Are the mechanical properties of Achilles tendon altered in CrossFit athletes? Reliability and accuracy of myotonometry

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Purpose: Tendons adapt to loads affecting them by changing tendons' mechanical and morphological properties. The aim of the study was to investigate the influence of involvement in sport activities in the form of CrossFit training by individuals of different age upon the mechanical properties of Achilles tendon. *Methods*: 231 people participated in the study. One group consisted of subjects who trained CrossFit as amateurs, the other group comprised subjects who were not physically active. Achilles tendon was studied for various positions of the ankle joint: 0° DF/PF, 10° DF, 20° DF, 20° PF and 40° PF. The following mechanical and viscoelastic tendon properties were measured using MyotonPRO: frequency [Hz], stiffness [N/m], decrement [log], relaxation time [ms] and creep [De]. The results have been compared in reference to physical activity, BMI, age and length of training history. *Results*: Both the tension and stiffness increased with degree of Achilles tendon stretching and decreased as it contracted. Higher values have been noted in the group of people in training and with higher BMI values. The elasticity of Achilles tendon decreased with plantar flexion increase. Lower elasticity has been recorded in the group in training and with higher BMI. No significant influence of age and length of training and accompanying mechanical load result in development of adaptation changes in Achilles tendon, in the form of its higher tone/tension and stiffness, as well as lower elasticity.

Key words: Achilles tendon, muscular rigidity, myotonometry, CrossFit athletes, sports activity

1. Introduction

Tendons adapt to loads affecting them, by changing tendons' mechanical features (namely: increased stiffness) and morphological properties (increased thickness and cross-sectional area – CSA), which may influence their load capacity) [22], [43]. It has been demonstrated that loading tendon fibres elicits adaptation response, in the form of increased collagen mRNA and inhibition of the activity of degradation enzymes. On the other

hand, absence of load results in increased activity of factors influencing their degradation: matrix metalloproteinases and collagenases [4], [5], [41]. Studies performed *in vivo* indicate, on the other hand, the increased concentration of collage synthesis markers within 24 after physical exercise [13].

Changes in tendon stiffness in reaction to load are initially a result of adaptation of the material to the function assigned. In long-term observation, however, the occurrence of morphological changes has been noticed. The reactions of tendons to loading, unloading,

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and aging have been presented, *inter alia*, in the literature reviewprepared by Magnusson et al. [25]. Increase in tendon cross-section enhances its load capacity, as the stress affecting the tendon gets reduced when the same force is applied (tension = force/CSA). A more stiff tendon thus accumulates more energy under a given load, it is also capable of withstanding greater loads before it gets injured. Although the mechanical and biological mechanisms that are the basis of adaptation changes in tendons under loads applied in training have not been completely understood, it has been demonstrated that mechanical loading triggers a biological cascade of reactions, which regulates the structural adaptation of tissues, including collagen synthesis and turnover [21], [24].

It remains unclear whether a tendon which is more stiff or more susceptible to deformations is more advantageous in the aspect of physical fitness, and whether the former or the later reduces the risk of injury. According to some authors, increasing the stiffness of tendons may be advantageous in the case of strength-based sports and the other way round [27], [34], a lower level of tendon stiffness may be advantageous for practicing endurance sports, which require optimization in tendon elasticity utilization to maintain a low metabolic cost [18].

Achilles tendon stiffness may be assessed by means of ultrasonography, on the basis of the stiffness index, defined as force to elongation ratio. That method is time-consuming, though, and relatively complicated. A better tool for examining mechanical properties of tendons is Shear Wave Elastography. It is an expensive method, however, and not so readily available, in comparison with other methods. It is still not commonly used for the examination of motor organ structures, due to diagnostics issues resulting from the layered structure of tissues with different density [6], [7].

Myotonometry is a promising and objective method for quantitative assessment of mechanical properties of tendons and muscles. It successfully replaces subjective digital palpation examinations and has been extensively described in w literature as being reliable and providing high repeatability of measurements. A device oftentimes used for taking measurements in research is MyotonPRO (Myoton AS, Tallinn, Estonia) [10], [22], [28], [30]–[32], [37]. It is a portable, affordable, and easy-to-operate myotonometer, which serves the purpose of recording the information on biomechanical and visco-elastic stiffness of tissues.

Difficulties encountered in the course of investigating mechanical properties of Achilles tendon stem from its considerable stiffness, which is, respectively, higher in comparison with smaller tendons. An additional hindrance is also the fact that measurements and studies have to be conducted in various conditions, including under a load. The values obtained by making myotometric measurements are subject to considerable deviations, then. Due to that, there is an risk that an error in measurement may occur and results of the experiment can be distorted. It is, therefore, necessary to conduct further research in order to arrive at reference values and improve the research protocols [11], [28], [44].

The results obtained in myotonometric examinations *in vivo* determine the biomechanical properties of tendons and muscles. They find applications both in diagnostics, and in assessment of results of treatment applied in the case of pathological states in soft tissues of motor organs. They should also serve as input data for establishing training programs, as well as for prophylactic/preventive measures.

The aim of the study has been to investigate the influence of involvement in sport activities in the form of CrossFit training by individuals of different age upon the mechanical properties of Achilles tendon. It was expected that the results obtained would be better, in comparison with individuals who are not physically active. A hypothesis has been formulated that the MyotonPRO device provides suitable reliability and accuracy of measurements that are indispensable for the execution of the study.

2. Materials and methods

2.1. Participants

231 people participated in the study (132 males and 99 females), their age range was 20-50 years (average age: 33.81 ± 8.46 years). The study subjects were divided into 2 groups. People assigned to group 1 (n = 131) were those who trained CrossFit as amateurs for minimum 1 year, at least 3 times a week. Group 2 (n = 100) comprised people who abstained from physical activities, who did not practice sports. Only healthy individuals were included in the study. Study participants were excluded from taking part in the case of finding orthopedic and/or neurological pathological conditions in them, this included surgical procedures which may have influenced the mechanical properties of Achilles tendon. The exclusion criteria also included the occurrence of pain in lower extremities on the day of examination.

2.2. Measurements

After arriving to the examination site, study participants were allowed a quarter of an hour for resting at room temperature. Measurements were taken on a day free from training. The subjects had been asked to abstain from exercising on the day preceding the examination as well as on the day of examination. Each study participant has been instructed about and asked to make efforts not to overload the tendon in any way. That also applied to the way they were getting to the examination site. Before taking measurements, neither warming up nor muscle relaxing procedures were applied. During testing, the subject was requested to assume a recumbent position on their belly, with the dominant lower extremity bent at the knee by 30°, the shank supported on a bolster and the foot outside the couch [37]. The subject did not engage their muscles to maintain the position for measurement. The point that was measured and marked on the Achilles tendon was at the distance of 5 cm from calcanean tuber, in accordance with the measurement method [10], [26], [28], [33] with the foot arranged at 0° of dorsiflexion/plantar flexion at the ankle joint. The foot that was loosely supported and outside the couch was set and stabilized in the desired position by the person taking the measurement. In such case, the subject did not engage their muscles to maintain the position for measurement, either. The researcher took the measurements with the use of MyotonPRO device (Myoton AS, Tallinn, Estonia) after previously arranging and holding, passively for the subject, the foot in the following positions, consecutively: 0° of dorsiflexion (DF)/plantar flexion (PF), 10° of dorsiflexion (DF), 20° of dorsiflexion (DF), 20° of plantar flexion (PF), and 40° of plantar flexion (PF). A low level of variation coefficient was taken care of, in order to secure a high reliability of obtained results. The measurements were repeated three times in one setting/position, then measurements were taken in another foot position. The results of measurements were averaged.

MyotonPRO probe [32], [38] was applied perpendicularly to the surface of skin at the examined site. The device automatically pre-compressed by exerting initial pressure on the tissue being examined, applying a force of 0.18 N, and when the conditions for taking a regular measurement were met, it automatically generated five times a short mechanical impulse force of 0.4 N and duration of 15 ms. The accelerometer registered the oscillations of the examined tissue. Five parameters were calculated. Three of those parameters correspond to biomechanical stiffness: (F) – frequency of oscillations [Hz], which determines the tissue tone, (S) – dynamic stiffness [N/m], which characterizes the tissue resistance to deformation, and (D) – decrement [log], which characterizes the reduction of tissue oscillations that get reduced freely. The drop/reduction in tissue oscillation inversely describes the elasticity. Two further parameters represent visco-elasticity: (R) – mechanical stresses relaxation time [ms] which indicates the time required for the tissue to return to its original shape after deformation, and (C) – the relation between relaxation time and deformation time, defined as creep which is accountable for gradual elongation of tissue over time, under constant load [37].

The criteria for interpretation of results were as follows: the higher the values of (F) – frequency of oscillations/vibrations [Hz] and (S) – dynamic stiffness [N/m], the higher the tension and stiffness of the examined tissue. The lower the value of (D) – decrement [log], the lower the dissipation of mechanical energy during oscillation and the higher the tissue elasticity of the tissue. The lower the value of (R) – relaxation time [ms] and (C) – (creep), the higher the tissue toneor stiffness [37].

The study was performed in the Didactic and Scientific Centre of Warsaw Medical Academy of Applied Sciences in Warsaw, Poland. The study was conducted in accordance with the Declaration of Helsinki and its protocol has been accepted by the Bioethics Committee at the Medical University of Mazovia in Warsaw, Poland (approval reference number: 2022/09/MUM-01).

2.3. Statistical analysis

Statistical analysis was performed using the software Statistica 13 package (Statsoft, Poland). The results have been presented by means of average values, standard deviation, and 95% confidence interval. The distribution of examined variables was analyzed by means of Shapiro–Wilk test. For testing of statistical differences in the studied parameters between specific groups, the Mann–Whitney *U*-test and Kruskal–Wallis test have been applied. The threshold of statistical significance was assumed at p < 0.05.

3. Results

3.1. Demographics

Descriptive statistics concerning demographic data: length of training history, age, height, body mass, and BMI, including division into groups, has been pre-

Table 1. General characteristics of participants

	Group $1 - \text{in training } (n = 131)$				Group 2 – not in training ($n = 100$)			
	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
Length of training history [years]	4.25	1	11	2.13	0	0	0	0
Age [years]	35.18	20	50	7.67	32.04	20	50	9.15
Height [cm]	175.55	152	193	8.53	173.33	154	198	10.11
Body mass [kg]	76.22	50	116	13.35	72.66	41	116	15.56
BMI [kg/m ²]	24.59	19.03	34.35	2.91	24.09	18.71	35.80	3.55

sented in Table 1. Both groups were characterized by substantial homogeneity.

3.2. Differences in myometric parameters of Achilles tendon at each angle

The results of measurements made have been compared between group 1 (n = 131) and group 2 (n = 100), using the Mann–Whitney *U*-test and are presented in Table 2. The values of frequency (*F*), which determine tissue tone were statistically significantly higher in group 1 for all 5 investigated setups of the ankle joint: 0°, (DF)10°, (DF)20°, (PF)20°, (PF)40°. The highest average value in group 1: 56.33 ± 5.39 Hz in comparison with 53.27 ± 6.12 Hz in group 2, (p < 0.001) was noted in case of the dorsiflexion of the foot (DF) 20°, with the lowest of 28.32 ± 2.21 Hz in comparison with 27.44 ± 2.25 Hz, (p = 0.003) in case of plantar flexion (PF) 20°. This implies that the tone of Achilles tendon increases with the increasing degree of dorsiflexion, and decreases with increasing plantar flexion. The tone turned out to be higher in group 1 for all the measurements taken.

The registered values of stiffness (S) were statistically significantly higher in group 1 as concerned 2 of

Table 2. The results of parameters recorded by MyotonPRO (group 1 vs. group 2)

	Group $1 - in training (n = 131)$				Group 2 – not in training $(n = 100)$					
	Mean	SD	-95% CI	95% CI	Mean	SD	-95% CI	95% CI	p^*	
$F = 0^{\circ}$	43.21	4.56	42.42	44.00	40.38	4.26	39.53	41.22	<i>p</i> < 0.001	
S 0°	1063.69	69.54	1051.67	1075.71	1049.99	114.34	1027.30	1072.68	0.476	
$D = 0^{\circ}$	0.41	0.17	0.38	0.44	0.43	0.17	0.40	0.46	0.234	
$R = 0^{\circ}$	4.83	1.33	4.60	5.06	4.69	0.99	4.49	4.88	0.195	
<i>C</i> 0°	0.39	0.18	0.36	0.43	0.35	0.13	0.33	0.38	0.283	
F (DF) 10°	49.29	5.34	48.37	50.21	46.22	5.60	45.11	47.34	<i>p</i> < 0.001	
S (DF) 10°	1122.58	119.05	1102.00	1143.16	1112.63	101.94	1092.40	1132.86	0.584	
<i>D</i> (DF) 10°	0.46	0.18	0.43	0.49	0.41	0.18	0.37	0.44	0.007	
<i>R</i> (DF) 10°	5.97	1.36	5.74	6.21	5.32	1.46	5.03	5.61	0.003	
C (DF) 10°	0.58	0.19	0.54	0.61	0.53	0.67	0.40	0.66	<i>p</i> < 0.001	
F (DF) 20°	56.33	5.39	55.39	57.26	53.27	6.12	52.06	54.48	<i>p</i> < 0.001	
S (DF) 20°	1308.22	213.55	1271.31	1345.13	1221.57	189.59	1183.95	1259.19	<i>p</i> < 0.001	
<i>D</i> (DF) 20°	0.76	0.29	0.71	0.81	0.61	0.30	0.56	0.67	<i>p</i> < 0.001	
<i>R</i> (DF) 20°	6.35	0.78	6.21	6.48	6.28	0.92	6.09	6.46	0.378	
C (DF) 20°	0.67	0.11	0.66	0.69	0.64	0.13	0.61	0.67	0.010	
F (PF) 20°	28.32	2.21	27.94	28.70	27.44	2.25	26.99	27.88	0.003	
S (PF) 20°	639.88	56.41	630.13	649.63	618.93	61.48	606.73	631.13	0.005	
D (PF) 20°	1.43	0.22	1.39	1.46	1.38	0.32	1.32	1.45	0.055	
<i>R</i> (PF) 20°	7.98	0.80	7.84	8.12	8.33	0.92	8.15	8.51	0.005	
C (PF) 20°	0.52	0.05	0.51	0.53	0.54	0.05	0.53	0.55	0.004	
F (PF) 40°	28.55	3.25	27.99	29.11	27.47	2.77	26.92	28.02	0.014	
S (PF) 40°	610.10	96.37	593.44	626.76	608.01	89.97	590.16	625.86	0.421	
<i>D</i> (PF) 40°	1.53	0.21	1.49	1.57	1.48	0.33	1.42	1.55	0.036	
<i>R</i> (PF) 40°	8.39	1.23	8.18	8.60	8.57	1.31	8.31	8.83	0.220	
C (PF) 40°	0.55	0.08	0.53	0.56	0.56	0.07	0.54	0.57	0.147	

F – frequency [Hz], S – stiffness [N/m], D – decrement [log], R – relaxation time [ms], C – creep [Deborah No.], DF – dorsiflexion, PF – plantar flexion, * Mann–Whitney U-test.

the 5 measurements taken. The highest average value measured in group 1: 1308.22 ± 213.55 N/m in comparison with 1221.57 ± 189.59 N/m in group 2, (p < 0.001) was noted in case of foot dorsiflexion (DF) of 20°, whereas the lowest statistically significant value of 639.88 ± 56.41 N/m in comparison with 618.93 \pm 61.48 N/m, (p = 0.005) was recorded in the case of foot plantar flexion (PF) of 20°, thus in the same ankle joint position settings as in the case of frequency (F). In the case of plantar flexion (PF) of 40° the stiffness value recorded in group 1 was even lower, amounting to 610.10 ± 96.37 N/m, yet the difference was not statistically significant (p = 0.421) in comparison with group 2: 608.01 ± 89.97 N/m. Both the values of frequency (F) which defined tone and the values of stiffness (S) increased as the Achilles tendon got more stretched, and decreased as the tendon contracted.

The values of decrement (D) revealed differences of statistical significance in 3 out of 5 measurements taken, and turned out to be higher in group 1. They increased with the increasing degree of plantar flexion, reaching highest average values in the plantar flexion (PF) of 40° in group 1: 1.53 ± 0.21 log in comparison with 1.48 ± 0.33 log in group 2; (p = 0.036). They decreased as the degree of dorsiflexion decreased, reaching the minimum at 0°. This means the elasticity of Achilles tendon decreases with increasing degree of plantar flexion, and increases with decreasing degree of dorsiflexion. Achilles tendon appears to be most elastic in the position of 0° and dorsiflexion (DF) of 10°. The elasticity reaches lowest values in plantar flexion.

The values of relaxation time (*R*) increased with increasing degree of dorsiflexion, and kept increasing ever more with increasing degree of plantar flexion, reaching their maximum levels in extreme foot positions. Those observations were valid for groups 1 and 2, yet the differences were not statistically significant. Relaxation time (*R*) in group 1 for dorsiflexion (DF) of 20° amounted to 6.35 ± 0.78 ms vs. 6.28 ± 0.92 ms in group 2; (p = 0.378). In the case of plantar flexion (PF) of 40° in group 1, it amounted to 8.39 ± 1.23 ms vs. 8.57 ± 1.31 ms in group 2; (p = 0.220).

The values of creep (C) reached the maximum level in case of dorsiflexion (DF) of 20° and, respec-

	BMI 18.5–24.99 kg/m ² ($n = 150$)				BMI 25–34.99 kg/m ² ($n = 81$)						
	Mean	SD	-95%	95%	Mean	SD	-95%	95%	p^*		
$F = 0^{\circ}$	41.32	4.42	40.60	42.03	43.22	4.81	42.15	44.28	0.012		
S 0°	1055.06	104.02	1038.28	1071.84	1062.75	63.08	1048.80	1076.70	0.969		
$D = 0^{\circ}$	0.40	0.14	0.38	0.42	0.46	0.20	0.41	0.50	0.061		
$R = 0^{\circ}$	4.70	1.10	4.52	4.88	4.89	1.35	4.59	5.19	0.937		
C 0°	0.36	0.15	0.34	0.39	0.40	0.18	0.36	0.44	0.865		
F (DF) 10°	47.33	5.60	46.42	48.23	49.14	5.60	47.90	50.38	0.044		
S (DF) 10°	1118.70	94.13	1103.51	1133.89	1117.48	139.50	1086.64	1148.33	0.391		
<i>D</i> (DF) 10°	0.39	0.15	0.37	0.42	0.52	0.21	0.48	0.57	<i>p</i> < 0.001		
<i>R</i> (DF) 10°	5.44	1.47	5.20	5.67	6.16	1.27	5.88	6.44	<i>p</i> < 0.001		
C (DF) 10°	0.49	0.21	0.46	0.52	0.68	0.71	0.52	0.83	<i>p</i> < 0.001		
F (DF) 20°	54.93	5.89	53.98	55.88	55.14	5.96	53.82	56.46	0.828		
S (DF) 20°	1260.25	213.41	1225.81	1294.68	1290.09	196.21	1246.70	1333.47	0.321		
D (DF) 20°	0.66	0.31	0.61	0.71	0.76	0.26	0.71	0.82	0.002		
<i>R</i> (DF) 20°	6.30	0.83	6.17	6.44	6.35	0.87	6.16	6.54	0.271		
C (DF) 20°	0.66	0.12	0.64	0.67	0.67	0.12	0.64	0.70	0.002		
F (PF) 20°	27.73	2.21	27.38	28.09	28.31	2.34	27.80	28.83	0.071		
S (PF) 20°	621.89	56.60	612.75	631.02	647.33	61.37	633.76	660.90	0.002		
D (PF) 20°	1.43	0.29	1.38	1.47	1.37	0.23	1.32	1.42	0.173		
<i>R</i> (PF) 20°	8.24	0.86	8.10	8.38	7.93	0.85	7.74	8.11	0.010		
<i>C</i> (PF) 20°	0.54	0.05	0.53	0.55	0.52	0.05	0.51	0.53	0.005		
F (PF) 40°	27.87	2.84	27.42	28.33	28.47	3.50	27.69	29.24	0.214		
S (PF) 40°	608.02	93.58	592.92	623.12	611.37	93.78	590.63	632.11	0.880		
D (PF) 40°	1.54	0.30	1.49	1.59	1.45	0.19	1.41	1.49	0.011		
<i>R</i> (PF) 40°	8.47	1.22	8.27	8.67	8.46	1.35	8.16	8.76	0.930		
C (PF) 40°	0.55	0.07	0.54	0.56	0.55	0.09	0.53	0.57	0.581		

Table 3. The results of parameters recorded by MyotonPRO (lower BMI vs. higher BMI)

F – frequency [Hz], S – stiffness [N/m], D – decrement [log], R – relaxation time [ms], C – creep [Deborah No.], DF – dorsiflexion, PF – plantar flexion, * Mann–Whitney U-test.

tively, reached the following level: 0.67 ± 0.11 Deborah No. in group 1 and 0.64 ± 0.13 Deborah No. in group 2 and differed at statistically significant level (p = 0.010).

The entire study material (both people in training and those not in training; n = 231) has also been divided into 2 groups in reference to BMI values: 1 – (normal values: 18.5–24.99 kg/m²; n = 150) vs. 2 – (overweight and obesity degree I: 25–34.99 kg/m²; n = 81). The comparison of measurement results is presented in Table 3.

Similar dependencies were noted in the analyzed results of studied parameters when comparing groups with higher and lower BMI as well as in case of comparing group 1 (people in training) with group 2 (people not in training). Statistically significant differences were noted only occasionally, however (comparison of Tables 2 and 3). Higher values of the parameters studied were noted in the group with higher BMI. The values of frequency (F), which defines tone and the values of stiffness (S) increased as the Achilles tendon stretched and decreased as it contracted. The values of decrement (D) increased with decreasing degree of plantar flexion, and decreased with decreasing

the degree of dorsiflexion, reaching minimum values in case of 0° . Relaxation time (*R*) increased with increasing dorsiflexion and kept on increasing with increasing plantar flexion. Also the values of creep (*C*) increased as dorsiflexion increased.

Table 4. Body Mass Index (BMI): frequency of values observed in groups

BMI [kg/m ²]	Group 2 [<i>n</i>]	Group 1 [<i>n</i>]	Total [n]
18.5-24.99	73	77	150
25-34.99	27	54	81
Total [n]	100	131	231

It was thus decided to compare, in order to find out whether in group 1 (people in training) there were more subjects with higher BMI (overweight and obesity degree I). The result was that in group 1 there were twice as many subjects with higher BMI, when compared with group 2, (54 vs. 27). The difference was statistically significant (p = 0.024; chi-square (χ^2) test. The results are presented in Table 4.

This entailed that higher BMI values occurring more frequently among people in training may influence

Table 5. The results of parameters recorded by MyotonPRO (group 1 and lower BMI vs. group 1 and higher BMI)

	In training + BMI 18.5–24.99 [kg/m ²] (<i>n</i> = 77)				In training + BMI 25–34.99 $[kg/m^2]$ (<i>n</i> = 54)					
	Mean	SD	-95%	95%	Mean	SD	-95%	95%	p^*	
$F = 0^{\circ}$	42.66	4.36	41.67	43.65	43.99	4.75	42.70	45.29	0.315	
S 0°	1064.47	72.18	1048.08	1080.85	1062.57	66.25	1044.49	1080.66	0.679	
$D = 0^{\circ}$	0.39	0.15	0.36	0.42	0.44	0.19	0.39	0.49	0.161	
$R 0^{\circ}$	4.76	1.27	4.47	5.04	4.93	1.41	4.55	5.31	0.714	
C 0°	0.38	0.17	0.34	0.42	0.41	0.19	0.36	0.46	0.440	
F (DF) 10°	48.82	5.50	47.57	50.07	49.96	5.09	48.58	51.35	0.294	
S (DF) 10°	1128.71	104.51	1105.00	1152.43	1113.83	137.74	1076.24	1151.43	0.384	
<i>D</i> (DF) 10°	0.42	0.15	0.38	0.45	0.53	0.21	0.47	0.58	0.003	
<i>R</i> (DF) 10°	5.69	1.43	5.36	6.01	6.38	1.14	6.07	6.69	0.001	
C (DF) 10°	0.54	0.20	0.49	0.58	0.63	0.16	0.59	0.67	0.001	
F (DF) 20°	56.53	5.36	55.32	57.75	56.03	5.47	54.54	57.52	0.483	
S (DF) 20°	1309.25	234.92	1255.93	1362.57	1306.76	180.88	1257.39	1356.13	0.743	
D (DF) 20°	0.73	0.32	0.66	0.81	0.79	0.23	0.72	0.85	0.194	
<i>R</i> (DF) 20°	6.29	0.81	6.10	6.47	6.44	0.73	6.24	6.64	0.063	
C (DF) 20°	0.67	0.12	0.64	0.69	0.69	0.10	0.66	0.71	0.025	
F (PF) 20°	27.98	2.02	27.52	28.44	28.80	2.40	28.14	29.45	0.056	
S (PF) 20°	628.87	51.81	617.11	640.63	655.57	59.41	639.36	671.79	0.013	
D (PF) 20°	1.46	0.22	1.41	1.50	1.39	0.23	1.32	1.45	0.036	
<i>R</i> (PF) 20°	8.12	0.78	7.95	8.30	7.78	0.80	7.56	8.00	0.026	
C (PF) 20°	0.53	0.05	0.52	0.54	0.51	0.05	0.50	0.52	0.016	
F (PF) 40°	28.29	2.92	27.63	28.96	28.92	3.67	27.91	29.92	0.353	
S (PF) 40°	605.35	95.56	583.66	627.04	616.87	98.01	590.12	643.62	0.920	
D (PF) 40°	1.58	0.23	1.52	1.63	1.46	0.16	1.42	1.51	0.003	
<i>R</i> (PF) 40°	8.40	1.09	8.15	8.65	8.38	1.42	7.99	8.76	0.861	
C (PF) 40°	0.55	0.06	0.53	0.56	0.55	0.10	0.52	0.57	0.620	

F – frequency [Hz], S – stiffness [N/m], D – decrement [log], R – relaxation time [ms], C – creep [Deborah No.], DF – dorsi-flexion, PF – plantar flexion, * Mann–Whitney U-test.

statistically significant differences in values of the studied parameters, noted between group 1 and group 2.

In order to verify the above hypothesis, a comparison was made of the results of measurements taken in group 1 (people in training) with additional breakdown into the following subgroups: 1 - subjects with higher BMI (25–34.99 kg/m²; n = 54) and 2 - subjects with lower (18.5–24.99 kg/m²; n = 77) BMI. The results are presented in Table 5.

The values of frequency (*F*) defining tone, and stiffness (*S*) values were higher in the group of subjects in training and with higher BMI only in the case of plantar flexion of ankle joint: (PF) 20° and 40°, that is in contracted Achilles tendon. The values of decrement (*D*) were lower, which corresponds to higher elasticity of tendon. The values relaxation time (*R*) and creep (*C*) in this foot position were lower, thus confirming the higher values of tone and stiffness, which is in agreement with the criteria for interpretation of Myoton-PRO measurement results.

The results of measurements taken were also compared in reference to the length of training history. Two subgroups were singled out in group 1 (subjects in training): A: training history <5 years (n = 75) and B: training history ≥ 5 years (n = 56). No statistically significant differences were noted (p > 0.05;Mann–Whitney *U*-test) for any of the studied parameters. A comparison was also made in case of subjects in training who had higher BMI (25–34.99 kg/m²; n = 54) as regards the length of their training history. Subgroup A was defined: subjects in training for <5 years (n = 25) and subgroup B: subjects in training for ≥ 5 years (n = 29). Likewise, no statistically significant differences were noted (p > 0.05) for the parameters studied.

Also the influence of age upon results of measurements were assessed. The entire study material was in this case divided into 3 subgroups: A: (20–29 years; n = 82), B: (30–39 years; n = 89), C: (40–50 years; n = 60). No statistically significant differences were noted (p > 0.05; Kruskal–Wallis test) for any of the studied parameters.

4. Discussion

Myotonometry is a promising and objective method which enables taking measurements and assessing the differences in tone, stiffness and elasticity regarding soft tissues. Thanks to myotonometry, we are able in a short, reliable and non-invasive manner to measure the mechanical properties of muscles, both in medicine and in sport environment as well as for the purpose of scientific research [29].

Myotonometry does not require specialist knowledge or costly equipment, as in the case of examinations with the use of shear wave elastography (SWE). At the same time, as had been indicated by the previously conducted scientific research, this examination method is comparably objective and repeatable. Research demonstrated a substantial correlation of stiffness measurements (inter-rater) taken by means of myotonometry and elastography [20]. A advantage that MyotonPRO has is also the possibility of examining both small and larger areas of tissues. A limitation, in turn, is that only superficially located tissues (muscles and tendons) may be measured that way. The possibility of diagnosing stiffness, with additional use of ultrasonography, enables also the differentiation between pathological stiffness condition which occurs as a result of mineralization and fibrosis in the course of trauma healing, and increased stiffness as adaptation reaction to loads [14].

Tendons transfer the force generated by contracting muscles upon the skeletal system, they also act as a mechanical buffer protecting a muscle against damage which may most frequently occur as a result of eccentric loading [17]. The observations made by Konow [16] demonstrate that a correct level of tendon elasticity is a major factor protecting tendons from injuries and degeneration. An elastic tendon is capable of storing and then giving back the elastic strain energy without unnecessarily transferring the forces to muscle fibres, by which it provides protection for muscles [8]. If some stiffness increase detected in myotonometric examination is accompanied by increased crosssection area (CSA) of the tendon without its swelling, as detected by ultrasonographic examination, this is the evidence of correct adaptation to loads and is not to be considered a risk factor for injury. In order to avoid injury, correct functioning of tendons should be within 3% tendon stretch level. In professional athletes, due to high level of training it should not be more than 4%. If the calcaneal tendon is extended over 8% of its capacity, the continuity of specific fibrils gets disrupted [2].

The results of our investigations indicate that people who train CrossFit manifested a clearly greater stiffness, in comparison with people who do not train it, as regards the angular settings which are most significant for dynamic workload in the scope of dorsal flexion DF 20° (group in training – 1308.2 N/m vs. group not in training – 1221.5 N/m p < 0.001) and plantar flexion PF 20° (group in training – 639.88 N/m vs. group not in training – 618.93 N/m, p < 0.005). This is

the evidence of proper reaction to CrossFit type of training and protection thus provided for Achilles tendon against the risk of injuries in the scope in which the tendon is subject to most substantial loads. In the case of plantar flexion of 20 degrees, the joint reaches half the scope of active movement, while the muscle is capable of generating the highest force in concentric movement, by which it is most prone to injuries, the scope of movement of 20 degrees in dorsal flexion is also half of the scope of passive movement. Also in this scope, during loading with body mass and inclusion of eccentric workload, the tendon is most susceptible to injuries. Increased stiffness guarantees better prevention of injuries and provides evidence of beneficial influence of training upon biomechanical parameters of the tendon [42]. In line with the studies reported by Barreto et al. [2], the increase of stiffness noted in the group of subjects in training, with simultaneous absence of pain symptoms, is a sign of increased tendon thickness as proper adaptation reaction resulting from training.

Similar results were reported by Fisker et al. [12] in their study on healthy volunteers training CrossFit, where Achilles tendon thickness and stiffness increased significantly after training. Because the study was carried out on asymptomatic subjects, its results may be explained by adaptation mechanism.

Changes in stiffness are not accompanied by analogous and unambiguous changes in tension, because those features develop in another physiological mechanism. Increase of tendon stiffness is a positive/desired response to training under load. It leads to hypertrophy of collagen fibers and elastin, which results in increased strength/resistance of tendons. Muscle tone may also increase as a result of training process, yet, it is not a result of training that is to be desired. It should be dealt with using various therapeutic techniques [15].

The best and most demanded effect of the training process is the increase of tendon stiffness, which makes it more resistant, at the same time the tension is maintained, which, in turn, provides proper blood supply, without over-compressing the capillary vessels [9].

The results of the study reported may be used for conducting further research on establishing norms pertaining to correct tendon tension at rest, as the tendon stiffness increases. Attention should be paid to tension parameters of the tendon examined in the position of flexion or extension in the joint (aside from neutral position). Tension may differ between individuals, because of heterogeneity of motor organs, with such differences as that concerning muscle length, joint structure (taking into account various post-traumatic changes) or different size of hypomochlions (fulcra), which are requisite of the angle at which tendons are attached to bone tissue. That is the reason why the most important parameter will be the measurement of tension when the joint is at rest in neutral position and at neutral setting. The best effect to be achieved in post-training process will be the increase of stiffness with constant tension maintained. It should be pointed out that tension should be determined from the tendons of muscles which have the correct norm of length at rest [1].

In the study with the use of MyotonPRO for the assessment of Achilles tendon properties under dynamic load in endurance-jumping trainin in Karate Shotokan, a substantial increase in stiffness after training was noted [33]. Our study has confirmed that observation, yet, not in direct comparison of stiffness before and after exercise, but by comparing subjects training CrossFit and those who do not train, with simultaneous increase of tone parameter. In both studies referred to above, the influence of training load upon tendon stiffness was noted, in the form of normal adaptation reaction. Sometimes, however, the increase of tendon cross-section area (CSA) after application of loads is not an adaptation change but a result of swelling in the course of tendinopathy [45]. Then, despite the increased cross-section area (CSA) in the injured tendon, reduction of its stiffness is observed [28], [41].

Stiffness and elasticity of tendons develop in dependence of training loads. In sport disciplines requiring strength, stiffness increases over a short period of time. In endurance sports, where a long-lasting muscle effort and moderate loads occur, elasticity increases [36], [40]. An abrupt change in training regime may lead to overloading and injuries [35].

In the study reported by Mrozik et al. [29], the authors assessed 7 volleyball players, who for 2 weeks followed a specifically designed plyometric training. Before training, as well as after 1st and after 2nd week of training, stiffness in the straight/rectus muscle of thigh was measured by means of MyotonPRO myometer. The measurements were taken in two points on the femoral straight muscle in supine position. After two weeks of training, statistically significant reduction of the femoral straight muscle was noted for both measurement points. On the other hand, after one week of training the change in stiffness value inthe femoral straight muscle was not statistically significant.

The results of our studies, as well as the observations of Pożarowszczyk et al. [33] indicate that, besides the strength-related type of training loads, also a higher body weight is a condition for the occurrence of higher stiffness values found on myotonometric assessment. The MyotonPRO device may be successfully applied for short – as well as long-term observations of mechanical lesions of soft tissue, in response to training loads. Conversely, in case of morphological assessment, that is the determination of tendon CSA, a longer time of observation is required. This practically entails a higher risk of not diagnosing function impairments at early stages, which results in the development of structural disturbances. That is why the knowledge concerning functional condition disturbances in Achilles tendon, for which direct information provided by the results of tone, stiffness, and elasticity is of such key importance in injury prevention [28].

Moreover, M. Bizzini [3] and team, in their studies performed with the use of myotonometer, recorded cohesive and reliable measurements of visco-elastic stiffness of muscles.

The results of measuring mechanical properties of tissues with the application of MyotonPRO device are comparable between studies [11], [23], [37]. This is an important factor which is a proof that device is useful indeed. The ever-increasing amount of scientific reports contributes to the determination of reference values for specific tissues.

5. Conclusions

The mechanical properties of Achilles tendon were examined in the study reported here. The subjects were people of different age, they comprised both the individuals who practice sport and those who do not do sports. The hypothesis assuming that better results would be noted in subjects doing sport was partly confirmed. Higher tone and stiffness values were noted in subjects in training, as well as those with higher BMI. Those values increased with tendon elongation/extension and decreased with tendon contraction. On the other hand, higher elasticity values were measured in subjects not in training and with lower BMI. Achilles tendon demonstrated its highest elasticity in intermediate position, which caused neither its extension nor contraction. Higher BMI values determine higher level of tone and stiffness in subjects in training only when the tendon is contracted. Thus, it has no influence upon those mechanical properties in case of tendon extension. The length of training history as well as age do not appear to influence the mechanical properties of Achilles tendon, measured by means of MyotonPRO device. Those properties were better in comparison with subjects who did not exercise.

The study confirmed the hypothesis that MyotonPRO is a useful tool for quantitative assessment of mechanical properties of Achilles tendon. The device provides high level of accuracy and repeatability of measurements. It can be complementary in clinical examinations, as a useful supplement of highly subjective digital palpation. To sum up, our study is a contribution to determination of reference values in myotonometry of Achilles tendon for healthy individuals of different ages, those who do sport on amateur basis, as well as for those who do not make full use of the opportunities to participate in selected physical or sport activities on everyday basis.

The study has certain limitations. Achilles tendon was examined in one point. It cannot be excluded that in other areas of the tendon the measurement results may differ from those reported in the study. Tendon properties were examined only under passive loads applied to Achilles tendon. Although the load magnitude may have varied only slightly, it provided higher level of repeatability than in the course of active movement where the forces applied are not controllable and body weight is the load applied. Moreover, the assumption of study authors was that the methodology used for examining Achilles tendon should not depart from typical physical examination. The composition of bodies of study subjects was not examined, thus is not known to what degree the higher level of BMI results from lean muscle mass.

The strength of the study include homogeneity of the group of subjects who do sports. All of them trained CrossFit in affiliated clubs, which assures a high repeatability of the physical exercises they were engaged in. All measurements were taken by the same person, trained in the methodology of examinations with the application of myotonometry.

The study was performed in the Didactic and Scientific Centre of Warsaw Medical Academy of Applied Sciences in Warsaw, Poland. The study was conducted in accordance with the Declaration of Helsinki and its protocol has been accepted by the Bioethics Committee at the Medical University of Mazovia in Warsaw, Poland (approval reference number: 2022/09/MUM-01). All participants gave written informed consent to participate in the study.

Conflict of interests

The author's declare no conflict of interests regarding this study.

Author contribution's

- SSz study design, data collection, data interpretation, manuscript preparation, literature search;
- JP study design, data collection, manuscript preparation, literature search;
- MD data collection, manuscript preparation;
- MZ data collection, manuscript preparation;
- GC data interpretation, manuscript preparation.

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