot of the subject
104 was fixed and supported by a customized ankle fixer with the plantar aspect of the foot against the
105 base of the ankle fixer so that the foot was 90° to the lower leg. A previous study on elastography of
106 Achilles tendon reported that the longitudinal scans achieved data with higher reproducibility than
107 transverse scans ²⁰. Therefore, longitudinal scans were used in the present study to assess the stiffness
108 of Achilles tendon. To increase the coupling efficiency of ultrasound and accurate measurement of
109 tendon stiffness, generous amount of ultrasound gel was applied over the Achilles tendon. During the
110 ultrasound examination, the transducer was placed over the ultrasound gel and effort was made to
111 avoid any tissue compression by the transducer since the tendon stiffness would be affected by the
112 additional load of pressure ¹⁰. The set-up for the ultrasound examination is shown in Fig 1.

113 In the ultrasound examination of Achilles tendon, grey scale ultrasound was initially used to
114 identify the Achilles tendon. Longitudinal scans were performed medially and laterally across the
115 Achilles tendon until the scan plane showing the Achilles tendon clearly and maximum tendon
116 thickness was obtained. To minimize anisotropic effect, the transducer was adjusted so that the
117 ultrasound beam was perpendicular to the tendon fibers. Once the optimal scan plane of the tendon
118 was identified, the SWE function was then activated. SWE was set at the penetration mode with the
119 measurable range of stiffness standardized at 0kPa to 800kPa. The SWE colour map (height x width:

120 1.4 x 1.3cm) was placed just above the superior border of calcaneum. Elastogram was obtained and
121 stored when the colour signals became steady for several seconds.

122 In the measurement of the tendon stiffness, archived elastograms were retrieved and the tendon
123 stiffness was measured with stiffness measurement tool - Q-boxTM. The circular measurement area
124 (Q-box) was set to 2 to 3mm in diameter dependent on the tendon size and was just covered the
125 Achilles tendon without including other adjacent soft tissues. Three Q-boxes were placed at the
126 superior, middle and inferior parts of the tendon within the SWE colour map (Figs. 2 and 3). For each
127 Q-box, the maximum, minimum and mean (in kPa) of stiffness (i.e. Young's modulus) of the tendon
128 were measured.

129 To evaluate the inter-operator variability of the measurement, each subject was scanned by three
130 operators and the same scanning protocol was used. The operators were sonographers with more than
131 one year of experience in shear wave elastography of Achilles tendon. The operators were blinded to
132 the measurement result of other operators. To evaluate the intra-operator variability of the
133 measurement, each operator scanned the subject 3 times in the same scanning session. To avoid bias,
134 in measuring the tendon stiffness using Q-boxTM on archived elastograms, the area of the monitor of
135 the ultrasound unit displaying the numerical stiffness measurement reading was masked by a small
136 piece of paper so that the operators were blinded to their measurements during the intra-operator
137 variability assessment. Images with the measurements were stored for subsequent data retrieval.

138 Data analysis was conducted using an average measurement of all the operators. All statistical
139 analyses were performed using the Statistical Package for the Social Science (SPSS 20.0 for Windows,
140 Chicago, IL, USA). The body mass index (BMI) of the subjects was calculated with the formula BMI
141 $= \text{weight (kg)} / \text{height (m}^2\text{)}$. Because of the small sample size of the frequent-exercise group (n=12),
142 two-tailed Mann-Whitney test was used to evaluate the level of significance of the difference in
143 Achilles tendon stiffness between frequent-exercise and infrequent-exercise groups. Wilcoxon
144 matched-pairs signed-ranks test was used to evaluate the significance of the difference in Achilles
145 tendon stiffness between dominant and non-dominant legs. Intraclass correlation coefficient models 2
146 and 3 [ICC2 (absolute agreement) and ICC3 (consistency) respectively] were used to assess
147 inter-operator reproducibility and intra-operator repeatability respectively. Coefficient of variation

148 (CV) was also used to evaluate the measurement variability.

149

150 **Results**

151 A total of 40 subjects were recruited (30 men and 10 women). Four subjects (3 men and 1
152 woman) had a VISA-A score less than 70 and were excluded from the study. The remaining 36
153 subjects were included in the study in which 12 were frequent-exercise subjects (33.3%) and 24 were
154 infrequent-exercise subjects (66.7%). None of the 36 subjects had substantial thickening of the
155 Achilles tendon or hypoechoic areas in the tendon. None of the subjects in the infrequent-exercise
156 group involved in high intensity exercise such as volleyball or basketball and majority of them just
157 performed activities of daily living. A total of 72 Achilles tendons were examined. There was no
158 significant difference in the body weight (median: 65.5 kg; interquartile range, IQR: 9.9 kg and
159 median: 61.3 kg; IQR: 9.2 kg respectively), height (median: 176.7 cm; IQR: 11.9 cm and median:
160 171.8 cm; IQR: 8 cm respectively) and BMI (median: 21.5; IQR: 1.9 and median: 20.6; IQR: 2.9
161 respectively) between the subjects in frequent-exercise and infrequent-exercise groups ($p > 0.05$)
162 (Table 1).

163 Regarding with the intra-operator repeatability, the individual ICC (3, k) of the three operators
164 were 0.845, 0.835 and 0.803 respectively and the CV of their measurements were 0.09, 0.1 and 0.11
165 respectively. For the inter-operator reproducibility, the ICC (2, k) among the three operators was
166 0.585 and the CV was 0.13.

167 For non-dominant ankles, result showed that the stiffness of Achilles tendon of the subjects in
168 frequent-exercise group (median: 320.1 kPa; IQR: 39.9 kPa) was significantly higher than that of the
169 subjects in infrequent-exercise group (median: 296 kPa; IQR: 48 kPa) ($p < 0.05$; effect size, $r =$
170 -0.352). However, for dominant ankles, there was no significant difference in the stiffness of Achilles
171 tendon between subjects in the frequent-exercise and infrequent-exercise groups (median: 322.1 kPa;
172 IQR: 64.7 kPa, and median: 294.5 kPa; IQR: 46.3 kPa respectively) ($p > 0.05$; $r = -0.218$).

173 In both frequent-exercise and infrequent-exercise groups, the Achilles tendon stiffness was not
174 significantly different between dominant and non-dominant legs ($p > 0.05$, $r = -0.115$ to 0.192).

175 **Discussion**

176 Results of the present study showed that the stiffness of Achilles tendon in non-dominant ankles
177 of frequent-exercisers is significantly higher than that of infrequent-exercisers. The result is consistent
178 with previous studies which found that after chronic mechanical loading like exercise Achilles tendon
179 became stiffer^{13,21}. A meta-analysis demonstrated that long duration of exercise is more efficient to
180 induce tendon adaptive responses than short duration²². It has reported that Achilles tendon stiffness
181 did not significantly increase after two months of exercise training but became statistically significant
182 by the end of the three-month training^{23,24}. However, information in the literature about causes of
183 exercise on Achilles tendon stiffness is limited. Some previous studies suggested that the increased
184 stiffness of the tendon may be associated with increased amount of type I collagen in the tendon^{25,26},
185 and the synthesis of type I collagen increases in response to prolonged exercise^{26,27}. Therefore, the
186 increased synthesis of type I collagen may account for the significantly stiffer Achilles tendon in the
187 non-dominant ankle of frequent-exercisers than infrequent-exercisers. In addition, Vilarta and Vidal²⁸
188 evaluated the effect of exercise on the alignment and number of collagen fibrils, and found that the
189 stiffness of Achilles tendon of rats increased after 30 days of exercise. They found that there were
190 more collagen fibrils in the Achilles tendon of the rats after the exercise and the collagen fibrils were
191 more aligned with each other. There are limited information in the literature about the effect of
192 prolonged exercise on human Achilles tendon, and the causes for the change in mechanical properties
193 of Achilles tendon due to exercise are still unclear. Further studies to investigate the association
194 between the mechanical property change of Achilles tendon and its histological variation after
195 prolonged exercise are suggested. The present study applied SWE to quantify the change in the
196 stiffness of Achilles tendon after prolonged weight-bearing exercise. It provides baseline information
197 about the feasibility of using SWE to assess the effect of exercise on Achilles tendon stiffness, and the
198 study result is useful for future ultrasound investigations on human musculoskeletal system.

199 In the present study, result showed that there was no significant difference in Achilles tendon
200 stiffness on dominant ankles between the frequent-exercise and the infrequent-exercise groups. The
201 insignificant finding may be due to the preferential use of one leg over another. In the present study,
202 basketball, football and high jump were the most common exercises performed by the subjects in

203 frequent-exercise group. For these types of physical activities, subjects tended to use the
204 non-dominant leg for supporting and jumping actions, and it is also the landing leg which exposes the
205 tendon to high loads of tensile force. Therefore, the mechanical loading on the two legs was unequal.
206 A meta-analysis revealed that high magnitude of mechanical loading is more effective to cause tendon
207 adaptation ²². Moreover, Arampatzis et al. ²⁹ found that the increase in Achilles tendon stiffness
208 occurred only in the leg with exercise at high strain magnitude, whereas low-strain-magnitude
209 exercise did not trigger adaptation effect on the Achilles tendon. Moreover, the strain magnitude
210 applied to Achilles tendon should exceed a threshold in order to trigger adaptation effect on the tendon
211 ²⁹. As the loading strain magnitude of dominant ankles in present study was lower than that in
212 non-dominant ankles, the mechanical loading on dominant ankles may not be larger enough to induce
213 adaptation effect on the Achilles tendon. This may explain the effect of long-term physical exercise
214 was found on non-dominant ankles but not on dominant ankles. Future studies using SWE need to
215 account for the differences in the non-dominant and dominant legs.

216 Shear wave elastography measurement of the Achilles tendon stiffness has been reported ¹²⁻¹⁶.
217 However, either the studies did not evaluate the measurement reliability ^{13,15} or they achieved a low
218 reliability with an ICC of 0.42-0.43 ^{14,16} or a coefficient of variation of 0.49 ¹². In the present study,
219 the measurement reliability (intra-operator repeatability, ICC=0.803-0.845 and inter-operator
220 reproducibility, ICC=0.585) was higher than that of previous studies. The higher measurement
221 reliability in the present study may be due to the use of ankle fixer for the ultrasound examination and
222 the standardized ultrasound scanning protocol. Nevertheless, some measurement errors still exist. In
223 the present study, the longitudinal scan of Achilles tendon for SWE measurement was obtained at the
224 scan plane which shows the maximum tendon thickness. The determination of the scan plane with
225 maximum tendon thickness by the operator was subjective, and this might associate with the
226 measurement errors between ultrasound scans of the same operator (intra-operator) as well as scans of
227 different operators (inter-operator). The consideration for future studies using SWE should be that one
228 operator is used.

229 The position of the ankle during ultrasound scanning would affect the reliability of the
230 measurement result. Peltz et al. ¹⁶ reported that the low measurement reliability in the study may be

231 due to the subtle differences in ankle joint position during the ultrasound scanning. Aubry et al. ¹⁴ also
232 stated it was difficult to achieve the desirable ankle position for scanning when the ankle joint position
233 is not stabilized. Therefore, the unstable and unstandardized ankle joint position during scanning in
234 previous studies caused the low reliability in SWE measurement of Achilles tendon. In the present
235 study, the repeatability (ICC=0.803-0.845) and reproducibility (ICC=0.585) of SWE measurement of
236 Achilles tendon were higher than those in previous studies ^{12,14,16}. The higher measurement reliability
237 of the present study is probably due to the use of the ankle fixer to maintain a stable and standardized
238 ankle joint position so that there was less movement of the ankle joint during ultrasound examination,
239 leading to higher measurement reliability.

240 Obesity is considered as a risk factor for Achilles tendonitis, and it has been reported obese
241 people had higher incidence of Achilles tendinopathy ¹⁷. In the present study, there was no significant
242 difference in the body weight and BMI of subjects between frequent-exercise and infrequent-exercise
243 groups. Therefore, the effect of the subjects' body weight and BMI on the Achilles tendon's
244 mechanical property between the two study groups is minimal. Central adiposity is a risk factor for
245 Achilles tendon pathology ³⁰. However, the waist circumference and waist hip ratio of the subjects did
246 not measure in the present study and thus the central adiposity of the subjects may be a possible
247 confounder of the study.

248 There were limitations in the present study. The sample size of the study was small with only 12
249 frequent-exercisers and 24 infrequent-exercisers. In the present study, the difference of the median
250 scores of non-dominant leg between frequent and infrequent-exercisers was smaller than that of
251 dominant leg, but significant difference was found in the non-dominant leg. This may be an artifact of
252 using median scores and non-parametric tests that will change with larger sample size and the use of
253 parametric tests. Moreover, we did not evaluate the difference in Achilles tendon stiffness among
254 different weight-bearing exercises. Therefore, further studies with a larger sample size and to
255 investigate the Achilles tendon stiffness of different athletes are suggested. Furthermore, the
256 association of different types and intensities of exercise with Achilles tendon stiffness did not evaluate
257 in the study. Further studies to investigate this association are suggested so that possible reasons for
258 the different Achilles tendon stiffness between study groups, and errors associated with measurement

259 reliability can be determined. In addition, this study is limited due to the cross-sectional design and
260 future prospective studies are needed. Also, the study only looked at current physical activity level of
261 the subjects and did not account for any physical activity in the past.

262

263 **Conclusion**

264 Shear Wave Elastography is feasible for assessing the Achilles tendon's stiffness in vivo with
265 high measurement reliability. SWE is useful to evaluate the effect of prolonged exercise on Achilles
266 tendon stiffness. The stiffness of Achilles tendon in frequent-exercise group is significantly greater
267 than that in infrequent-exercise group on non-dominant ankles but not on dominant ankles.

268

269 **Practical Implications**

- 270 • The new ultrasound elasticity imaging technique (i.e. shear wave elastography) is reliable for
271 measuring Achilles tendon stiffness with CV varies between 0.09-0.13. Note the inter-operator
272 reproducibility was 0.585 (CV=0.13), follow up scans should be performed by the same operator.
- 273 • The Achilles tendon in the non-dominant ankle of people who do exercise frequently is stiffer
274 than that of people who do exercise less frequently. However, this variation was not found in the
275 dominant ankle. Note the small sample size of the present study, further study with a larger
276 sample size is suggested to verify the finding.
- 277 • Sport medicine scientists and professionals should aware of the Achilles tendon stiffness
278 difference between dominant and non-dominant ankles as well as between frequent-exercise and
279 infrequent-exercise people for better management and prevention of related injuries.

280

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356 **Figure Legends**

357 Fig 1. The set-up for the shear wave elastographic examination of Achilles tendon. The subject's
358 foot was fixed by the ankle fixer. Generous amount of ultrasound gel was applied over the
359 posterior aspect of the ankle for the scanning. The transducer was placed over the ultrasound
360 gel to avoid any compression on the tendon.

361

362 Fig 2. Longitudinal shear wave elastogram in a frequent-exercise subject shows the Achilles tendon
363 in the non-dominant leg. The tendon has relatively higher stiffness values than the tendon in
364 the infrequent-exercise subject as shown in Fig. 3.

365

366 Fig 3. Longitudinal shear wave elastogram in an infrequent-exercise subject shows the Achilles
367 tendon in the non-dominant leg. The tendon has relatively lower stiffness values than the
368 tendon in the frequent-exercise subject as shown in Fig. 2.

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