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Changes in stiffness induced by hindlimb suspension in rat Achilles tendon

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Abstract The aim of this study was to measure the effects of hindlimb suspension on mechanical properties of the rat Achilles tendon. Adult male Wistar rats were randomly assigned to groups to be either suspended, or a control. After 21 days, Achilles tendons were removed for mechanical analysis. Classical tests of tensile performance were made, and mechanical parameters were derived from a stress-strain relationship. The tendons of animals that had been suspended presented values for maximal stress and tangent modulus which were 37.5% ($P < 0.01$) and 41% ($P < 0.01$), respectively, lower than the tendons of the control rats. In a similar way, the energy absorption capacity had largely decreased in animals that had been suspended. However, the maximal strain was similar in the two groups. These results showed that hindlimb suspension in rats has an important detrimental effect on mechanical properties of the Achilles tendon. Differences in tendon stiffness obtained here, along with those found by other investigators, encourage the hypothesis that homeostatic responses of soft tissues are due to changes in limb loadings. This study may be useful in providing a better understanding of the adaptation of human skeletal muscle when exposed to microgravity.

Key words Achilles tendon · Hindlimb suspension · Mechanical properties · Hypoactivity · Tendon stiffness

Introduction

Muscle-tendon complexes are responsible for joint movements and have properties which depend on the mechanics of both active and passive structures of the complex. According to the classical model of Hill (1938), the skeletal muscle-tendon complex consists of contractile, series elastic (SEC) and parallel elastic components. It has now been demonstrated that the SEC includes both active (cross-bridges) and passive structures (Shorten 1987). Because the major part of SEC is located in the tendinous tissues, a knowledge of the mechanics of both muscle and tendons has to be considered. Moreover, it has been shown that SEC adapts its properties according to the functional demand. With the lack of gravity during spaceflight, functional demand greatly decreases in postural muscles, and considerable functional modifications may occur. Such changes are a risk for those in space because they limit physical activity and work and are followed by difficulties in readaptation to the terrestrial environment.

The hindlimb suspension method developed by Morey (1979) has proved to be a useful animal model for the simulation of the decrease in mechanical loading (hypodynamia) and motor activity (hypokinesia) in the muscle system. As with spaceflight, hindlimb suspension has been found to produce a significant reduction in hindlimb muscle mass, with the greatest changes occurring in antigravitary muscles (Thomason and Booth 1990). With respect to the elastic properties of SEC, it has been established that the suspension method led to a decrease in stiffness of the rat soleus muscle (Canon and Goubel 1995). These changes in elastic properties were attributed to modifications occurring in both the active and passive parts of SEC. Furthermore, changes in the morphological and biochemical characteristics of the Achilles tendon have been observed after suspension: a smaller surface area of collagen fibres (Nakagawa et al. 1989) and a lower concentration of collagen (Vailas et al. 1988) have been described. These could be factors in the mechanical changes in the passive part of SEC. However, to our knowledge, no data are available concerning the effects of hindlimb suspension on the mechanical properties of the tendons. Thus, the aim of the present study was to quantify the effects of a period of

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suspension on the mechanical properties of rat Achilles tendon to demonstrate that the passive part of SEC is also affected by simulated microgravity.

Methods

Animals

The experiments were performed upon 11 male Wistar rats weighing 213 (SEM 8) g and 50 days old. Rats were randomly assigned to either a hindlimb suspended group ($n = 4$) or a control group ($n = 7$). The Achilles tendons were removed form each limb: this led to an analysis of 8 tendons, from the group that had been suspended and 14 tendons from the control group. Each population was housed in a 38 cm (wide) × 38 cm (deep) × 40 cm (high) plexiglass cage in which temperature and light were controlled. The rats were suspended by their tails for 3 weeks according to the procedure of Morey (1979). This method of suspension consisted in elevating the hindlimbs at an angle of about 40° from the floor and preventing their contact with any of the cage walls. However, the attachment by the tail allowed the rat to move freely on its forelimbs. Water and rat food were provided ad libitum. The protocol was approved by the Hygiene, Safety and Ethics Committee of the University.

Tissue preparation

After 21 days, rats in the control and suspended groups were anaesthetized initially with 30 $\text{mg} \cdot \text{kg}^{-1}$ of sodium pentobarbital (IP) with supplemental doses if needed. An incision was made along the posterior surface of the legs and all extraneous soft tissues were removed from the calcaneus-Achilles tendon complex. Care was taken during soft tissue dissection not to cut any part of the tendon. The free tendon was excised by cutting the proximal part beyond the muscle-tendon junction and the distal part beyond the calcaneum. After their Achilles tendon dissection, the animals were sacrificed by giving an overdose of pentobarbital sodium. The calcaneus-Achilles tendon complexes from each animal were stored in plastic tubes and kept at −22°C until tested. On the test day each tendon-bone complex was thawed at room temperature and all surrounding excess tissue was carefully removed. During the mechanical tests performed at room temperature, the tendons were kept moist using physiological solution, but were not immersed to avoid swelling of the tissue (see Bennett et al. 1986).

Mechanical tests

The tendon-bone complex was clamped and mounted vertically on a conventional mechanical test machine (Rhéo TA XT2 2.0). The clamping system was composed of metal blocks (2.5 × 3.5 cm) with the external part having serrated grips with smooth surface teeth. To strengthen the interface between the grips and the preparation, the proximal (muscle-tendon junction) and distal parts (tendon-bone complex) were secured between two pieces of rubber using a touch of gel super glue. During this procedure, care was taken to ensure that the gel did not spread over the tendon itself.

After a period for equilibration, a preload of about 2×10^{-2} N was applied, and the length observed between the two attachment grips was considered as the reference length (l_0). The specimen was preconditioned using ten loading-unloading cycles having an amplitude of 10% l_0 and a extension rate of 0.1 $\text{mm} \cdot \text{s}^{-1}$. After preconditioning, the specimens were stretched to failure at the same rate. Force was measured continuously using a strain-gauge load-cell and recorded together with specimen displacement. The specimen displacement was measured from crosshead movement during the tensile testing. From these data, stress-strain curves were established and the following parameters were calculated: maximal strain, maximal stress, tangent modulus, and maximal strain energy, i.e. the energy absorption capacity. Stress was obtained by dividing tensile force (in Newtons) by the cross-sectional area (in metres squared) and expressed in megapascals (1 MPa = $10^6 \text{ N} \cdot \text{m}^{-2}$).

Tendon cross-sectional area (CSA) was assessed using a saline displacement method (Loren and Lieber 1995). A volume of physiological solution at room temperature was placed in a 10^3-µl graduated cylinder etched at 10-µl increments. The tendon specimen was submerged in the cylinder after gentle blotting with gauze and the final volume was recorded; three volume measurements were made. They were divided by specimen length, and averaged to obtain the mean tendon CSA.

Extra deformation due to the rubber interface of the fixing system was calculated by comparing strength-deformation curves obtained with:

1. A wire and rubber-covered grips
2. A wire directly held within the grips.

A linear relationship was found between strength and deformation in excess in 1 thus allowing a correction of measured deformation at any level of strength.

The strain values corrected for the extra compliances were expressed as percentages dividing the displacements by the clamp-to-clamp distances (reference length). No evidence of substantial slippage of the tendon within the clamps was observed over the range of the displacement applied.

Tangent modulus was calculated as the slope of the linear region of the stress-strain curve and maximal strain energy was measured as the area between the curve and the x-axis.

Reliability of measurement techniques

Every study of the mechanical properties of soft tissues has to overcome the difficulty of obtaining precise measurements of stress and strain. Thus, prior to discussing our results, some experimental features need to be detailed. The small size of rat Achilles tendons makes the measurements of tensile properties difficult. The mechanical tests were performed using a conventional testing machine and the strain values were expressed by considering the clamp-to-clamp distance as a reference length. At the distal end, the angle between the calcaneum and the Achilles tendon was not measured. However, care was taken when aligning the specimen in the test machine to ensure the tendon was vertical. This method assumed that most of the tendon fascicles were equally stressed and stretched by pure tension. However, the strain measurement could have been affected by artefacts arising from the measurement of reference length, and to changes in strain characteristics along the tendon substance. It has been found that strain measurements obtained by using a clamp-to-clamp distance are larger than those obtained by using markers together with a video dimension analyser (Yamamoto et al. 1992).

Another factor deserves special attention: clamping with rubber sheets adds a compliant element, so much of the length increase between clamps was probably due to the shear of the rubber sheets, which may explain the unusually high strain and low stress reported here. In spite of the corrections for the extra compliances, the values for maximal stress and tangent modulus remained rather lower than those in the literature. Thus, the effect of including rubber in the grips was probably not totally compensated for by the correction method. On the other hand, another reason for lower values of tensile strength in this study was the fixing of the proximal end of the tendon with glue. Using glue, all the tendon fibres could not be stuck in the same way. If only the outer part of the tendon was secured, it was probable that an underestimation of the tension strength remained.

Statistical analysis

A Wilcoxon-Mann-Whitney test was applied to assess differences between tendons, at the 1% level of significance.

Results

At sacrifice, the mean body masses of the rats were 323 (SEM 15) g and 393 (SEM 14) g for the animals in the suspended and control groups respectively, indicating a reduction of 17.6% in the rats that had been suspended. The mean CSA of the tendons in the group that had been suspended was 2.55 (SEM 0.26) mm^2, and the mean of the control group was 2.34 (SEM 0.43) mm^2. Thus, no significant differences in CSA of the Achilles tendon between the two groups was observed.

Figure 1 illustrates a typical stress-strain curve of rat Achilles tendon. Each curve presented this classic nonlinear shape. As usual, this relationship could be divided into two regions: an initial region characterized by a very low stiffness, and a linear region where, after a rapid increase, the slope became almost constant. The initial *toe* region, associated with the straightening of the collagen fibres, extended to a strain of about 4% where the linear region of the relationship started. Then, the maximal stress was obtained. An additional increase in deformation resulted in the failure of the tissue associated with a drastic decrease in stress.

Mean stress-strain curves established for Achilles tendons of both populations are given in Fig. 2. It can be seen that the tendons of the control animals had strength characteristics generally higher than those of the suspended animals. In their *toe* regions the two curves approximated closely. It is noteworthy that the slope of the linear region was found to be significantly ($P < 0.01$) lower for the suspended rats as attested by tangent modulus mean values: 180.5 (SEM 14.7) MPa and of 308.8 (SEM 24) MPa (Fig. 3) for the suspended and control groups, respectively.

Thus, in the rat Achilles tendon, suspension resulted in significant reductions in mean maximal stress and tangent modulus of 37.4% and 41.5%, respectively (Fig. 3). However, the mean failure strains for the two groups were similar 19.5 (SEM 1.5)% and 19.3 (SEM 0.89)%, respectively (n.s.).

The area under the stress-strain curve up to the failure of the Achilles tendons showed a significant decrease after 3 weeks of suspension (Fig. 3), indicating that the mean maximal strain energy for the group that had been suspended was about 42% lower ($P < 0.01$) than that for the controls.

Discussion

The purpose of this study was to examine the effects of a period of suspension on the mechanical properties of rat Achilles tendon. All the specimens used had failures in the midsubstance of the tendon during tension testing. The stress-strain curves obtained in this study showed a non-linear shape which has been shown to be typical for collagenous tissues (Woo et al. 1981, 1982; Bennett et al.

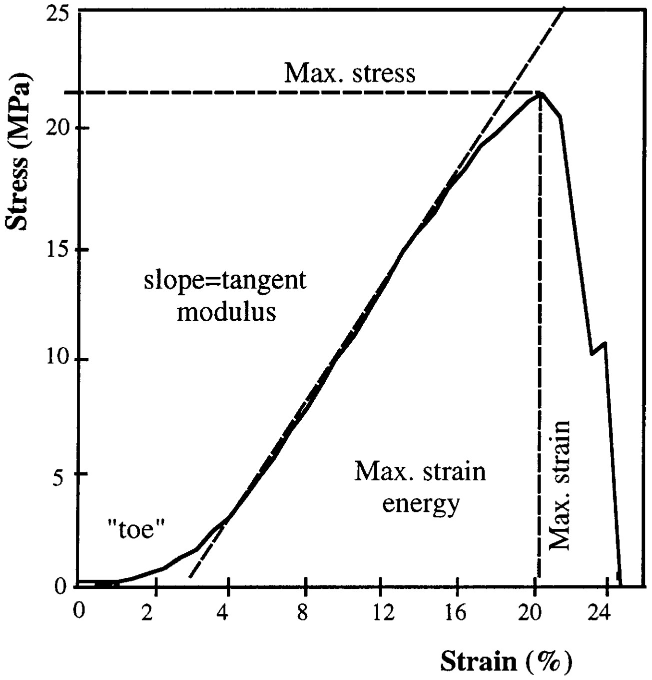

Fig. 1 A typical stress-strain curve of the rat Achilles tendon. The maximal stress (*Max. stress*) and maximal strain (*Max. strain*) were computed from these data. Stiffness (tangent modulus) was measured as the slope in the linear region. The maximal strain energy was measured as the area between the curve up to maximal stress and the *x*-axis

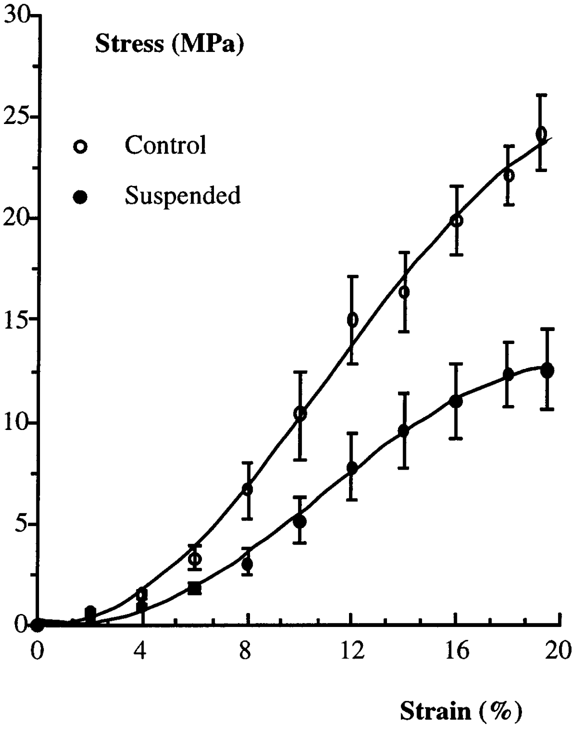

Fig. 2 Comparison of stress-strain curves of the Achilles tendons from the two populations of rats. Results are expressed as mean and SEM

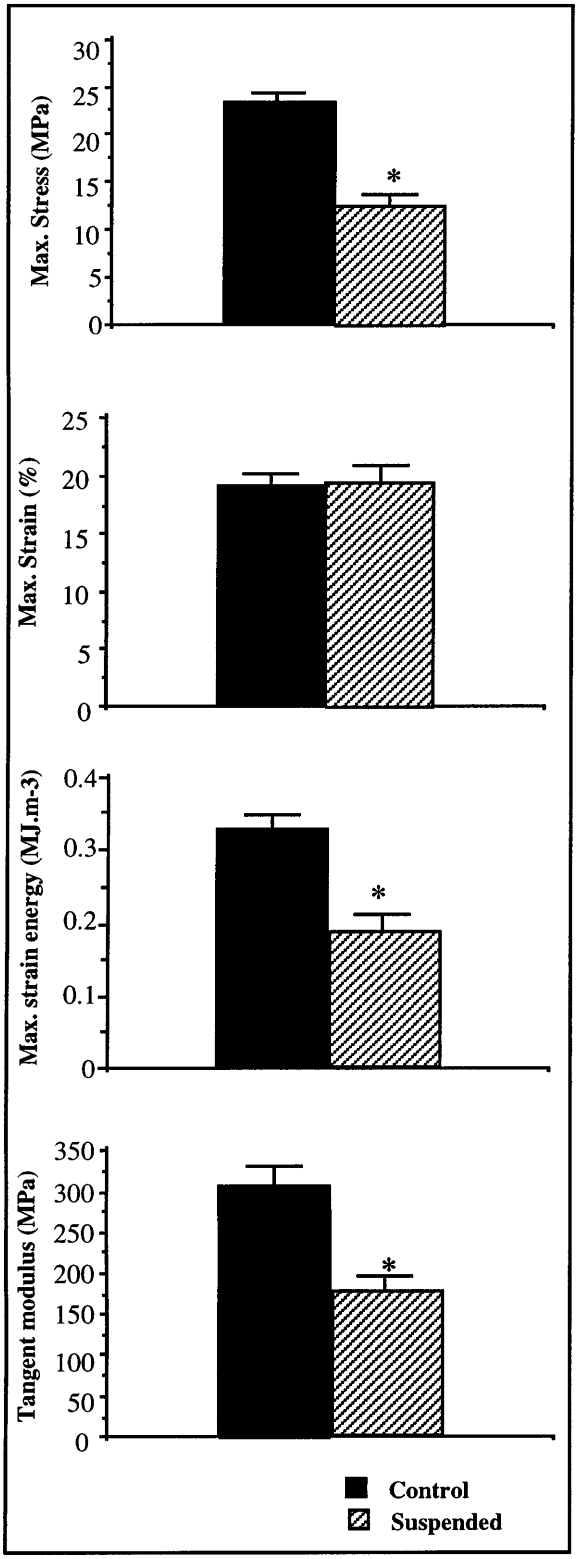

Fig. 3 The effect of 3 weeks of suspension on the mechanical properties of rat Achilles tendon. The values of the mechanical parameters are given as the mean and SEM. *Statistical significance $P < 0.01$

1986). These curves reflected not only the elastic behaviour but also the viscous and failure behaviours of a biological material. The absolute values of the tensile properties in Achilles tendon obtained here were much lower than those found in the literature.

To our knowledge, the tensile properties have not been reported to vary significantly among tendons from animals of different body mass, in a wide range of adult mammals (Pollock and Shadwick 1994). It is known that discrepancies in tensile measurements of biological materials depend on the various techniques used by different investigators. However, the present study aimed at quantifying changes in tendon mechanical characteristics with hypokinesia/hypodynamia with relative measurements of tensile properties of the rat Achilles tendon rather than absolute values. Thus, the same protocol was applied to both control and treated tendons. Possible errors were similar in both cases and the contrasting results seem of interest.

As far as we know, this is the first report documenting the influence of suspension on the mechanical properties of rat Achilles tendon. Our findings indicated that 3 weeks of suspension resulted in major changes in the mechanical properties of the tendon. Suspension led to a significant decrease in maximal stress (35%) and tangent modulus (26.7%). Similarly, with a strain of about 20% (strain until failure), the energy absorption capacity decreased (42%) in the suspended animals in a major way. All these results indicated an important decrease in stiffness as a result of suspension.

In the last few years many investigators have characterized the mechanical properties of soft tissues in response to stress changes. Numerous studies have reported age-dependent changes in mechanical properties in mammalian tendons. An increase in stiffness during growth has been observed in the digital tendons of miniature swine (Woo et al. 1982; Shadwick 1990). Similarly, an increase in stiffness has been observed in the Achilles tendon of rabbits (Nakagawa et al. 1996). What factors can determine these differences? On the one hand, it may be suggested that the differences in mechanical properties can be conditioned by differences in the sizes of collagen fibrils (Parry et al. 1978). On the other hand, the differences can be determined by changes in physiological function associated with biological aging. This assumption agrees with the finding of a greater age-dependent increase of stiffness in flexor than in extensor muscle tendons of miniature pigs (Shadwick 1990).

Studies using models for a decrease in functional demand have demonstrated that a short period of immobilization results in a profound alteration in biochemical and mechanical properties of soft tissues. Immobilization has been associated with a decrease in collagen mass and an increase in collagen degradation in the collateral ligament of the rabbit (Amiel et al. 1983). With regard to mechanical properties, a reduction in tensile strength has been observed in rat Achilles tendon after a period of immobilization (Murrel et al. 1994). However, with classic methods of immobilization the exact amount of stress deprivation is difficult to quantify.

To analyse precisely the effects of a stress deprivation in the tendon, Hayashi et al. (1996) have developed a technique for the determination of total stress deprivation (100% stress deprivation) in patellar tendons of the rabbit. Their studies have shown a rapid and marked decrease in the tensile strength of the rabbit patellar tendon. Thus, it is clear that immobilization has a highly detrimental effect on soft tissues. However, the opposite results have been reported concerning immobilization methods for short lengths. It has been shown that prolonged immobilization for these lengths produces an increase in stiffness and a decrease in the resting length of the whole muscle tendon complex (Herbert and Balnave 1993; Herbert and Crosbie 1997). These studies probably illustrate the basis for the clinical phenomenon of muscle contracture seen following prolonged immobilization of joints.

It is believed that differences in the mechanical behaviour of tendons may be attributed to their respective biochemical components. For instance, changes in the mechanical properties of tendons and other collagenous tissues that occur with aging and stress changes have seemed to be correlated with morphological and biochemical changes (Vogel 1978; Viidik 1982). Previous studies performed by other authors have shown that a period of suspension produces a decrease in the surface of collagen fibres and reduction in collagen concentration in the rat Achilles tendon (Vailas et al. 1988; Nakagawa et al. 1989). These findings have confirmed that ground reaction forces are an important factor in the maintenance of tendon homeostasis. They have suggested that such changes would affect the biomechanical properties of the tendon. Our study confirmed this hypothesis.

In this study, we found no difference in the average CSA of the Achilles tendons between the control animals and those that were suspended. It has been shown that changes in CSA cannot be attributed solely to changes in collagen component (Riemersma and Schamhardt 1985; Loren and Lieber 1995). Identical CSA obtained in out study probably indicates that 3 weeks of suspension induced remodelling of collagen fibres, but could not produce changes in other components of the extracellular matrix of tendon.

This study may also be useful in providing a better understanding of the adaptation of muscle SEC induced by a change in mechanical stress. In the rat soleus muscle, an increase in stiffness has been observed after an endurance training programme (Goubel and Marini 1987) and a decrease in stiffness after a pliometric training programme (Almeida-Silveira et al. 1994, 1996). Such changes can arise in the active fraction (muscle fibre) or in the passive fraction (tendon) of SEC. In the rat soleus muscle, it was shown that stiffness changes with training could be attributed in part to a change in the percentage of fibre types (active fraction of SEC), consistent with the hypothesis that different types of fibres may present different elastic characteristics (Petit et al. 1991). Therefore it can be asserted that slow twitch fibres are stiffer than fast twitch fibres.

On the other hand, with respect to the passive fraction of SEC (tendinous structure) there has been less evidence of changes in elastic properties with hyperactivity. It has been reported that an increase in tendon stiffness requires a very long period (12 months for swine tendon) of exercise training (Woo et al. 1981). The opposite elastic change (i.e. decrease in stiffness) of the tendon with training has never been reported. Changes in SEC characteristics have also been observed after a programme of hypoactivity by suspension. In the rat soleus muscle, a decrease in stiffness has been associated with an increase in the percentage of fast-twitch fibres (Canon and Goubel 1995). Nevertheless, the data should not be interpreted only in terms of changes in the active fraction of SEC because the magnitude of the changes observed was large. Thus, the decrease in SEC stiffness observed after a suspension period is likely to have at least two origins: an increase in fast-twitch fibres and a change in tendon elements. The method used in this study for characterizing the mechanical properties of the isolated rat Achilles tendon clearly showed a decrease in tendon stiffness after a period of hypoactivity by suspension. Thus, our data were consistent with the hypothesis of Canon and Goubel (1995), in which the decrease in stiffness of the rat soleus muscle determined by suspension has been suggested to be at least partially due to changes in the elastic properties of the tendon.

Finally, considering the role of the tendon, the decrease in tendon stiffness reported here could markedly limit force transmission to the periphery. Concerning mechanical adaptation of tendons following exposure to microgravity in humans, there has been no evidence in the literature of such an adaptative response. However, a generalization of the present results in relation to astronaut during spaceflight would indicate that countermeasures should be developed on board to minimize this loss in peripheral force which would counter the well-documented reduction in muscle strength due to atrophy.

References

Almeida-Silveira MI, Pérot C, Pousson M, Goubel F (1994) Effects of stretch-shortening cycle training on mechanical properties and fibre type transition in the rat soleus muscle. Pflügers Arch 427:289–294

Almeida-Silveira MI, Pérot C, Goubel F (1996) Neuromuscular adaptations in rats trained by muscle stretch-shortening. Eur J Appl Physiol 72:261–266

Amiel D, Akeson WH, Harwood FL, Franck CB (1983) Stress deprivation effect on metabolic turnover of the medial collateral ligament collagen: a comparison between nine- and 12-week immobilization. Clin Orthop 172:265–270

Bennett MB, Ker RF, Dimery NJ, Alexander RM (1986) Mechanical properties of various mammalian tendons. J Zool Lond 209:537–548

Canon F, Goubel F (1995) Changes in stiffness induced by hindlimb suspension in rat soleus muscle. Pflügers Arch 429:332–337

Goubel F, Marini JF (1987) Fibre type transition and stiffness modification of soleus muscle of trained rats. Pflügers Arch 410:321–325

Hayashi K, Yamamoto N, Yasuda K (1996) Response of knee joint tendons and ligaments to mechanical stress. In: Hayshi K, Kamiya A, Ono K (eds) Biomechanics – functional adaptation and remodeling. Springer Berlin Heidelberg Tokyo New York, pp 185–211

Herbert RD, Balnave RJ (1993) Effect of position of immobilisation on resting length, resting stiffness and weight of the soleus muscle of rabbit. J Orthop Res 11:358–366

Herbert RD, Crosbie J (1997) Rest length and compliance of non-immobilised and immobilised rabbit soleus muscle and tendon. Eur J Appl Physiol 76:472–479

Hill AV (1938) The heat of shortening and the dynamic constants of muscle. Proc R Soc Lond [Biol] 126:136–195

Loren GJ, Lieber RL (1995) Tendon biomechanical properties enhance human wrist muscle specialization. J Biomech 28:791–799

Morey ER (1979) Spaceflight and bone turnover: correlation with a new rat model of weightlessness. Bioscience 29:168–172

Murrell GAC, Lilly EG, Goldner RD, Seaber AV, Best TM (1994) Effects of immobilization on Achilles tendon healing in a rat model. J Orthop Res 12:582–591

Nakagawa Y, Totsuka M, Sato T, Hirota K (1989) Effects of disuse on the ultrastructure on the Achilles tendon in rats. Eur J Appl Physiol 59:239–242

Nakagawa Y, Hayashi K, Yamamoto N, Nagashima K (1996) Age-related changes in biochemical properties of the Achilles tendon in rabbits. Eur J Appl Physiol 73:7–10

Parry DAD, Barnes GRG, Craig AS (1978) A comparison of the size distribution of collagen fibrils in connective tissues as a function of age and a possible relation between fibril size and mechanical properties. Proc R Soc Lond [Biol] 203: 305–321

Petit J, Filippi GM, Emonet-Denand F, Hunt CC, Laporte Y (1991) Changes in muscle stiffness produced by motor units of different types in peroneus longus muscle of the cat. J Neurophysiol 63:190–197

Pollock CM, Shadwick RE (1994) Relationship between body mass and biomechanical properties of limb tendons in adult mammals. Am J Physiol 266:R1016–R1021

Riemersma DJ, Schamhardt HC (1985) In vitro mechanical properties of equine tendons in relation to cross-sectional area and collagen content. Res Vet Sci 39:263–270

Shadwick RE (1990) Elastic energy storage in tendons: mechanical differences related to function and age. J Appl Physiol 68:1033–1040

Shorten MR (1987) Muscle elasticity and human performance. Med Sport Sci 25:1–18

Thomason DB, Booth FW (1990) Atrophy of the soleus muscle by hindlimb unweighting. J Appl Physiol 68:1–12

Vailas AC, Duluna DM, Lewis LL, Curwin SL, Roy RR, Alford EK (1988) Adaptation of bone and tendon to prolonged hindlimb suspension in rats. J Appl Physiol 65:373–376

Viidik A (1982) Age-related changes in connective tissue. In: Viidik A (ed) Lectures on gerontology. Academic Press, London, pp 173–211

Vogel HB (1978) Influence of maturation and age on mechanical and biochemical parameters of connective tissue of various organs in the rat. Connect Tissue Res 6:83–94

Woo SLY, Gomez MA, Amiel D, Ritter MA, Gelberman RH, Akeson WH (1981) The effects of exercise on the biomechanical and biochemical properties of swine digital flexor tendons. J Biomech Eng 103:51–56

Woo SLY, Gomez MA, Woo YK, Akeson WH (1982) Mechanical properties of tendons and ligaments. The relationships of immobilization and exercise on tissue remodeling. Biorheology 19:397–408

Yamamoto N, Hayashi K, Kuriyama H, Ohno K, Yasuda K, Kaneda K (1992) Mechanical properties of the rabbit patellar tendon. J Biomech Eng 114:332–337