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TENSILE STRENGTH PROPERTIES OF ACHILLES TENDON SYSTEMS IN TRAINED AND UNTRAINED RABBITS

By

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Tendons consist of parallel-fibred dense connective tissue that is extensible and of great tensile strength, properties investigated by numerous authors since *Wertheim* 1847. Its tensile strength has been estimated at 50 to 100 newton (N) per mm² (cf. *Viidik* 1966). The stress-strain curve commences with a toe part that is convex towards the strain axis (e.g. *Reuterwall* 1921, *Stucke* 1950, *Morgan* 1960) and considered to be caused by stretching the wavy formation of the collagenous bundles seen in a relaxed preparation (*Reutervall* 1921, *Rigby et al.* 1959). The next part of the curve is fairly linear. The failure may occur in this part of the curve or the curve may level off somewhat towards the stress axis before failure occurs (*Viidik et al.* 1965).

The metabolic activity in such tissue is very slow once the latter is mature (*Neuberger & Slack* 1953, *Thompson & Ballou* 1956). In young animals, however, it responds, at least morphologically, to altered functional conditions. *Ingelmark* (1945) trained growing rabbits in a treadmill and found that the cross-sectional areas of the primary bundles in their Achilles tendons increased more than the number of cells. In studies on white mice *Ingelmark* (1948) found that training enlarged the Achilles tendons in young animals but only the corresponding muscles in mature animals. Thickening of a tendon does not necessarily mean improved performance of the whole functional bone-tendon-muscle-tendon-bone system, as a healthy tendon is considered hardly ever to fail. When such a functional system is subjected to increasing stress, failure occurs most often as a tear-off fracture at the insertion of the tendon into the bone (*McMaster* 1933, *Stucke* 1950,

Davidsson 1954, 1956). Other failure sites have also been reported. *McMaster* (1933) described the musculo-tendinous junction and the muscle belly to be other possible sites and *Fink & Wyss* (1942) reported consistent failures in the muscle belly, in the middle or near the musculo-tendinous junction.

Therefore, to achieve maximum information on the mechanical behavior of tendons it is necessary to test (1) the complete system to ascertain the properties of the tendon until failure point of the weakest component of the system and discover which one it is, and (2) the isolated tendon itself to evaluate its properties until failure of the collagen fibres themselves.

This paper reports a series of rabbits trained in a running machine together with control rabbits of the same stock, together with results on tensile strength testing of their complete. Achilles tendon systems, *i.e.* calcaneus, Achilles tendon, gastrocnemius muscle and femur. The results of tensile strength tests of isolated hind limb tendons and of tensile strength and elasticity experiments on anterior cruciate ligament preparations from knee joints of the same series of animals will be the subjects of future papers.

MATERIALS AND METHODS

Fifteen male white rabbits, about three months old and of the same stock trained in a running machine three times daily, every week-day for forty weeks (normally 5 days a week). The speed and running time were adjusted to the maximum capacity of the rabbits. The scheme is shown in Figure 1, where the upper curve (ordinate A) gives the speed in meters per minute and the lower curve (ordinate B) gives the running time on each training occasion. During the total training period they covered a distance of about 100 kilometers. At the same time, thirteen rabbits of the same stock were kept as controls and permitted to move about freely in standard cages (floor 69 × 45 cm) of the same type as those used for the trained animals. The weights of the animals were checked weekly (Figure 2). The control animals tended to gain slightly more in weight than the trained ones, but the difference was not statistically significant.

An earlier group consisting of four similar rabbits trained in the same way, and four control rabbits showed no statistically significant difference in weight gain, compared with the main group. It was therefore included in the material presented in this study after a confirmative analysis of variance of the results of the mechanical testing in both groups.

At the beginning of the training period the rabbits were immature but at the time of the mechanical testing, their hind limbs were mature. The criterion of maturity was cessation of skeletal growth, as indicated by calcified epiphyseal plates. This was checked in roentgenograms of the knee joints. An immature animal is shown in Figure 3 and the same animal, but after having reached maturity, in Figure 4.

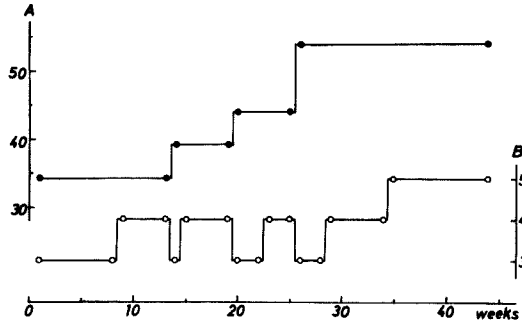


Figure 1. The training scheme. The upper curve (ordinate A) shows the running speed in meters per minute and the lower curve (ordinate B) the time in minutes of each running period (three times a day every week-day).

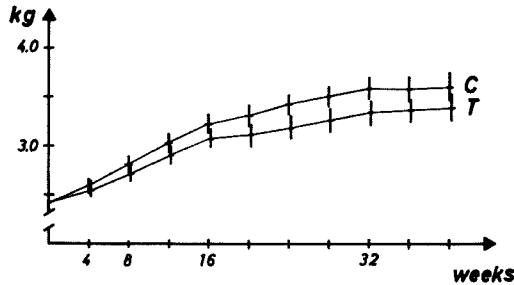


Figure 2. The weight of the trained (T) and control (C) animals during the training weeks. (Mean values \pm standard errors).

Preliminary studies showed that the tensile strength of the muscle tissue varied with the intensity of training before sacrifice and the interval between death and the test. If training had been intense or if a few hours had elapsed between death and testing, the bone-tendon-muscle-tendon-bone system quite often failed in the muscle belly at rather lower loads. To avoid these drawbacks the following method was employed and then true muscle rupture never occurred. The animal was anaesthetised with an intravenous injection of allypropymalum (5 mg per kg body weight), whereafter asphyxia was induced with an injection of tubocurarine (1 mg per kg body weight i.v.). The mechanical tests were performed within less than an hour of death.

The system consisting of calcaneus, Achilles tendon, gastrocnemius muscle and femur was dissected out after the distal tendons of the peroneal and tibialis posterior muscles had been removed from the limb. Care was taken to keep the knee joint intact. The calcaneus was freed from other structures than the Achilles tendon and fitted into a contour-shaped clamp with a rounded outlet for the tendon. A thin layer of polyethylene was placed between the metal of the clamp and the bone to improve the fit and to make the distribution of the holding forces more even. The femur was also fastened into a contour-shaped clamp without sharp edges, where only the anterior and posterior edges of the femoral condyles and a short length

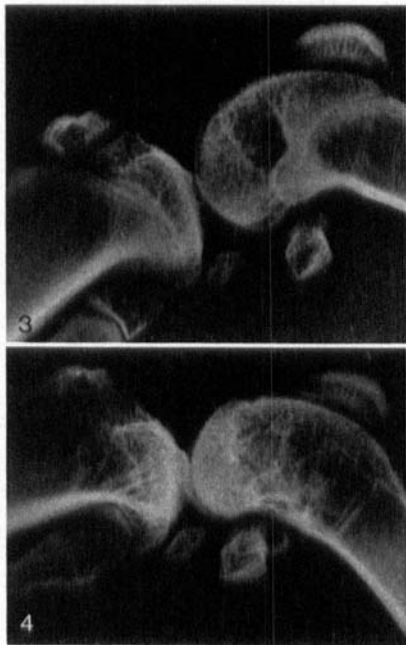


Figure 3. Roentgenogram of the right knee joint of an immature animal at the start of the training period.

Figure 4. Roentgenogram of the same knee joint as in Figure 3, but seven months later, when the epiphyseal plates have calcified.

of the femoral shaft were required for fixation, with the muscle passing out between the two fixation sites. Care was taken to avoid cutting the muscle when dissecting it free from surrounding tissues. The soleus part of the triceps muscle was cut loose from its attachment. The loose connective tissue covering the Achilles tendon was removed to enable a more accurate estimation of the site of the musculo-tendinous junction and to prevent slipping of the registration marker.

The clamp with the calcaneus was fastened to the immobile end of a material testing machine and the clamp with the femur was fastened to the piston that was operated by a hydraulic system regulated by valves and powered by an electric motor. The load was registered continuously by a tensile force pick-up (Philips PR 9226/02, max. force 2000 N). As the elongation of the tendon and not of the whole system was of interest, a marker was fastened at the musculo-tendinous junction.

The marker of stainless steel consisted of a ring with four bars in notches and at right angles to each other, movable towards the center of the ring and fixable with screws. The blunt ends pointing towards the center had rough surfaces and they were pressed against the tendon to hold it firmly without damage. A flexible steel blade with a considerably stiffer U-shaped end was placed against this ring thus causing the blade to bend in proportion to the tendon's elongation (cf. Figure 5). The behavior of the musculo-tendinous junction, the marker and the U-shaped blade were checked with cinematography in the preliminary experiments. No slipping or oblique movements were noticed in analysis of the pictures, both sequentially and individually. The set-up is shown schematically in Figure 5. The force is applied to the femur (A) and transmitted via the gastrocnemius muscle (B) to the tendon (D). The calcaneus (E) is fastened to the immobile end of the apparatus via the

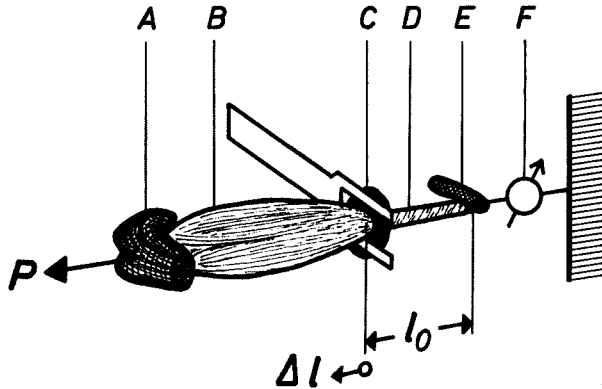


Figure 5. Schematic drawing of the mechanical experiment. Force (P) is applied to the femur (A) and transmitted via the gastrocnemius muscle (B) to the Achilles tendon (D) and resisted by the calcaneus (E) at the immobile end of the set-up, where force is recorded by a pick-up (F). The deformation of the specimen from the immobile end, Δl , i.e. that of the tendon, is recorded with a ring at the musculotendinous junction (C) and a steel blade with a U-shaped end.

force pick-up (F). The elongation of the tendon, Δl , is registered by bending of the steel blade with the U-shaped end caused by the ring (C) at the musculotendinous junction. This bending was registered with strain gauges (Philips PR 9810) cemented on the blade and coupled in a half-bridge. Both the force pick-up and the strain gauges were coupled to direct reading measuring bridges (Philips PT 1200), the strain gauges via a zero adjustment- and switching equipment (Philips PT 1210). The elongation was performed at an almost constant speed, which was the same for all experiments. The signals from the two bridges were recorded continuously by (1) a Siemens Oscillomink jet ink recorder with load and deformation on separate channels, and (2) a Tectronix 502 oscilloscope that was x-y-coupled and the tracing was photographed.

Both load and deformation measuring systems were calibrated each day before and after the series of experiments. The readings were found to be directly proportional to load and deformation, respectively, in the measuring range used. The error of measurement, calculated as the coefficient of variation from multiple measurements of known forces and deformations, respectively, was found to be 0.55 and 0.90 per cent.

Mathematical and Statistical Methods

The oscilloscope photographs were subjected to cursory inspection and the following parameters were analysed (cf. Figure 6):

(1) $\tan \alpha$, the coefficient of inclination of the linear portion of the load-deformation curve distance A to B in Figure 6, corresponding to the elastic stiffness of the tissue,

(2) W_f , the failure energy, measured as the area between the curve and the de-

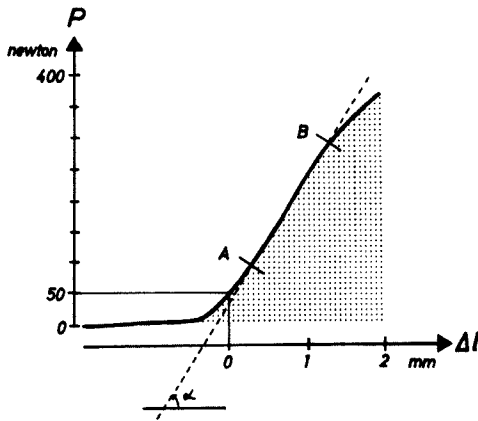


Figure 6. Schematic drawing of an oscilloscope photograph from a tensile strength experiment on an Achilles tendon system. The elongation is on the abscissa and the deformation on the ordinate. For explanation see mathematical methods, p. 5-6.

formation axis from the starting point to the point of maximum load (shaded area in Figure 6).

(3) P_{\max} , the maximum load.

(4) $\Delta l_{P_{\max}}$, the elongation at the maximum load point, measured from the point of elongation where the load was 50 N.

(5) P_{lin} , the point at which the curve ends its linear portion and commences a convexity towards the load axis (B in Figure 6).

The $\tan \alpha$ and W_f values were calculated by a computer. The load and deformation recordings from the Oscillomink were read for every millimeter changes in the load curve and the corresponding deformation values were read to the nearest quarter of a millimeter. These data together with calibration data were processed by the computer. First the mean $\tan \alpha$ value for a whole group was estimated, assuming that the linear parts of the load-deformation curves should have a common starting point. Then individual values were estimated by the method of least squares. The standard error of the mean $\tan \alpha$ for a group was estimated from all individual measuring points on the load-deformation curves. When calculating the failure energy values the load-deformation curve was approximated to be linear between the points of measurement.

RESULTS

The load-deformation curves in both the trained and control groups started with a toe part, concave towards the load axis. Then a fairly linear portion ensued after which, the curve tended to level off from the load axis until maximum load was reached. After this point some of the curves levelled off an additional portion from the load axis before failure, while others failed at once. In both varieties failure could occur in one step or in several in rapid succession.

Analysis of variance was performed for the two groups of animals. No difference was found between the two trained and the two control groups. The results in both series were therefore pooled.

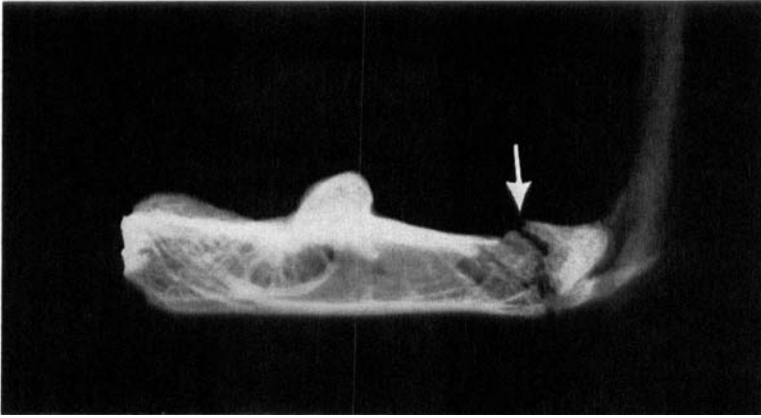


Figure 7. A roentgenogram of a calcaneus from a test specimen after the tensile strength test is completed and a tear-off fracture has occurred (see arrow).

Table 1. Tensile strength parameters on the complete functional system of calcaneus, Achilles tendon, gastrocnemius muscle and femur. All values are from the Achilles tendon except P_{max} , which is for the weakest point in the whole system. Mean values \pm standard errors are given. N = newton is the force unit in the International System of Units and 1 N = 0.102 kiloponds = 7.23 poundals.

Parameter n	Denomination -	Trained 37	Controls 33
$\tan \alpha$	N/mm	176.8 \pm 12.3	156.7 \pm 10.1
W_f	Nmm	577.0 \pm 46.6	552.1 \pm 37.8
P_{max}	N	388.2 \pm 19.8	377.4 \pm 17.5
$\Delta l_{P_{max}}$	mm	2.42 \pm 0.12	2.43 \pm 0.11
P_{lin}	N	295.7 \pm 16.9	285.3 \pm 18.6

The results of computer analysis are given in Table 1. The $\tan \alpha$ tended to increase with training, but the increase was not statistically significant at the 5 per cent level ($2P = 0.204$). The W_f and P_{max} values showed a very slight increase in the mean values. For all parameters the variation within groups was considerable.

Parameter calculation was also performed with the deformation values reduced to units of original length measured as the distance between the calcaneus clamp and the ring (cf. Figure 5). The coefficients of variation of the different parameters were not reduced by this procedure. To enable summation of different parameters their values were, by classification, normalized to a standard variable with the

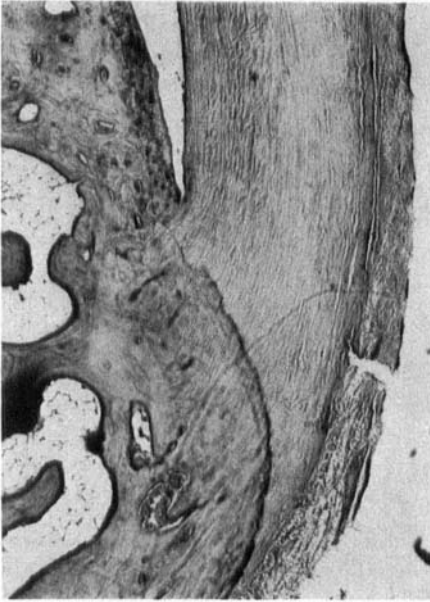


Figure 8: Sagittal section through the insertion of the Achilles tendon into the calcaneus. Note the thickness of the tendon and the cortical bone. (Decalcified section, hematoxylin-eosin stained 32 × magnification).

mean value of zero and the standard deformation of unity, so that four almost equally sized groups were obtained in the control group. This classification was performed on the basis of the various mean values and standard deviations of the parameters for the control animals. The distributions were compared by χ^2 -test. This showed that the trained group differed significantly from the control group. The difference was, however, due to considerable scattering in the trained group and not to overweight of positive values.

The sites of rupture of the test specimens were recorded. In the trained group 34 specimens out of 37 failed as tear-off fractures of the calcaneus. Of the remaining specimens, two failed at the muscle's attachment to the femur and in one the failure started by a tearing off of the lateral gastrocnemius belly's insertion into the calcaneus, whereafter the specimen failed in the muscle. Three of the specimens in the control group failed in the same way, one failed at the muscle's short tendinous attachment to the femur and one as a tear-off fracture of the femur. The remaining 28 of the 33 specimens in the control group failed as calcaneal tear-off fractures. Such a fracture is shown roentgenographically in Figure 7.

DISCUSSION

From a general biological point of view it is of great interest to know how the mechanical properties of collagenous tissue react to increased functional demands. The morphological picture has been evaluated earlier and the tissue has been found to react to these demands when young, but not when adult (*Ingelmark* 1945, 1948). In this study rabbits were trained in a running machine during the middle and later part of their growth period and by the time of mechanical testing the bones of their hind limbs were mature. The trained animals tended to be slightly lighter than the untrained ones. As both groups were fed ad libitum this may have been due to a somewhat smaller body fat mass. No studies were, however, performed to check this possibility.

This study confirms the opinion of earlier investigators that the tendon is not the weakest point in a bone-tendon-muscle-tendon-bone system and shows that this fact is not changed by training the animal. Here it was also found that the insertion of the Achilles tendon into the calcaneus is the weakest point of the system and that the strength of the system is not altered by training. This finding is in agreement with those of earlier investigators who used living, anaesthetised animals, *i.e.* *Stucke* (1951) and *Davidsson* (1954, 1956). On the other hand, *McMaster* (1933) reported more diverse failure sites using animals sacrificed at some unstated previous time. This is in agreement with the present author's experience from preliminary experiments.

Much discussion has been devoted to ruptures of the human Achilles tendon, their mechanism and etiology. Numerous authors believe in a pre-existing degeneration *e.g.* *Arner et al.* (1959), *Schönbauer* (1964), *Viernstein & Galli* (1964) and much interest has been focused on a locus *resistentii minoris* due to vascular deficiency (*Schnorrenberg* 1962, *Minne* 1967). Attempts have been made to correlate the clinical picture with experimental work, but there is, as pointed out by *Frings* (1961), a profound difference in the results, in that in the clinical cases the tendon fails about 5 centimeters proximal to the calcaneus, while in experimental work the failure occurs as a tear-off fracture. In the experimental animal some rather violent attempts have also been made to simulate the histological picture of clinical degeneration (*Borsay et al.* 1951). When making comparisons we must bear in mind the obvious anatomical differences between human beings and animals: the Achilles tendon of the rabbit is inserted into a rather small part of the dorso-plantar section of the calcaneus (Figure 8) and, furthermore,

the shape of the calcaneus of the rabbit (Figure 7) is proportionally longer and narrower than that of a human and it has a rather thin layer of cortical bone, into which the tendon fibres are inserted.

Information on the mechanical properties of the tendon itself in the range above the system's failure point cannot be evaluated this way. It is therefore necessary to perform mechanical tests on isolated tendons to ascertain whether the mechanical properties of the tendon itself have been altered by training or not. Such tests on the distal tendons of the tibialis posterior, peroneus longus, tertius and quartus muscles from the same animals will be the subject of future papers.

SUMMARY

19 rabbits were trained in a running machine, while 17 rabbits of the same stock served as controls. At the end of the training period of 40 weeks the calcaneus-Achilles tendon-gastrocnemius muscle-femur systems were tested regarding their tensile strength. The shapes of the load-deformation curves were inspected and the following parameters calculated for the tendon until failure at the system's weakest point: (1) the slope of the linear portion of the load-deformation curve, (2) the failure energy, (3) the maximum load, (4) the elongation at the maximum load, (5) the point of load where the linear part of the load-deformation curve ends. The failure site was also recorded.

It was concluded that the slope tended to become steeper with training but that no other parameters were altered. The failure site proved to be the insertion of the Achilles tendon into the calcaneus. It was concluded that studies on the properties of isolated tendons were needed to ascertain whether tendons change their mechanical properties in response to training or not.

RESUME

19 lapins ont été entraînés dans une machine à courir, alors que 17 lapins de la même souche ont été gardés comme sujets de contrôle. A la fin de la période d'entraînement qui fut de 40 semaines le système du calcaneum - tendon d'Acille - muscle gastrocnemius - femur a été soumise à des tests de force de tension. Les courbes de la déformation de la forme à la charge ont été étudiées et les paramètres suivants ont été calculés pour le tendon jusqu'à rupture au point le plus faible du système: 1) l'inclinaison de la portion linéaire de la courbe de déformation à la charge, 2) le manque d'énergie, 3) la charge maximum, 4)

l'élongation à la charge maximum, 5) le point de charge où la partie linéaire de la courbe de déformation à la charge se termine. La localisation de la rupture a également été notée.

Il est conclu que l'inclinaison devient plus raide à l'entraînement mais qu'aucun paramètre n'est autrement modifié. L'endroit de la rupture s'est montré être l'insertion du tendon d'Achille sur le calcaneum. Il est conclu aussi que des études sur les propriétés de tendons isolés sont nécessaires pour établir si les propriétés mécaniques des tendons se modifient en relation avec l'entraînement ou non.

ZUSAMMENFASSUNG

19 Kaninchen wurden in einer Laufmaschine trainiert, während 17 Kaninchen desselben Stammes als Kontrollen behalten wurden. Am Ende der Trainings-Zeit von 40 Wochen wurde das Calcaneus-Achillessehne-Gastrocnemius Muskel-Femursystem einer Probe auf Dehnbarkeitsstärke unterworfen. Die Form der Belastungsdeformierungskurven wurden besichtigt und die folgenden Parameter für die Sehne bis zum Versagen am schwachsten Punkt des Systemes errechnet: (1) Die Neigung des linearen Teiles der Belastungsdeformierungskurve, (2) die Versagungsenergie, (3) die maximale Belastung (4) die Verlängerung der maximalen Belastung, (5) der Punkt der Belastung wo der lineare Teil der Belastungsdeformierungskurve endet. Die Stelle des Versagens wurde ebenfalls aufgezeichnet.

Die Schlussfolgerung war, dass der Abfall der Kurve beim Trainieren die Neigung hatte steiler zu sein, dass aber kein anderer Parameter verändert war. Es erwies sich, dass der Sitz des Versagens die Insertion der Achillessehne in den Calcaneus war. Es wurde geschlossen, dass Untersuchungen der Eigenschaften isolierter Sehnen notwendig waren, um festzustellen ob Sehnen ihre mechanischen Eigenschaften als eine Trainingsreaktion verändern oder nicht.

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