ABSTRACT

Objective: Analyze in vitro the mechanical response of bovine calcaneus tendons subjected to static stretching in three different intervals (15, 30, 45 s). Methods: Six groups of bovine calcaneus tendons (n=10) were formed according to the static stretching protocol: three different intervals (15, 30, 45 s) and initial stretching percentage (2.5% and 3.5%). The control group (n=10) did not perform prior stretching. At the end of the stretching tests, the specimens were subjected to stress rupture tests. Results: The values for force relaxation presented stability after the 30th second (p<0.0001) at both levels of deformation. Greater force relaxation (p<0.0026) and the least tensile strength (p=0.0123) was observed in the group that was subjected to the highest stretch percentage (3.5%). No difference was observed between the rupture parameters of the stretch and control groups. The variables, stretch duration and percentage did not demonstrate interaction. Conclusion: In relation to force relaxation, the 30 second interval seems to be the most effective when stretching tendons. This fact should be considered when establishing new clinical stretching protocols.

INTRODUCTION

The importance of regular physical activity is widely recognized and confirmed by countless scientific articles. It has a positive effect not only on the locomotor system but also improves functional performance of the cardiovascular and immunological systems where influencing quality of life. In this context, stretching exercises play an important role as they help regulate the functional balance of the musculoskeletal system.

Some studies have suggested that stretching can help prevent lesions, reduces tissue stiffness, and improve range of movement; it is also recommend by the American College of Sports Medicine as essential for those who regularly practice physical activities.

However, the parameters that govern the practice of stretching have still not been established. The duration of stretching proposed in the literature is highly variable. Clinical studies, in general, analyze stretching intervals between 15 to 120 seconds, while in vitro studies consider 100, 600, or even 1,800 seconds. Another parameter studied is the initial stretch percentage. Studies have shown that greater the initial deformation (stretch), greater is the tissue relaxation. Wren et al. state that initial deformation has a direct effect on injury, and is the main parameter to predict rupture.

Physiologically, when biological tissues are submitted to stretching they present progressive decrease in tensile strength resistance, until they reach a plateau and, consequently, the functional balance of the tissue. This phenomenon is called force relaxation.

Accordingly, the aim of this study was to analyze in vitro the mechanical force relaxation response, in tendons submitted to static stretching, considering intervals commonly used in clinical practice and verify the possible influence of stretching on the injurious event (rupture).
MATERIAL AND METHODS
The study material consisted of 70 specimens of bovine calcaneal tendons, from Nellore males, with average age of three years. The material was obtained after death, according to the ethical principles in animal experimentation. The research project was approved by the Committee of Ethics in Animal Use (CEUA/Unicamp), protocol 2005-1/2009. After harvesting, the tendons were packed in plastic bags, wrapped in gauze dampened in a 0.9% saline solution. The tests were carried out within 5 hours after their obtainment at ambient temperature (28°C). For the performance of the tests, each specimen was carefully dissected, removing all the structures around the tendon, including the paratendon. Then rectangular strips were removed from the tendons using a scalpel.

The test were conducted with a LOYD TA 500 model (Fa-reham UK) texturometer type press, with a 500N load cell, force resolution of 0.01% and charging speed control between 0.0017 and 17mm/s.

Test protocol
Six groups of ten specimens of bovine calcaneal tendons were defined according to the static stretching protocol: intervals (15, 30, 45 seconds) and initial stretching percentages (2.5 and 3.5%). At the end of the stretching test, all the specimens were immediately submitted to the stress rupture test. The control group (n=10) only performed the stress rupture test.

Cross-sectional area
After the fixation of the gripper system to the Texturometer (Univer-sal Testing Machine), the measurements of length, width and thickness were recorded with a digital caliper (Starret, model 727-6 A150; 0.01mm of precision). Each measurement was taken three times, considering the average of these to calculate the cross-sectional area (CSA).

This procedure favors the use of the tension concept (force/CSA), as it standardizes the force values in relation to the dimensions of the test body.

Mechanical tests
The specimens were submitted to uniaxial tensile tests, conducted at a speed of 10% of the initial length of the test specimen per second. Once the pre-established stretching was reached, force decreases were recorded at one second intervals, within the timeframe considered. Immediately after the end of the stretching, all the specimens were submitted to the uniaxial tensile test until rupture at a speed of 100% of the initial length per second.

Data acquisition system
The data acquisition system consisted of a standard interface, coupled to the texturometer, and composed of a Pentium Pro computer and NEXIGEN 3.0 software. Force and deformation were recorded continuously at the frequency of 10 Hz. The values of force (N) and time (s), were used as a basis to calculate: the percentage of force relaxation, for the stretching tests; and the tension (FR/CSA), the relative deformation (L/L0) and the rupture energy per cross-sectional area (FR*LO/CSA) for the rupture tests.

Statistical analysis
A descriptive analysis was used with presentation of position and dispersion measurements for numerical variables. Rank transform ANOVA was used to compare parameters among the factors studied, while the Kruskal-Wallis test was used to compare the independent groups. We used the SAS System for Windows program (Statistical Analysis System), version 9.2. SAS Institute Inc, 2002-2008, Cary, NC, USA. The confidence interval was set at 95%, and the comparison parameter was p = 0.05.

RESULTS
Table 1 presents the dimensional parameters of the specimens. At both levels of deformation studied, the force relaxation value in the 30-second interval was significantly higher than in the 15-second interval, yet without any differences when compared with the 45 second group (p<0.0001). When comparing two different levels of deformation (2.5% and 3.5%) it was observed that the higher value resulted in a significant increase (p<0.0026) in force relaxation. (Table 2) The analysis of interaction between the parameters time and stretching percentage showed that the effects are independent (p=0.1510). (Table 2)

In the rupture tests it was possible to observe a significant decrease of the tensile strength required for rupture in the group that performed stretching with a higher rate of deformation (3.5%) (p = 0.0123). (Figure 1)

<table>
<thead>
<tr>
<th>Groups (% stretching/time)</th>
<th>2.5/15s</th>
<th>2.5/30s</th>
<th>2.5/45s</th>
<th>3.5/15s</th>
<th>3.5/30s</th>
<th>3.5/45s</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA (mm²)</td>
<td>27.62±7.27</td>
<td>24.67±7.98</td>
<td>24.42±14.13</td>
<td>26.12±6.17</td>
<td>22.46±8.76</td>
<td>23.6±14.94</td>
<td>27.0±8.18</td>
</tr>
<tr>
<td>L0 (mm)</td>
<td>14.51±4.3</td>
<td>15.4±3.89</td>
<td>16.39±5.88</td>
<td>14.78±3.43</td>
<td>11.89±2.65</td>
<td>14.55±3.75</td>
<td>14.3±5.24</td>
</tr>
</tbody>
</table>

CSA: Cross-sectional area; L0: Initial length.

<table>
<thead>
<tr>
<th>% stretching</th>
<th>15 s</th>
<th>30 s</th>
<th>45 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>31.57±6.14 Ab</td>
<td>38.86±5.57 Aa</td>
<td>38.60±4.84 Aa</td>
</tr>
<tr>
<td>3.5</td>
<td>34.53±4.09 Bb</td>
<td>43.27±9.21 Ba</td>
<td>48.05±5.43 Ba</td>
</tr>
</tbody>
</table>

N.B.: Measurements with the same letter do not differ at the probability of 5%, with rank transform ANOVA. Lower case letters compare the means on the horizontal axis, while upper case letters compare the means on the vertical axis.

The statistical analysis did not identify any influence of the stretching times (15, 30 and 45 seconds) and of the percentages of stretching (2.5% and 3.5%) in relation to the mean values of tensile strength, relative deformation, and rupture energy by cross-sectional area (p>0.05) compared with the control group, without previous stretching. (Table 3)

**DISCUSSION**

The most important finding of this study was to identify that the parameters of time and stretching percentage influence the mechanical response of the tendon. Although several experimental studies indicate an influence of time and of the degree of stretching, in tendon response, there is not yet enough evidence to allow us to establish standardization for its performance.

In literature, the lack of uniformity in the execution of stretching is probably a product of the methodological diversity presented in the studies. Within this context, the study of the mechanical behavior of tendons to stretching is essential, as it can help to establish more effective training strategies and possibly has a positive impact on injury prevention. The approach to this topic involves clinical and laboratory studies, where in vitro mechanical tests represent the initial line of research. These studies have concentrated on analyzing the mechanical response of the tissues (viscoelasticity), in order to establish conducts to be tested, and subsequently, applied in everyday practice. Fung defined that 5% of relative deformation in the tendon represents the permissible upper limit for normal human activities. When analyzing the medial collateral ligament of rats, Provenzano et al. demonstrated that 5.14% of deformation caused damage to the tissue.

In the present study, the tendons were submitted to percentage stretching of 2.5% and 3.5%, as these values do not exceed the physiological limit of the tendon. It is worth emphasizing that non-destructive tests (viscoelastic) are essential, as they can impose mechanical demands on the tendons, similar to those that occur in stretching exercises. In practice, these exercises are based on the pursuit of physiological and functional balance. During the mechanical tests, balance is defined in the stress X time diagram by the force relaxation stabilization plateau. Screen analyzed, under a laser microscope, the phenomenon of force relaxation of isolated Wistar rat tendon fascicles. The author notes that although about 50% of the relaxation occurs even after the 200th second.

In this study, the force relaxation plateau was identified in the three stretching time intervals considered (15s, 30s and 45s). However, the 30-second interval was more effective as it provided greater tissue relaxation than the 15s interval, at a level similar to that observed for the 45s interval (p<0.0001).

The tissue relaxation response is also influenced by the stretching percentage. Swedlik and Lanir and Duewald et al. emphasize that the greater the initial stretching, the greater the tissue relaxation. According to Wren et al., this initial deformation is the main parameter to predict rupture, and to influence the occurrence of injuries. These data corroborate the findings of this study, while stretching of 3.5% presented a decrease in tensile strength (p = 0.0123), compared with the group with 2.5%, and also a higher tissue relaxation (stress relaxation) rate (p=0.0026). Other studies demonstrated the existence of an increase in tendon compliance after stretching, suggesting a probable tendineal ability to resist tensile stress during activities. On the other hand, in this study, previous stretching did not influence the tendineal response in the rupture test (injury event). This fact is consistent with the lack of consensus observed in the literature, on the effectiveness of stretching in injury prevention. It is also worth emphasizing that although in vitro studies represent the initial line of research in the study of the mechanical response of tendons to stretching, the results obtained should be ratified by clinical trials.

**CONCLUSION**

The data obtained in this study allowed to conclude that the 30-second stretching interval was most effective, as it presented tissue relaxation superior to the 15-second interval, and similar to that observed for the 45-second interval, a fact to be considered when establishing of new clinical protocols for stretching.
REFERENCES


