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Effect of immobilization in a lengthened position on mechanical properties of the Achilles tendon in growing rats

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The purpose of the study was to examine the effect of long-lasting immobilization in a lengthened position on mechanical properties of immature tendon in a rat model. One hindlimb of growing rat was immobilized for two weeks with the Achilles tendon in extension. Tensile failure test was performed *post-mortem* at a rate of 10 mm/min without any preconditioning. Immobilization caused reduction of the elastic range of deformation both considering force and extension. The stiffness of tendons was decreased, especially at small strains in an inelastic "toe" region. However, the plastic range was broader and the maximum load similar compared to these of normal tendons.

It was revealed that immobilization, even under constant tension, changes significantly mechanical properties of tendons in the range of small strains encountered during physiological loading. The changes may alter the behaviour of a joint after restoration of a normal activity.

Key words: immobilization, the Achilles tendon, mechanical properties

1. Introduction

Mechanical properties of tendons are determined based on their microstructural parameters including collagen fiber content, fiber orientations, and cross-link density. Tensile strains that tendons are normally subjected to control the collagen production and alignment of the collagen fibers in directions of principle tensile strain. Tendon as each living tissue adapts its structure and mechanical properties to suit conditions different than normally encountered. Adaptation to the level of loads in immobilized and unloaded tendons and ligaments changes their biochemistry, composition and morphology [1]–[5]. Removal of loadings leads to disruption of collagen fiber alignment and cross-linking and to degradation of the extra-cellular matrix resulting in significant modifications of mechanical characteristic of the tendon, in particular, a decrease of strength and stiffness [6]–[9]. The changes are responsible for an increased risk of tendon injuries and a decrease of efficiency of load bearing in muscosceletal system. Hence, one of consequences of tendon dysfunction is deterioration of bone mechanical properties, for example a slow and incomplete recovery of bone after osteoporosis induced by temporary immobilization [10]–[12].

Investigations of immobilized muscles and ligaments revealed that the kind and range of the adaptational changes depend to a great extent on immobilization procedure and in particular on position of unloading, i.e. the tissue is either lengthened or shortened during immobilization [3], [13], [14]. Immobilization in a shortened position was followed by a more marked atrophy and a greater decrease in tensile properties than immobilization in a lengthened position.

The purpose of this study was to examine the effects of long-lasting immobilization in a lengthened position on mechanical properties of immature Achilles tendon in a rat model.

2. Material and methods

Growing male Wistar rats were used in the study. 11 week-old animals with mean body weight of 200 (23) g were randomly divided into 2 groups: experimental group (ten rats) and control group (eight rats). Right hindlimbs of the experimental animals were immobilized against the abdomen using bandages and padded tape. The knee was at flexion and the ankle was at about 30-degree dorsiflexion, so that the Achilles tendon was in a lengthened position. The fixation was checked daily and the right hindlimb was exercised for few seconds with passive range of motion to limit the joint contractures. After two weeks, the rats were killed by cardiac puncture in ether anesthesia. Both the Achilles tendons from the experimental group (R, L) and the right Achilles tendon from the control (C) group were excised by cutting the proximal part beyond the muscle–tendon junction and the distal part beyond the calcaneum. Care was taken not to cut any part of the tendon during dissection. The experiment was approved by the local committee of ethics for animal experiments.

The mechanical tests were performed using a testing machine (Lloyd, type LRX)*. The clamping system was composed of metal blocks with serrated grips with smooth-surface teeth. The tendon–calcaneus complex was mounted vertically with calcaneus at the upper clamp and the muscle tendon junction at the lower clamp. The distance between the two attachment grips at a load of 0.1 N was considered as the reference length. The diameter of the middle part of the tendon was measured with calipers. Each tendon was stretched to failure at a rate of 10 mm/min without any preconditioning and the load–deflection curve was recorded. The following parameters were analyzed: the highest registered force ($load_{max}$), the force at proportional limit ($load_{H}$), strain at maximum load ($strain_{max}$), strain at proportional limit ($strain_{H}$), energy absorbed to

* All mechanical tests were performed in the Institute of Agrophysics, Polish Academy of Sciences, Lublin.

maximum load ($energy_{max}$), energy absorbed to the proportional limit ($energy_{H}$), and stiffness of the tendon ($sitffness_{max}$, $sitffness_{average}$). Maximum stiffness was calculated as the slope of the linear portion of the load–extension plot after the characteristic nonlinear "toe" response and before the first sign of deflection from proportionality. Given the low elongation rate resulting in a large toe region, "the average stiffness" was also measured for 0.2 mm increments of extension from the start to the limit of proportionality. Strain values were expressed by considering the elongation at a given point and the reference length. During measurements tendons were kept wet in saline solution.

One-way ANOVA was used to analyze the differences in mean values of parameters between three groups of tendons: immobilized ($Exp\text{-}_{Right}$) and loaded ($Exp\text{-}_{Left}$) from the experimental group and the right tendon from the control group (Control). Right-to-left differences in the experimental group were analyzed using paired *t*-test.

3. Results

In figure 1, average values of mechanical parameters for three groups of tendons are presented. Results of statistical analysis of differences between groups are given in the table. Mean values of loads in groups did not differ significantly (ANOVA), though the load at proportional limit in the right (immobilized) tendons was significantly lower than that in the contralateral tendons (in terms of paired *t*-test). Strain at the maximum load in the right tendons of experimental rats was significantly higher

Table. Significance of the differences between groups in terms of *p*-values.
ANOVA and post-hoc test for three groups: experimental right (R – tendon immobilized for two weeks), experimental left (L – tendon loaded during the experiment), and control
(C – right tendon from the control group). Paired *t*-test for experimental right versus experimental left

Parameter	ANOVA	Post-hoc	Right vs. Left Paired *t*-test
Load $_{max}$	NS	–	NS
Load $_{H}$	NS	–	0.006
Strain $_{max}$	0.002	R-L: 0.003 R-C: 0.003 L-C: NS	0.05
Strain $_{H}$	0.004	R-L: NS R-C: 0.007 L-C: 0.005	NS
Stiffness $_{max}$	NS	–	NS
Stiffness $_{average}$	0.002	R-L: 0.05 R-C: 0.010 L-C: NS	0.005
Energy $_{max}$	NS	–	NS

Energy $_H$	0.002	R-L: NS R-C: 0.001 L-C: 0.006	0.006

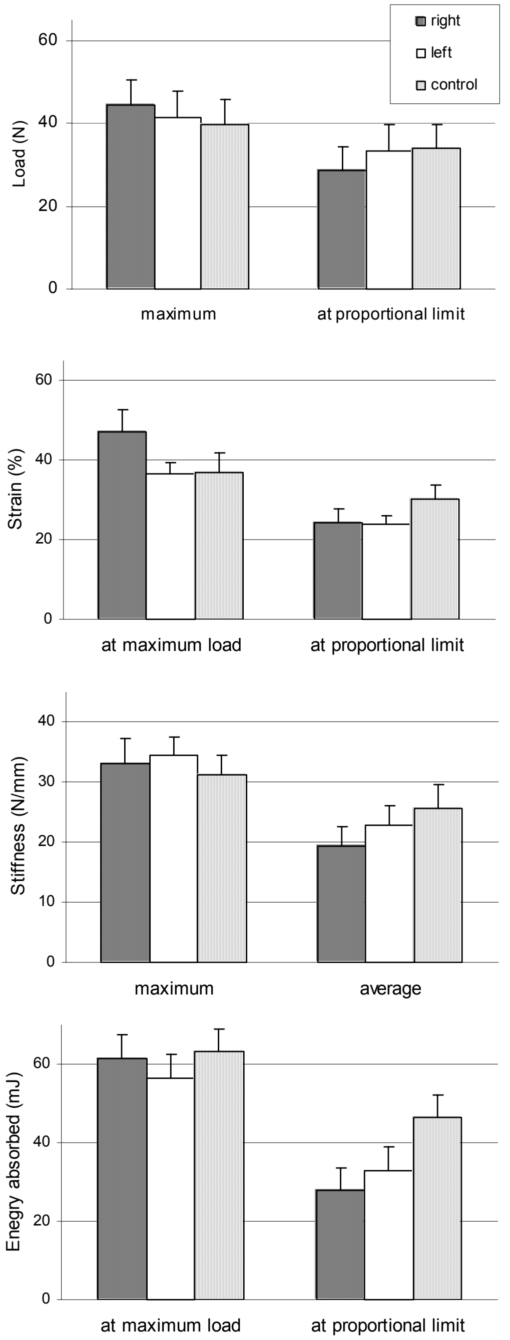

Fig. 1. Average values of mechanical parameters in three groups of tendons: experimental right – tendon immobilized for two weeks, experimental left – tendon loaded during the experiment, and control – right tendon from the control group

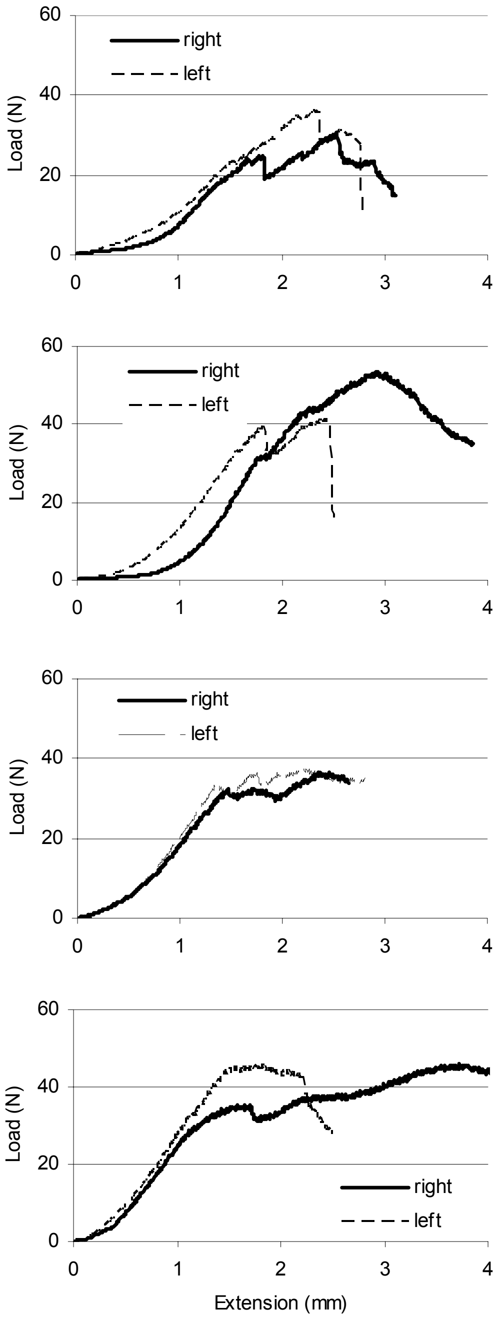

Fig. 2. Load–extension curves for pairs of the Achilles tendons of four experimental rats:
experimental right – tendon immobilized for two weeks,
experimental left – tendon loaded during the experiment

than that in the left and than that in the controls. Strains at proportional limit in both experimental groups of tendons were similar and significantly lower than these in the controls. Stiffness in the proportional range did not differ between groups. However, the average stiffness including the nonlinear "toe" region was significantly lower in immobilized tendons compared to the control and contralateral, loaded tendons.

The post-yield, plastic range of the load–extension curve in immobilized (right) tendons was usually broader than that in the left tendons from the same rat. Figure 2 illustrates load–extension curves of both Achilles tendons from four experimental rats. The curves differing in the shapes of post-elastic region were chosen for presentation.

After 14 days of immobilization in a lengthened position no significant change in tendon length as well as no difference in the length between immobilized and loaded tendons were observed, so the differences in extension between immobilized and loaded tendons corresponded to the differences in strain. There was no significant change in tendons diameter either, though the estimation of diameter was rather rough and for that reason the stress was not calculated.

4. Discussion

Mechanical properties of tendons are determined by their structural parameters [15]. The changes in elasticity of tendon are largely due to the changes in the amount and cross-linking of collagen fibrils in tendon fascicles which run in parallel to form a tough and rather inelastic structure. The crimps of fibers in fascicles are responsible for a low modulus of the "toe" region near the origin of load–deflection curve. Stresses between fibrils are transferred through the extracellular matrix of very low stiffness.

As stated previously immobilization of tendons and ligaments increases the collagen turnover [1], [4]. Accelerated collagen turnover in the immobilized tissue results in less mature collagen fibers which could be more pliable and less stiffness under tensile loads. Moreover, because of the absence of a normal control imposed by physical forces on the orientation of matrix, the newly synthesized collagen fibers in the immobilized tendon are laid down in a haphazard manner causing the longer "toe" region in such tendons. However, immobilization-induced changes in collagen turnover in a tendon being under constant, resting tone during immobilization were small in comparison with the changes in fully relaxed ligaments [2].

Previous studies revealed that in tendons suspended or immobilized in a shortened position, without any tension during the period of immobilization, a significant decrease in failure load, strength and stiffness occurred. Especially, the "toe" region was influenced [6], [8], [9]. However, in tibialis anterior tendon subjected to continuous passive motion during immobilization, there was no difference in linear loads between immobilized and control tendons [7].

The results of the present study revealed that immobilization of the Achilles tendon in a lengthened position reduces the elastic range of deformation both in the case of force and extension. The stiffness of tendons was significantly decreased at small strains in the inelastic "toe" region. Consequently, the energy absorbed to the limit of elasticity was significantly lower than the energy in the loaded tendons, though the stiffness in the range of proportional deformations was only slightly influenced. However, the plastic range in immobilized tendon was broader, sometimes with higher maximum force than in normally loaded tendon. Pattern of mechanical changes established in this study could indicate that immobilization of a lengthened tendon did not influence considerably the collagen structure in fibers but rather organization of fibers within the crimps. Moreover, both the lowered proportional limit of loads and larger plastic range of deformation could reveal that relative amount of fibers to extracellular matrix was disturbed causing a change in the transfer of stresses along the tendon.

Results of the present study indicate that immobilization, even under permanent tension, changes significantly mechanical properties of tendons in the range of small strains encountered during physiological loading. The changes may lead to an elongation of previously immobilized tendons and consequently alter the behaviour of a joint after restoration of loadings even in the physiological range of normal activity.

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