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Mechanical and chemical factors in tendon healing

Effects of indomethacin and surgery in the rabbit

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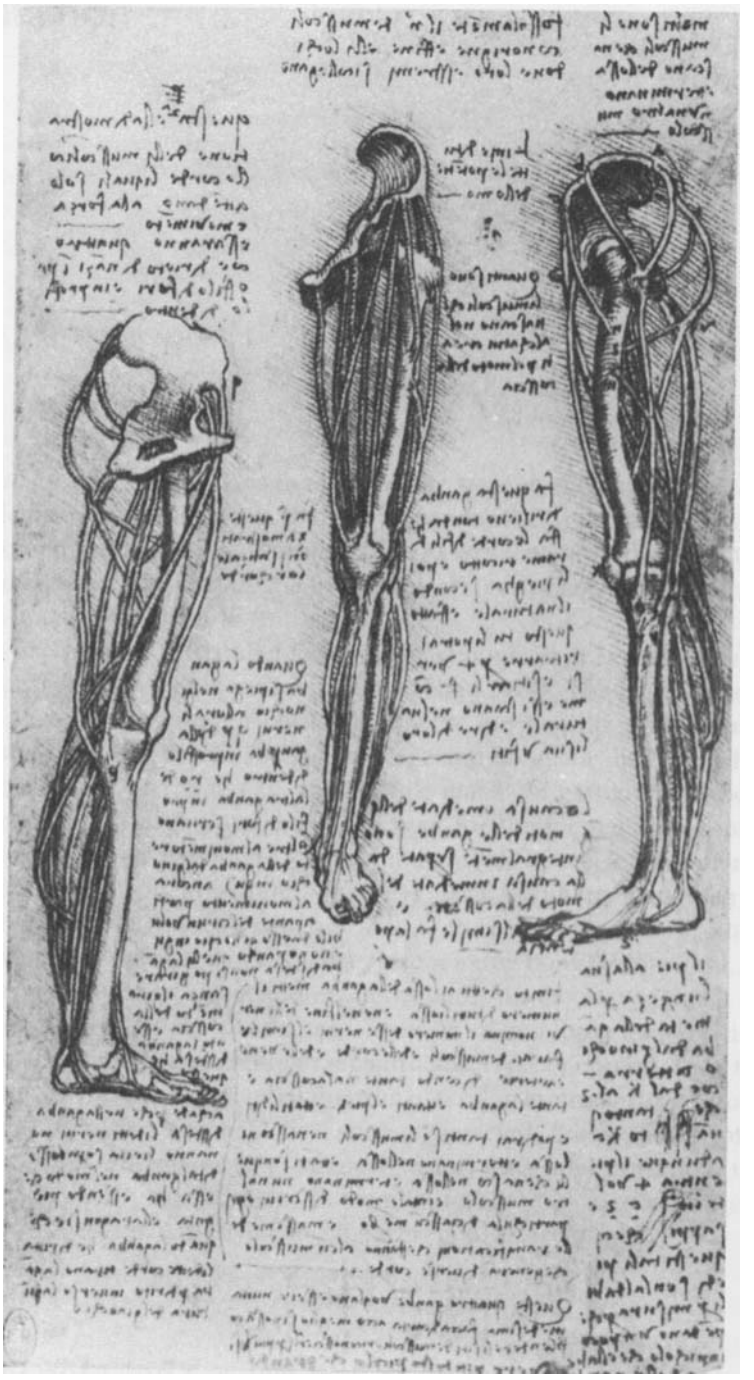


LEFT LEG IN THREE POSITIONS TO SHOW BONES AND TENDONS
(Leonardo Da Vinci 1517)

Introduction

Tendons are an important link in the locomotive system, the main physiological function of which is to transmit forces that elicit characteristic mechanical responses. The tendon also eliminates the need for any unnecessary length of muscle between origin and insertion and enables the muscle belly to be at a convenient distance from the joint over which it acts.

Tendons and ligaments (parallel-fibered collagenous tissue) consist mainly of collagen (99% of the dry weight in rat tail tendons; Dale 1974). The mechanical stability of collagen is thus the most important factor for the mechanical strength of the tissue. Other dense connective tissues such as cartilage and bone may be biomechanically regarded as composite materials, i.e. the organic matrix of cartilage and bone consists mainly of collagen. The importance of collagen for the biomechanical properties of bone has recently been discussed (Jonsson et al. (1985). Collagen in other connective tissues such as vessels, heart, ureters, kidneys, skin, and hepar, has also primarily a mechanical supportive function which is unique to these molecules. Collagen constitutes approximately one-third of the total protein in the body (White et al. 1964).

Injuries and disturbances in tendons are common. In the management of these injuries, understanding of the mechanical function of tendons, their homeostatic responses, their physiology and ability to repair themselves are important. In the past and also currently the treatment and rehabilitation of tendon injuries is based mainly on empirical ground. By using biomechanical studies, however, the possibility of evaluating effects of different treatments (e.g. surgical repair, drug administration, immobilization, etc.) is achieved. Furthermore by combining these biomechanical studies with biochemical studies, the underlying mechanisms may be revealed.

Historical notes on tendon repair

Hippocrates called the Achilles tendon "neura megula" and concluded that if the tendon was injured or cut it would cause acute fever, convulsions, unconsciousness and finally death (Couch 1936). The ancient and medieval surgeons were handicapped not

only by lack of knowledge of wound infection, but also by the fact that it was not until the middle of the eighteenth century that a clear distinction was made between tendon and nerve. However, Galen in the second century had already demonstrated the differential anatomy of tendons, nerves and ligaments. The catastrophic possibilities of this lack of knowledge were enough to discredit most of the early operative repair of tendon wounds. Reports of tendon repair are meager although there must have been many lacerating injuries of the hands and feet with which the older surgeons had to deal. Although Galen demonstrated the differential anatomy of tendons, nerves and ligaments, he warned against the poor results of tendon suture. This warning arose from the fact that he considered tendons a mixture of nerve and ligament and that suture within nerve substance was likely to be followed by severe pain, twitching and convulsions. The influence of Galen persisted for nearly sixteen centuries.

However, in the tenth century the Arabian physician Avicenna strongly advocated tendon suture, and in the twelfth century the Italian surgeon William of Falicet concluded that nature was unable to unite divided tendons, and that surgeons could bring the tendon ends together better than nature. In the fourtheenth century the French surgeons Guy de Chauliac, under the influence of Italian surgeons, attempted with a modicum of success to defend the closure of tendon wounds.

Ambrosius Paré in the sixteenth century reported instances of successful tendon suture (Paré 1641), but there was no general acceptance of surgical procedures on tendons. In 1641 he reported the first case of subcutaneous Achilles tendon rupture. He treated these ruptures with a bandage immersed in wine and spices and concluded that complete healing was not to be expected.

In the seventeenth century Lanzweerde in experimental work on dogs reported successful Achilles tendon sutures after surgical division. Petit (1722, 1728) described three cases of subcutaneous Achilles tendon ruptures and advocated treatment using a bandage to keep the foot in plantar flexion. This treatment was widely accepted and used. In the middle of the eighteenth century Haller demonstrated the specificity of tendon tissue and gradually operative procedures on tendons gradually became generally accepted. Hunter (1767) studied the natural process of tendon repair experimentally in dogs and concluded that tendon heals by the formation of callus produced in much the same manner as a bone cullus. In a case from 1776, Hunter reported an excellent result of healing a subcutaneous Achilles tendon rupture in himself using a plantar flexion bandage. Tendon repair was also studied by Billroth, who asserted that the exudated between the tendon ends hindered growth of the scar, thus delaying union, and that absorption must first occur. With the advances of antiseptics and anesthesia at the end of the nineteenth century, surgical treatment of tendon ruptures first became a viable alternative. Polaillon (1888) first described a case of surgical treatment of a subcutaneous Achilles tendon rupture. Numerous studies of tendon repair have been performed since Billroth to the classical study of Mason and Shearon (1932) and different and conflicting values of the exudative, the tendon ends, tendon sheath and the surrounding connective tissue and blood have been reported.

The treatment of total subcutanous Achilles tendon ruptures was still an open question when Quénu and Stoianovitch (1929) reviewed from the literature 39 conservatively treated and 29 surgically treated subcutaneous Achilles tendon ruptures, but they

concluded that "les malades attempts d'une rupture du tendon d'Achille doivent etre opéres" (the sufferer of an Achilles tendon rupture should be surgically treated). Until 1970 serveral works on the treatment of subcutaneous Achilles tendon ruptures had been presented, but these mostly have discussed etiology, partial ruptures and different surgical techniques. However, during the last few decades, conservative treatment has once again attracted serious consideration. Several authors (Gillies and Chalmers 1970, Lea and Smith 1972, Nistor 1981) have reported good results with non-surgical treatment (plaster cast only).

The tendon

Structure of the tendon

A tendon is composed of densely packed bundles of collagen fibers. Each fiber is in turn composed of thinner fibrils (Fig. 2). On the surface of the tendon there is a white glistening synovia-like membrane, the epitenon. The fibers are bound together by a thin layer of connective tissue, the endotenon, which also contains blood vessels and lymphatics (Edwards 1946). In the space between the fibrils there are rows of cells, fibroblasts, which are flattened and comparatively few in number. The tendon is surrounded by a loose areolar tissue, the paratenon, which is specialized to form a tendon sheath where the tendon bends. Within this sheath the mesotenon carries important blood vessels to the tendon (Nisbet 1960).

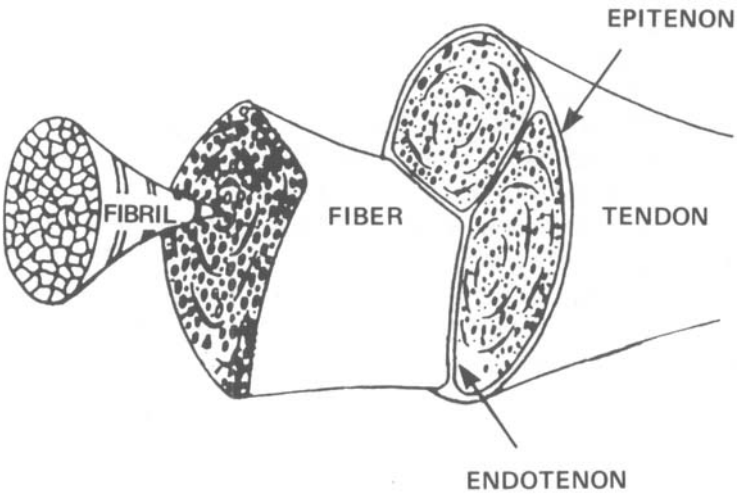


Figure 2. Principal morphology of the tendon (modified from Kastelic et al. 1978).

At the musculo-tendinear junction the perimysium becomes continuous with the endotenon. At the tendo-osseous junction the collagen fibers continue into the bone as the perforating fibers of Sharpey, and the endotenon becomes continuous with the periosteum.

These two junctions and the mesotendon are the three sources of vascular supply to the tendon inside the sheath. The longitudinal vascular system mentioned above is connected to the vessels which enter the tendon at each end. The longitudinal vessels are also connected to the mesotendal vessels which perforate the tendon in a segmental fashion all on the same side of the tendon. The longitudinal system contains an artery accompanied by veins. Several transverse anastomoses exist (Biesalsky and Mayer 1916, Edwards 1946, Nisbeth 1960, Smith 1965, Bengljung 1968, Schatzken and Brånemark 1969). Outside the sheath there are no specialized mesotendons, and the segmental vessels can reach the tendon from any part of the circumference.

Tendon matrix molecules

As with other connective tissue, tendons consist of relatively few cells (fibroblasts) and an abundant extracellular matrix. In both tendons and ligaments the matrix consists mainly of coarse collagen fibers, with only minor amounts of elastic fibers, ground substance and water. In rat tail tendons, for example, almost 99 % of the dry weight consists of collagen (Dale 1974).

Collagen

The most common collagen molecule, type I collagen, is composed of three peptide chains (α chains) each in a left-hand coiled superhelix (Rich and Crick 1955) with about 100 amino acids each, giving it a total molecular weight of about 340,000 (Ramachandran and Kartha 1954; Rich and Crick 1961). Two of the peptide chains are identical (called α1 chains) and one differs slightly (the α2 chain). The collagen molecules are combined in a right-handed triple helix, giving the molecule a rod-like shape. Its length is about 280 nm and its diameter about 1.5 nm (Rich and Crick 1955, White et al. 1964, Hall 1965, Diamant et al. 1972, Ham 1974).

Every third amino acid in the α chain is glycine and this repetitive sequence is essential for the proper formation of the collagen helix. Other amino acids commonly present are proline (15 %) and hydroxyproline (15 %) (Ramachandran 1963). Thus almost two-thirds of the collagen molecule consists of these three amino acids. Hydroxyproline is a derivative of the incorporated proline and is almost unique to collagen. Another amino acid unique to collagen is hydroxylysine. The content of hydroxylysine in collagen is small, about 1.3 %. This triple helix is stabilized mainly by hydrogen bonds. A stable biological unit is formed by many collagen molecules which are aggregated in a quarternary structure (Fig. 3). This structure is based upon the fact that the collagen molecules

are opposed to those with an opposite charge. Due to a regular arrangement of mainly basic or acid amino acids, collagen fibers are formed. The fibrills show a periodicity of 64 nM and have a characteristic undulated form.

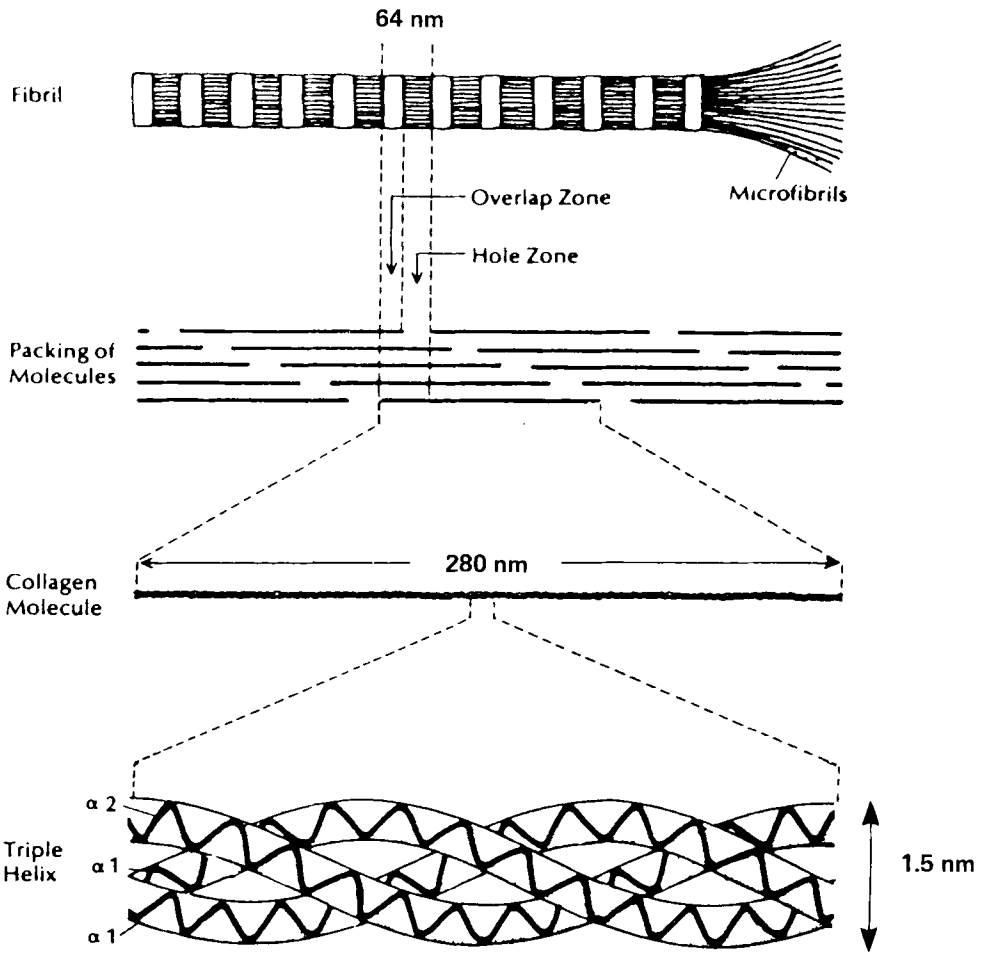


Figure 3. Collagen microstructure, the collagen molecule consists of 3 α chains in a triple helix. Several collagen molecules are aggregated into a staggered parallel array. This staggering causes the coss-striation visible in the collagen fibril. (Modified from Prockop and Guzman 1977.)

Elastin

Elastin is scarcely present in extremity tendons. However, in elastic ligaments such as ligamentum flavum and ligamentum nuchae, the proportion of elastic fibers is more

abundant. In a study by Nachemson and Evans (1968) the proportion of elastin to collagen fibers was found to be 2:1 in ligamentum flavum. Elastin is extremely insoluble. Thus, a standard chemical procedure for elastin determination involves autoclaving and/or extraction with strong protein denaturants, defining the insoluble residue as elastin.

Ground substance

The ground substance consists of proteoglycans, glycosaminoglycans, structural glycoproteins, plasma proteins and a variety of small molecules. The waterbinding capacity of these macromolecules is considered important; for example, the hyaluronate molecule takes up a hydrodynamic volume 1000 times the space occupied by the chain itself. There are also data that suggest that the microelasticity seen in the toe-part of a stress-strain curve is mainly due to interfibrillar sliding and shear of the interfibrillar gel (for review, see Viidik et al. 1982). It is possible that the proteoglycans and glycosaminoglycans are important for stabilizing the collagenous skeleton of connective tissue. However, due to the small amount of these molecules in tendon, their importance for the biomechanical properties has been questioned (Viidik 1973).

Metabolism of collagen

The collagen molecule is synthetized by the fibroblast (Branwood 1963, Porter 1964). The collagen molecule is synthetized within the cell as a larger precursor (procollagen) which is then secreted and cleaved extracellularly to become collagen (Fitton-Jackson 1965). The synthetic activity of mature tendon fibroblasts is, however, uncertain.

By using tritium-labelled hydroxyproline or glycine and autoradiographic methods, the metabolic turnover of collagen may be studied. In adult animals the half-life of collagen is comparable to the entire life span. Neuberger et al. (1951) reported that in young animals the turnover is elevated and may reach the same magnitude as in skeletal muscle.

Newly formed soluble collagen and breakdown of stable collagen results in urinary excretion of hydroxyproline. The breakdown itself is an effect of rapid turnover of the collagen which usually occurs in connection with increased collagen synthesis when the tissue remodels (Woessner 1968). The correlation between the amount of soluble collagen in the body and the total excretion of hydroxyproline is a measure of collagen metabolism (Prockop and Kivirikko, 1967). In the mature animal under normal condi-

tions, the collagen in liver and bone has the fastest turnover and thus is the dominating source of urinary hydroxyproline (Neuberger 1965, Woessner 1968).

Collagen metabolism is disturbed by a variety of connective tissue disease; for example: In lathyrism the hydroxyproline excretion is increased as a result of both increased breakdown and increased synthesis of collagen (Smith and Shuster 1962). In ideopathic scoliosis there is also an increased excretion of hydroxyproline as a result of increased collagen turnover in bone (Benson 1965). In scurvy, despite a decreased breakdown of collagen, there is still an increase of hydroxyproline in the urine due to a net loss of collagen (an extreme decrease of syntheses). In rheumatoid arthritis, orthogenesis imperfection, Paget's disease, thyroid disturbances, Marfan's syndrome, Ehlers-Danlos syndrome and pregnancy there is also an increased hydroxyproline excretion (Sjoerdsma et al. 1958, Woessner 1968).

The oxygen consumption in tendons is very low, estimated by Peacock (1957) to be 0.1 μl O_2 per mg dried tissue per hour. The blood flow in tendons (assessed with a method based upon ^{52}Cr-labelled erythrocytes) is 0.10 cm^3 per gram tissue per minute as compared to 0.16 for bone and 0.27 for skeletal muscles (White et al. 1964).

Physical properties of collagenous tissue

Mechanical properties of soft collagenous tissue are mainly dependent on the architecture and properties of the collagen fibers and of the proportion of elastin. Tendons consist mainly of type 1 collagen and only minor amounts of ground substances. The physical properties of collagen are highly dependent on cross-links within and between the collagen molecules. During maturation of collagen the number and quality of the cross-links increases, resulting in increased tensile strength and decreased collagen solubility (Piez 1968, Vogel 1978, Viidik et al. 1982).

The small amino acid glycin is necessary for the tight helical packing of the collagen molecule and also adds to its stability by forming hydrogen bonds to the other two chains in the superhelix. Hydroxyproline and proline form hydrogen bonds or hydrogen-bonded water bridges to the other two chains. Examples of cross-links between the collagen molecules are lysino-norleucines hydroxylated to varying degrees. Hydroxylycine-norleucine forms a more stable cross-link from a physiological point of view. There are also several other, more stable and less well-known cross-links in collagen; for a review see Viidik et al. (1982). Newly formed collagen is soluble in neutral salt solutions and in acid solutions and, as the cross-links are relatively few and more easily denaturated by heat. Mature collagen is not soluble in neutral salt solutions or acid solutions, survives a higher denaturation temperature and has a stronger heat denaturation isometric contraction. After maturation when aging, collagen reaches a plateau in respect to mechanical properties after which the tensile strength begins to decrease (Rollhäuser 1950, Yamada 1970, Vogel 1978, Viidik et al. 1982).

Factors affecting the physical properties of collagen

Maturation: During maturation the number and quality of the cross-links within and between the collagen molecules increases, resulting in an increased tensile strength and decreased collagen solubility (Piez 1968, Vogel 1978, Viidik et al. 1982).

Aging: In the post-maturation phase the biomechanical properties reach a plateau, after which a slow decrease in biomechanical properties begins. The decrease in biomechanical parameters is correlated to a decrease in the amount of insoluble collagen and of the total collagen content (Vogel 1978, 1983, Viidik et al. 1982). However, an increase in stiffness occurs (Viidik 1980b, Viidik et al. 1982). The increase in stiffness of type I collagen may contribute to the increased compliance and residual volume of the aging lung (Viidik 1979).

Training: Training results in increased tensile strength in tendons (Tipton et al. 1967, Viidik 1967a, 1979, Woo et al. 1980, 1982) as well as in the ligament-bone interface (Tipton et al. 1967, Viidik 1968. Cabaud et al. 1980, Woo et al. 1981a). Parallel to an increased tensile strength, an increased collagen content is observed (Woo et al. 1980, 1982). Other investigators (Tipton et al. 1967, Laros et al. 1971, Noyes 1977) have also demonstrated a relationship between the degree and duration of motion, on the one hand, and resulting changes in tissue properties and mass on the other hand.

Immobilization: Immobilization results in a decrease of tensile strength in tendons (Noyes 1977, Amiel et al. 1982) and an increased collagen turnover (Amiel et al. 1982). The increased collagen turnover results in an increased amount of reducible cross-links (Akeson et al. 1977).

Glucocortical treatment: Short-term treatment of prednisolone increases the strength of muscle tendons (Oxlund et al. 1981). Kühn et al. (1964) and Oxlund (1983) showed that prednisolone treatment accelerated the conversion of soluble collagen to insoluble in skin and that the total amount of collagen increased. Prednisalone treatment also results in increased thermal stability in rat tail tendons (Vogel 1969). Long-term glucocorticoid treatment (prednisolone) results in reduced dry weights of muscle tendons and no change in collagen content or stress-strain parameters. However, the elastic stiffness after mechanical exhaustion was increased (Oxlund 1982).

The influence of local injections of corticosteroids:

Short-term local administration: Local administration around the peroneal tendons of rats for 24 days resulted in increased tensile strength (increased maximal load and stiffness) with no change in collagen content (Oxlund 1980). However, local administration of prednisalone in knee joints for 24 days resulted in decreased strength in the ligament-bone interface (PCL). The systemic effects of this local cortisol treatment on the skin were decreased thickness and fat content, increased collagen concentration and higher tensile strength and failure energy.

Long-term local administration: In a study by Oxlund (1982) rats received cortisone injections 10 mg/kg into the hind limb around the perineal tendons every third day for 55 days. The local cortisone treatment did not alter the mechanical properties of the tendons, even though the dry weight and hydroxypoline content were reduced. Two different effects of corticosteroids on collagen tissues are suggested to act: 1) within the first one or two weeks, corticosteroids induce a relatively fast increase in the stability of the collagenous tissue; followed by 2) a progressive thinning and reduction in collagen of the tissue mainly caused by an inhibition of the collagen synthesis. Glucocorticoids inhibit the synthesis of collagen (Kühn et al. 1964, Kirvirikko et al. 1965, Manthorpe et al. 1974). Thus the strength of the tendons is not reduced even though the collagen content is reduced.

Clinical reports of bilateral rupture of the Achilles tendon in patients on oral glucocorticoid therapy has been reported (Cowan and Alexander 1961, Lee 1961, Smaill 1961, Melmed 1965). However, in these cases the patients had been treated for months with glucocorticoids and the inhibited collagen synthesis had been presented for a long time, which may have resulted in decreased collagen content and decreased tensile strength. Local injections around the Achilles tendon (Lee 1957) and the patellar tendon (Ismail et al. 1969) have resulted in ruptures of the tendonous tissue.

Diabetes: Andreassen and coworkers (1981) report that in experimentally induced diabetes (steptozotocin diabetic rats) the maximum strength and stiffness of the rat tail tendons was increased after 10 days of diabetes and even more after 30 days. Insulin treatment prevented all changes.

Pregnancy and the postpartum period: The tensile strength of muscle tendons and the public symphysis in rats was found to be decreased the end of pregnancy and during the postpartum period (Rundgren 1974). In the early postpartum period, a decrease of the stiffness is observed followed by a restoration. In adrenalectomized rats there is no such initial decrease. This indicates that relaxation during pregnancy had been less pronounced (Oxlund et al. 1980). However, the strength of muscle tendons from adrenalectomized animals is decreased during the postpartum phase.

Lathyrism is a condition produced by ingestion of seeds of the sweet pea (*Lathymus odoratus*) which contain beta-aminopropionitril. This results in an absence of intra- and intermolecular cross-links in collagen caused by a blocking of lysyl oxidase leading to a decrease of tensile strength (Levene and Gross 1959, Fry et al. 1962, Zweymüller and Plenk 1968). The collagen also naturally becomes more easily soluble. D-penicillamine blocks the action of lysyl oxidase and splits certain cross-links. This drug has been used, for example, in scleroderma to prevent excessive formation of connective tissue (Viidik et al. 1982). Vitamin C deficiency (scurvy) causes an increased breakdown and diminished synthesis of collagen. Copper deficiency leads to a reduced tensile strength of collagen (Coulson et al. 1965).

Non-steroidal Anti-inflammatory Drugs (NSAID): Vogel (1977) found that indomethacin treatment resulted in increased tensile strength in rat tail tendons. An increase of the proportion of insoluble collagen and in the total collagen content was also observed. Ohkawa (1982) found an increased tensile strength in periodontum in rats after indomethacin treatment.

Biomechanics of tendons and ligaments

The main physiological function of tendons and ligaments is to transmit forces. Tendons and ligaments are non-linear visco-elastic materials (Viidik 1968b). One of the simplest way to analyze the biomechanical properties is to subject the specimen to tensile deformation using a constant rate of elongation. The tissue is then elongated until rupture and the resulting force (load) (P) is plotted (Fig. 4).

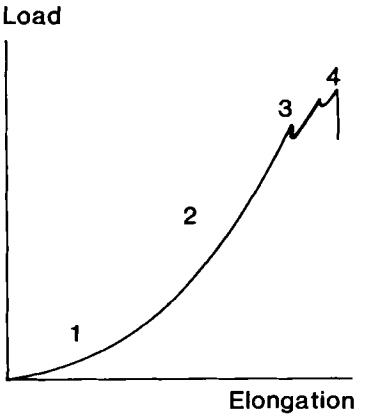


Figure 4. Load-elongation curve, (1) primary or "toe" region, (2) secondary or "linear" region, (3) end of secondary region (P_{lin}), (4) maximum load (P_{max}).

The first region is concave and is usually called the "toe" region. This region is believed to be due to a straightening of the wavy pattern of the fibers to a more straightened pattern (Gustavsson 1956, Elliott 1965, Viidik et al. 1965, Viidik 1966, 1967b, Abrahams 1967, Diamant et al. 1972, Hirsch 1974). In this region little force is required initially to elongate the tissue, but the stiffness increases and greater forces are required for equivalent elongations. The end of this region has been reported to have a strain value between 1.5 % and 4 % (Cronkite 1936, Rigby et al. 1959, Abrahams 1967, Diamant et al. 1972, Haut and Littel 1972, Viidik 1973).

The second region represents the response of collagenous tissue to further elongation. This region is often more or less linear and is consequently called the linear region. Fibers are more parallel and have lost their wavy appearance (Gratz 1931, Rigby et al. 1959, Viidik et al. 1965, Viidik 1966, 1973, Abrahams 1967, Diamant et al. 1972). In the end of this region small force reductions ("dips") can sometimes be observed in the loading curves for tendons and ligaments. These dips can be caused by early sequential failure of a few greatly stretched fiber bundles (Butler et al. 1978). This is not generally observed when collagen bundles are tested (Galeski et al. 1977).

After the second or "linear" region, major failure of fiber bundles appears in an unpredictable manner. When maximum load is attained, complete failure occurs rapidly and the load-supporting ability of the tendon is lost.

The elongation is usually expressed as strain (ε) in percent of the original length (in mm) of the tested specimen:

$$\varepsilon = \frac{l - l_0}{l_0} \times 100$$

where l = length of specimen

l_0 = initial length of specimen.

The load may be expressed as stress (σ) (load per unit cross-sectional area).

$$\sigma = \frac{P}{A}$$

where P = load

A = cross-sectional area.

The simplest way to present biomechanical results is to present original curves of the biomechanical tests. Another common procedure is to register the stress or load at certain strain values and to calculate the mean stress/load value at each strain value and in this way achieve a mean stress/load-strain curve for a group of curves. Such mean curves are often used for illustration, analyses are made between discrete points.

1) Ultimate tensile strength (maximum load; P_{max}). When testing whole tendons, this point varies considerably. This variation is caused by major failure of tendon fibers that appear unpredictably and possibly by technical problems in the clamping of the tendon ends (see below). Furthermore P_{max} is of less interest from a functional point of view, as tendons and ligaments under physiological conditions in vivo are only subjected to stress of a magnitude about one-third of P_{max} (Viidik 1980b). Irreversible injuries are already appearing during the second or linear region.

The variation in P_{max} (or P_{lin}, see below) may also be due to variations in cross-sectional area. However, the difficulty in calculating stress lies in determining the cross-sectional area of the specimen (for a review, see Ellis 1969). A significant technical error is added when small areas (less than approx. 10 mm^2) are measured. One way to minimize this technical error is to use the wet or dry weight per unit specimen length as measures for average cross-sectional areas and subsequently express "stress" in newtons per weight per length (e.g. N mm mg^{-1}) (Viidik 1967b). The effect of increasing cross-sectional area are illustrated in Fig. 5.

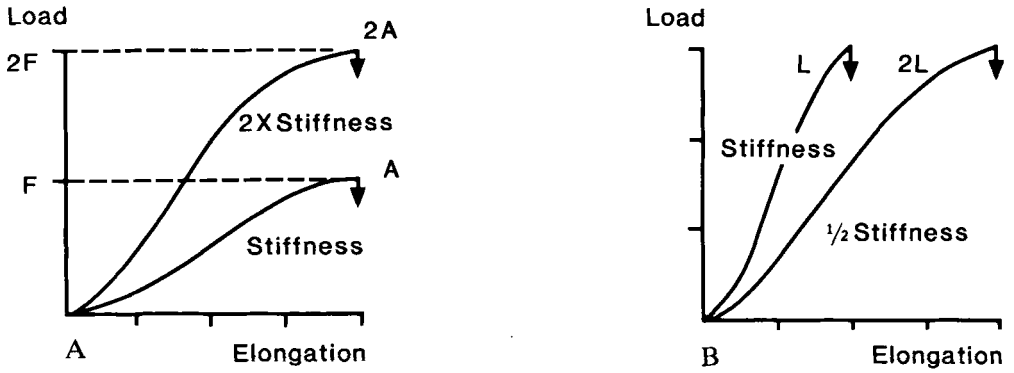


Figure 5. Effect on increasing cross-sectional area (A) and original length (B) on shape of load-elongation curves. (Modified from Butler et al. 1978.)

2) The load value at the end of the secondary or linear region (P_{lin}), i.e. where the curve levels off towards the strain axis (Viidik 1968c). This point is naturally less varying, as the problems of determining P_{max} mentioned above have not yet appeared.

3) Tanα, the coefficient of inclination for the linear portion of the curve corresponding to the elastic stiffness of the material (Viidik 1968c). This parameter is easily calculated when the curve contains a pronounced linear region.

4) The strain at rupture ($\varepsilon_{P_{max}}$) or the strain at P_{lin} ($\varepsilon_{P_{lin}}$). Strain is the deformation related to the original length (see above) expressed in percentage or units of original length. The main difficulty in strain measurement is determining the original length of the specimen.

In the in vivo resting state these tissues are subjected to stress. Thus the length of the specimen as compared with the specimen after excision is not known. The wavy form of the fiber bundles in the relaxed tendon is straightened out by tensile loading (Viidik 1972, Gathercole and Keller 1978).

The original length of the specimen may be defined at a specific pre-load, which is the smallest clearly recordable load. With most testing equipment this load is 0.5–1.0% of P_{max} (Viidik 1980b).

When analyzing the comparing different load-strain curves, care must be taken that the same pre-load is used throughout the experiment. Naturally the same length of specimen must be excised throughout the experiment; otherwise, the effect will be as illustrated in Figure 5.

5) W_f, the failure energy, or $W_{P_{lin}}$, the energy to P_{lin}: These parameters are represented by the area between the load-strain curve and the deformation axis up to respective load values.

The "modulus of elasticity" for different connective tissues (i.e. tendons and ligaments) has been determined by many investigators. This is based on Hooke's law and a linear relationship between load and deformation:

$$E = \frac{\sigma}{\varepsilon}$$

Where E = modulus of elasticity
σ = stress
ε = strain

However, Fung (1967, 1972) has pointed out the meaninglessness of calculating the modules of elasticity as it changes with increasing strain. In the toe-phase the modules increase gradually. If the load/stress-strain curve contains a fairly linear secondary region, then the "modulus" stabilizes (tanα). In order not to confuse this "modulus" (tanα) with the modulus of elasticity used in engineering sciences, it may be called "elastic stiffness" (Viidik 1980b).

Until now the nonlinearity of tendons and ligaments has been discussed. However, tendons and ligaments are also visco-elastic materials with a certain degree of plasticity. This is demonstrated and analyzed in more elaborate tests.

1) Cyclically tested with loading-unloading, the stress-strain curve is displaced to the right along the deformation axis because of a presence of a plastic component. There is also an increase in elastic stiffness (Viidik 1968c, 1979).

2) With an increased strain rate the linear part becomes steeper (Frisén et al. 1969a, b, Viidik 1979).

3) Stress-relaxation test: A stress-strain test is halted safely below the linear region of the stress-strain curve and the strain is kept constant. Then the stress will decrease at first rapidly and then gradually more slowly. When this stress-relaxation test is repeated cyclically, the decrease in stress becomes gradually less pronounced.

4) Creep-test: A stress-strain test is halted safely below the linear region of the stress-strain curve and the stress is kept constant. The strain at first increases relatively quickly and then more and more slowly. If this test is performed cyclically, the increase in strain becomes less pronounced.

The stress-strain curves of cyclic tests coverge gradually. This phenomenon can be used when repeated tests are planned on the same specimen (preconditioning) (Rigby et al. 1959). For a comprehensive review of these tests, see Viidik (1980b).

Biomechanical factors of collagenous tissue are also affected by environmental factors:

1) Influence of humidity on the specimen: With drying, the stiffness of the specimen increases (Morgan 1960, Galante 1967). Different methods of keeping the tissue from drying have been presented. The most effective is to keep the tissue in a humidity of 100% (Hirsch and Galante 1967, Tkaczuk 1968). Immersion in different fluids (i.e. water, Ringer's solution, plasma) causes swelling of the collagen fibers and causes changes in biomechanical properties (Viidik and Lewin 1966, Galante 1967).

2) Postmortal changes: It is concluded by Viidik et al. (1965) that no biomechanical changes appear in the collagenous tissue for 24 hours if it is not removed from its normal surroundings in the expired body.

3) Deep-freezing: In a study by Hirsch and Galante (1962) rapid freezing and thawing did not change the biomechanical properties of the tissue. In a study by Matthews and Ellis (1968), when cat tendons were frozen to only $-10\,°C$ for two weeks there was a decrease in elastic modulus.

4) When testing isolated tendons, clamping into the testing machine has posed difficulties: slipping from the clamps; or tendon injuries caused by the edge of the clamp. Consequently care must be taken to increase the friction between tendon and clamp and that the clamping is as "atraumatic" as possible. If isolated tendon samples are tested a slow strain rate is to be used; otherwise failure or slippage will occur at the tendon-clamp interface (Welsh et al. 1971, Matthews et al. 1975).

Several authors have determined numerical values for tensile properties of tendons, such as ultimate tensile strength (P_{max}) and modulus of elasticity (E) (McMaster 1933, Cronkite 1936, Rollhäuser 1950, Stucke 1949/50, Abrahams 1967, Yamada 1970). However, the numerical values found differ considerably due to several reasons.

Mathematical models (continuum formulations)

To facilitate statistical analysis and correlation to other parameters, for example biochemical and morphometrical parameters, a variety of empirically derived mathematical models describing stress/load-strain (load-deformation) relationships has been presented. For a comprehensive review of previous works, see Viidik (1980b).

When applying a mathematical model to a load-strain curve, several factors must be considered.

1) The function must fit the data (load-strain curve) very well so as not to lose information when making the data reduction. One measurement of the goodness of the fit is the fraction of explained variance (FEV):

$$\mathrm{FEV} = 1 - \frac{\sigma(\mathrm{yd} - \mathrm{ym})}{\sigma(\mathrm{yd})}$$

where
σ = variance
yd = y data
ym = y model (according to mathematical model).
Thus, with a perfect fit, FEV = 1.

2) The number of parameters in the mathematical model used should be as few as possible. A model with numerous parameters can always fit the experimental data, but the uncertainty in the parameters will increase. This uncertainty is expressed by the condition number, which should be less than 1,000.

3) The dependency between the parameters should be as low as possible. This dependency is expressed by a dependency parameter; if this parameter is 0.95 or higher, then the dependency between the parameters is significant.

The first model was related by Wertheim in 1847, who presented a power function for tendons:

$$\varepsilon^2 = c_1\sigma^2 + c_2\sigma$$

where ε is strain, σ stress and c_1 and c_2 constants. Morgan (1960) presented the empirically derived function

$$\varepsilon = c_3\sigma^{0.812}$$

for tendon fibers, and Elden (1968)

$$\sigma = c_4\varepsilon^2$$

for tendons. Ridge and Wright (1965) presented two empirically derived functions to describe load-deformation sequences in connective tissue (skin).

$$E = c_5 + c_6 \log L$$

for the initial phase (toe region) and

$$E = c_7 + c_8 L^{c_9}$$

for the second phase (the "linear" phase) where E is extension, L is load, c_5, c_6, c_7, c_8 and c_9 are constant.

Empirically found expressions have also been used to describe stress-relaxation and creep phenomena. Viidik (1968a, c) presented the formula

$$F(t) = F_0\exp(-\beta t) + F_a[1 - \exp(-\beta t)]$$

where F_0 is initial load, F_a is the asymptotic value of F(t) when time tends to infinity and β is the shape parameter (relaxation speed) indicating the speed by which the asymptote is reached, calculated with the method of least squares.

An extensive theoretical framework for mathematical characterization of soft tissue was presented by Fung in 1967. He especially studied cyclic loading stress-relaxation and creep within the toe part of the stress-strain curve of rabbit mesentery and formulated a "quasi-linear" visco-elasticity low for soft biological tissues:

$$\sigma(t) = f\{\varepsilon(t)\} + f'\{\varepsilon(t-\tau); t, \tau\}$$

where $\sigma(t)$ is stress and $\varepsilon(t)$ is strain at time t, f, a function of the strain at time t and f' a function for the entire strain history.

It has also been shown by Fung (1967) that the most important parameter for rabbit mesentery is the slope of $dT/d\lambda$ vs. T ("tangent modulus") where T stands for "Lagrangian" stress and λ stands for extension rate (deformed length divided by original length of the specimen). Within the "toe-part" the relationship is linear. It has also been shown by recent investigators that there is a linear relationship between the "tangent modulus" vs. strain (Danielsen 1982).

Rheological models

Discrete-element models have also been utilized to mimic the behavior of non-linear visco-elastic materials. The elements are linear in character and must therefore be combined to compose models. The three basic model components are the linear spring, the dashpot and the frictional element (Fig. 6).

Several discrete-element rheological models have been developed which are com-

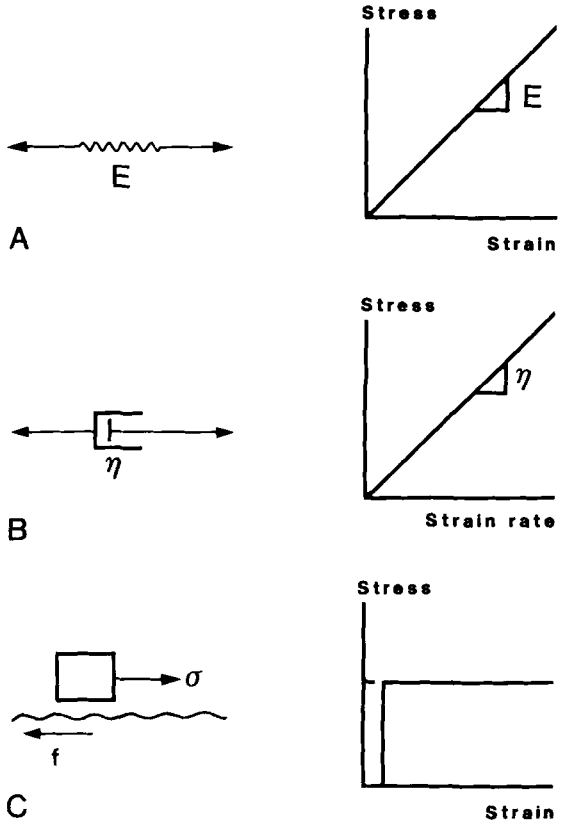


Figure 6. Basic rheological elements: (A) linear spring, (B) dashpot, (C) frictional element, and stress-strain curves. (Modified from Butler et al. 1978.)

posed of simple springs, dashpots and frictional elements (Burstein and Frankel 1968, Hirsch and Sonnerup 1968, Frisén et al. 1969a, b). Viidik (1968b) presented a discrete model which provides an easy method to analyze the visco-elasticity during the linear part of the curve.

Tendon healing

The substrate phase

Tendon healing, as with healing elsewhere in the body, begins with the formation of a blood clot and an inflammatory reaction including outpouring of fibrin and inflammatory cells. A fiber clot is formed between the two tendon ends. The clot is then invaded by cells very much resembling fibroblasts and migrating capillary buds. Different opinions exist over the exact mechanism of tendon healing. One early view is that (1) regeneration takes place by outward growth from the cut (injured) ends of the tendon (for

review, see Van der Meulen and Leistokow 1977). (2) The union is first effected by proliferation of the surrounding connective tissues, followed by proliferation of the tendon itself (Mason and Shearon 1932, Mason and Allen 1941, Flynn et al. 1960, Birch and Lindsay 1964). (3) The tendon healing is dependent on the surrounding tissues and that the tendon itself plays no significant role (Peacock 1959a, b, Lipscomb and Wakin 1961, Potenza 1962a, 1963, 1964). However, in recent years an intrinsic tendon cell proliferation capacity has been postulated (Matthews and Richards 1974, 1975, Eiken et al. 1975, Lundborg 1976).

Lundborg (1976) showed that sutured free tendon grafts of rabbit flexor tendons healed without adhesions when kept in a vascular synovial milieu of the suprapatellar bursa. This was also confirmed by Matthews (1976) and Rank et al. (1980). These findings confirm the intrinsic tendon healing capacity. It is also difficult to understand why tendons should lack properties of regeneration since they are well vascularized and possess circulation and a measurable respiratory activity (see above).

The fibroplasia phase

On the second or third day fibroblasts appear in the wound. These fibroblasts are presumed to arise from locally resident cells in the perivascular tissue (Grillo 1963, Ross and Odland 1968, Ross and Everett 1971). Type I collagen is synthesized and extruded into the extracellular space as procollagen, which is converted to type I collagen by the enzyme procollagenase. Collagen assembles into fibril (Peach et al. 1961, Fernando and Movat 1963, Ross and Odland 1968, Lindner 1973). In this stage of fibroplasia, fibroblasts synthesize collagen, mucopolysaccharides and glycoproteins. The fibrils are formed by several types of cross-linking mechanisms (Gustavson 1956). Finally the fibrils organize into fibers forming connective tissue. By this process adhesions are also formed.

The remodelling phase

Restoration of gliding function of the tendon depends on the effectiveness of dissolution and reforming of the collagen fibers during the scar remodelling phase, which starts about the fifteenth day. By 28 days most of the fibroblasts and collagen between the tendon stumps are oriented longitudinally (for a review see Ketchum 1977, Van der Meulen and Leistikow 1977; for an excellent review of the earlier works, see Mason and Shearon 1932).

Collagen synthesis

Using tritum-labelled proline, Birdsell et al. (1966) found a six-fold increase in the collagen synthesis in sutured tendons, beginning after the first week and reaching a maximum after about 4 weeks. After three months there was still a three- to four-fold increase. Histological studies indicate that collagen maturation and remodelling beginning in the third week continues up to one year after injury (Mason and Allen 1941, Buck 1953, Greenlee and Pike 1971).

Enzymatic changes

Characteristic changes of repair are produced throughout the entire length of the tendon if the tendon is injured at one point. During the first 12–36 hours following tenotomi glycolysis, the pentose shunt, and the citric acid cycle enzymes are stimulated throughout the tendon. The most marked changes are in the level of lysosomal enzymes and the changes are most marked near the site of tenotomy (Strömberg et al. 1977).

Vascular reaction

It has been demonstrated using microangiographic techniques that during the first week sprouting buds of capillaries grow in the direction of the tendon wound. These sprouting buds arise from the surrounding connective tissue. To a lesser extent, capillaries also come from the tendon ends. Organization follows and after five weeks the vascular anatomy is practically normal (Bergljung 1968). The vascular supply of the normal tendon is covered in the proximal third from vessels from the muscle and in the distal quarter via vessels from the bone and in between from segmental vascular supply (Peacock 1959a). However, if the vascular supply is disconnected due to an injury, new communications are in general established along with adhesion formation. This process leads to an increased vascularity during the first 4–6 weeks (Nichols et al. 1954, Peacock 1959a, Bergljung 1968).

Biomechanics of tendon healing

Paget (1853) was the first to biomechanically study tendon healing. He reported the breaking strength of two healing rabbit Achilles tendons. Many studies in order to quantify tensile properties of healing tenotomies have been performed Carstam 1953, Cowan and Courtemanche 1959, Forward and Cowan 1963, Gonzalez 1949, Hirsch 1974, Ketchum 1971, Ketchum et al. 1977, Levine et al. 1966, Mason and Shearon 1932, Mason and Allen 1941, Urbaniak et al. 1975, Wrenn et al. 1954. In most studies, breaking strength of rabbit or dog sutured tenotomies was determined by direct clamping of the tendon which was then subjected to tensile force. In the classical experimental study performed by Mason and Allen (1941), they found that the tensile strength exhibited three phases parallel with the phases of healing: (1) A phase of exsudation and fibrinous union. In this phase tensile strength diminishes as a result of wound edema, which lasts about five days. The strength is primarily due to the suture. (2) A phase of fibroplasia. The tensile strength now increases, reaching a plateau on about the 16th day. (3) A phase of maturation, organization and differentiation. This phase probably begins between the 19th and 21st days. The tensile strength continues to increase for an undetermined period of time (the time studied was 72 days). Parallel to the deposition and maturation of collagen in the healing wound is the rise in tensile strength (Harkness 1968). There are also quantitative changes in acid mucopolysaccharides that occur prior to and accompany the collagen and tensile strength change (Dunphy and Udupa 1955).

It was also demonstrated by Bryant and Weeks (1967) that the ratio of wound collagen to mucopolysaccharides was the best determinant of the gain in tensile strength. Other studies have investigated the tensile strength as a function of stuture method (Cowan and Courtemanche 1959, Forward and Cowan 1963, Hirsch 1974, Levine et al. 1966, Urbanik et al. 1975, Ketchum 1977).

In an experimental study by Steiner (1982) using bone-muscle-tendon-bone complexes to investigate the healing of unsutured cat Achilles tendons, there was a faster relative return of stiffness before strength. This has also been demonstrated in skin wounds and in ligaments following immobilization (Noyes 1977, Zingg 1975). Mechanical properties of connective tissue are strain-rate dependent. However, using a low strain rate, Hirsch (1974) found that after four weeks of healing of sutured rabbit tenotomies, the strength was 15–20% compared to the normal tendon, and after 26 weeks of healing it had reached 50%.

Factors affecting tendon healing

Immobilization

Immobilization is necessary after tendon surgery to prevent suture insufficiency (Mason and Allen 1941).

Influence of stress

Tensile strength increases rapidly after mobilization when preceded by immobilization (Mason and Allen 1941, Cowan and Courtemanche 1959). Mechanical stress provokes an orientation of the collagen fibrills (Peacock 1965) and for remodelling of the collagen scar tissue into mature tendon tissue, tensile forces are necessary (Potenza 1962a, b, Flynn 1965, Peacock 1965, Viidik 1967a).

Controlled passive mobilization

Immediate controlled mobilization was introduced by Kleinert et al. (1973). The effect of controlled passive mobilization was biomechanically investigated by Woo et al. (1981b). The rate of tendon repair was significantly improved (faster regain in ultimate tensile load). The gliding function was also improved. This was also confirmed by Gelberman et al. (1982).

Mobilization

After the end of immobilization, there is a factor gain in tensile strength (Mason and Allen 1941, Cowan and Courtemanche 1959). Mason and Allen (1941) have shown that a tendon which is immobilized for three weeks and then is mobilized triples its tensile strength in two weeks.

Suture technique

"Plaited" sutures like the Bunnell (1948) criss-cross suture have the highest tensile strength. However, the Bunell criss-cross suture is a particularly invasive technique (Berglung 1968), i.e. a suture can constrict the microcirculation of the tendon. Irrespective of the suture technique used, tension on the tendon in the area of repair can constrict the microcirculation of the tendon (Ketchum et al. 1977). Tendon-to-bone sutures are found to be stronger than tendon-to-tendon sutures (Forward and Cowan 1963, Levine et al. 1966). However, a basic requirement in successful tendon repair is to approximate and not to strangulate the tendon ends and to minimize the trauma to the sheath (if present) (De Klerk and Jonick 1982).

Delayed primary closure (DPC)

Delayed primary closure of rat skin incisions was studied by Fogdestam (1980). The DPC wounds had developed a higher relative failure energy after both 10 and 60 days as compared to primary closure.

Diabetes

Experimental diabetes results in decreased mechanical strength in healing experimental incisions in the stomach of the rat (Gottrup 1983).

Corticosteroids

Large doses of corticosteroids inhibit wound healing (Sandberg 1964, Ehrlich and Hunt 1968). However the effect of small and moderate doses is conflicting. Corticosteroids are also found to diminish adhesion formation (Carstam 1953, Wrenn et al. 1954) and to lower the tensile strength of sutured tendons. After 3 weeks of healing untreated tendons were 40 % stronger (Wrenn et al. 1954). This was also confirmed by Vogel (1970), who after both small and large doses of cortisone found a reduced tensile strength in skin wounds in rats.

However, a moderate dose increased the tensile strength after the twelfth day. Oxlund et al. (1979) found increased stiffness for 10-day wounds but reduced failure energy after 20 days of healing in skin wounds in rats subjected to cortisone. The latter is in accordance with Gottrup (1983) who after long-term cortisone treatment of rats found a decreased breaking strength and breaking energy in healing experimental stomach incisions.

Special problems in tendon surgery

Adhesion formation

As tendon healing is accomplished, at least partly, by fibroblasts derived from the surrounding tissues, it is obvious that at least some adhesion formation occurs during the tendon healing. Adhesion formation has been regarded as a necessary factor for tendon healing. On the other hand, excessive adhesion formation has an adverse effect on tendon function. Investigations concerning this problem are directed to prevention or reduction of the adhesions.

Controlled passive mobilization: Duran (1975) has shown experimentally that controlled passive motion produces elongated adhesions. Kleinert et al. (1973) have shown that during limited active extension in the hand, there is reciprocal relaxation of flexor tendons which allows passive extension of the repaired flexor tendon. The fingers are protected from full extension by rubberband traction. This technique is effective both experimentally and clinically in decreasing the tethering effect of adhesions. The improvement of gliding function after controlled passive mobilization was also experimentally confirmed by Woo (1981b) (see above).

ß-aminopropionitril (BAPN). Peacock and Madden (1969) have used BAPN experimentally which interferes with the cross-linking of collagen molecules (see above) and thus prevents adhesions. However, BAPN also alters the healing tendon in an unfavorable way by increasing the visco-elasticity.

Blocking agents. Several methods of wrapping a cut tendon in an artificial sheath have been made to avoid adhesions. (Potenza 1962b, 1963). However, all these attempts have resulted in prevention of tendon healing.

Cortisones: These are found to suppress adhesion formation. Unfortunately, the tensile strength of the healing tendon is, however, reduced. This may cause spontaneous rupture (Carstam 1953, Douglas et al. 1967).

Instillation of Triamcinolone (a cortisone analog) has isolated the healing tendon from the paratendonous tissue as the inflammation response has been altered. Healing has proceeded more slowly. However, at 6 weeks there was adequate tensile strength and the gliding was significantly better (Ketchum 1971). Somatotropine in combination with thyrotropine (Brummer 1966), liquified human fat (Zeumer 1967). Furthermore, the use of artificial tendon substitutes and induction of a sheath has been used; for a comprehensive review see Van der Meulen and Leistikow (1977).

Tendon end separation

Mason and Allen (1941) observed separation of the tendon ends after surgical repair. They also observed increased adhesion formation in association with the separation. Early active mobilization improves increased tension on the suture line and gradual gap formation, possibly ischemia, tenomalacin and finally tendon rupture. The gap is filled with granulation tissue (tendon callus; see above). Adhesion formation is stimulated and remodelling will take a longer time. Tendon repairs mobilized early have greater tendon strength, provided no rupture occurs (for review, see Ketchum 1977). Separa-

tion of the tendon ends also results in a lengthening of the tendon (Sgarlato 1975). Other factors that might result in increased tendon end separation are the suture material, suture technique, tension of the tendon repair, maceration of the repair (by hematoma) (Ketchum et al. 1977). Repairs done with greater tension resulted in greater separation.

Traumatic surgical technique

Traumatic handling of flexor tendons and surrounding structures has been associated with poor functional results (Mason and Shearon 1932). Traumatic handling results in increased adhesion formation (Lindsay and Thomson 1959/60).

Conservative (non-surgical) treatment of tendon ruptures

Tendon ruptures are usually treated with suture and immobilization (i.e. plaster cast). Immobilization is mandatory or suture insufficiency will occur (Mason and Allen 1941). In hand surgery, tendon elongation (tendon end reparation) is the deciding factor for the range of motion (ROM) of the fingers and consequently tendon elongation is unfavorable (Ejeskär 1981). However, in other anatomical regions where tendon elongation is less deleterious for the functional result, conservative treatment (plaster cast only) has successfully been used. Several authors (Gillies and Chalmers 1970, Lea and Smith 1972, Nistor 1981) have in clinical investigations reported good results with conservative treatment of Achilles tendon ruptures.

Nyström and Holmlund (1983) have shown in an experimental study of the Achilles tendon in the rabbit that both conservative and surgical treatments result in equal tendon elongation, although the elongation patterns were different (two types of biphasic separation). Conservative treatment resulted in large early (during the first week) elongation and small secondary (during the third week) elongation. Surgical treatment resulted in the contrary. Nyström and Holmlund (1983) have also suggested that conservative treatment and the resulting large early elongation causes an impaired tendon healing.

Non-steroidal anti-inflammatory drugs (NSAID)

NSAIDs or aspirin-like drugs are frequently used in the treatment of various painful conditions in the musculoskeletal system such as rheumatoid arthritis, osteoarthritis and ankylosing spondylitis (for a review see Ekstrand 1981). NSAIDs are also widely used in the treatment of soft-tissue injuries, for example inflammatory disorders and partial ruptures of tendons and ligaments and their surrounding structures. NSAIDs were sold for approximately 1 billion dollars in the U.S.A. during 1984 (Clive and Stoff 1984). For a recent review of the effect of NSAID on bone tissue, see Törnkvist (1984).

The first drug in this family is the salicylates whose therapeutic quality was first described by Stone (1763) (for a pharmacodynamic and pharmacokinetic review, see Woodbury 1970). As a result of systematic research for alternatives to salicylates, a group of organic acids with similar properties was discovered (NSAID). The first was phenylbutacon, found in the early 1950s. Indomethacin, introduced in 1959, still has a very wide use and has been shown to possess several biochemical effects (Woodbury 1970). Indomethacin was followed by a large number of NSAIDs which are now in clinical use. Among the most common side effects of NSAIDs are dyspepsia and gastrointestinal bleeding. They are now also used for pain relief (kidney stones, Holmlund and Sjödin 1978, and dysmenorrhea (Lundström 1981), and to accelerate the healing of ductus arteriousus (Sharpe et al. 1974, Thorén 1981).

Vane (1971) described the inhibitory effect of NSAID on prostaglandin synthesis. This effect is found in all species examined (Samuelsson 1974). NSAIDs interfere with cyclooxygenase and thus inhibit the oxygenation of arachidonic acid to prostaglandins. Prostaglandins are extremely short-lived and act as local hormones regulating cellular mechanisms. Different types of prostaglandins and different concentrations of the same prostaglandin may have opposite effects (Falconer et al. 1980). Release of prostaglandins is stimulated by trauma or by changes in the extracellular environment (Staszewska-Barczak and Vane 1975).

Indomethacin. Indomethacin (Indocin) 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid is an anti-inflammatory agent with antipyretic and mild analgesic effect. It was first synthesized by Shen et al. (1963). Its pharmacological properties have been described by Winter et al. (1963).

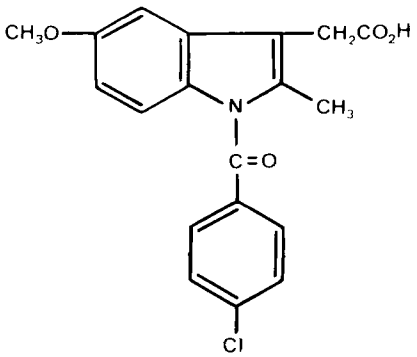


Figure 7. Molecular structure of indomethacin.

Indomethacin is rapidly and well absorbed after oral dosing (Emori et al. 1976) with peak plasma concentration within 2 hours in man (Alvan et al. 1975). Indomethacin given clinically to humans (25 mg × 3) results in a plasma concentration around 1 μg/ml (Alvan et al. 1975). Indomethacin is highly bound to plasma proteins leaving only a small active and free amount (the plasma determination includes both the free and protein-bound amount). Of the indomethacin given as an oral dose, 5–10% is found un-

metabolized in the urine. Hepatic biotransformation mainly eliminates indomethacin. Elimination halflife varies considerably (1–16 hours) among individuals (variations within the individual also occur). The metabolites of indomethacin are believed to be inactive (Duggan et al. 1975).

Effect of prostaglandins on bone and collagen

Prostaglandins (mainly E and F types) are well-known powerful bone resorptive agents in vitro (Klein and Raisz 1970, Tashijan et al. 1974, Katz et al. 1981, 1983). A major component of the bone resorptive response induced by PGE_2 is characterized by hyperplasia of the osteo clasts (Shelling et al. 1980). Extensive hypercalcemia was found in clinical practice after injection of prostaglandin F (Kuppe and Wetter 1974). It has been reported that collagen synthesis is both stimulated and inhibited by prostaglandin E and F (Blumenkrantz and Søndergaard 1972, Raisz and Koolemans-Beynen 1974, Bennet and Harvey 1981, Parnham et al. 1982).

During inflammation prostaglandin E is known to contribute to vascular events (Staszewska-Barczak and Vane 1975). Prostaglandin E also dominates initially in inflammatory sites (i.e. fractures, arthritic conditions (Floman et al. 1977, Dekel et al. 1981). Elevated levels of prostaglandins have also been found in periodontal diseases and dental cysts (Harris et al. 1973, Goodson et al. 1974), osteomyelitis (Corbett et al. 1979, Dekel and Francis 1981), and skeletal tumors (Powles et al. 1973, Dowsett et al. 1976, Minkin et al. 1981).

Effect of indomethacin on bone and collagen

It has been shown in several studies that orthopic and heterotopic bone formation in association with fractures and surgery is inhibited by indomethacin (Almåsbakk and Rösland 1977, Rø et al. 1978b, Sudmann 1975, Sudmann et al. 1979a, b, Sudmann and Bang 1979). In an experimental study Törnkvist (1984) found that indomethacin exerts a mild inhibitory effect on the early stages of bone formation. He also found that the inhibitory effect of indomethacin on heterotopic bone formation and fracture healing does not appear to be mediated through a toxic effect on bone-forming cells. It has also been shown that indomethacin treatment leads to a decrease in mechanical strength in healing bone (Rø et al. 1976, Törnkvist et al. 1984). However, indomethacin has been reported to have no effect on normal skeletal growth during development (Sudmann et al. 1982). Rø et al. (1978a) and Elves et al. (1982) have found after indomethacin treatment of rats increased hydroxyproline synthesis and increased fibrogenesis, respectively, in association with fractures.

NSAIDs and indomethacin in particular have been suspected of promoting rapid deterioration of the joint cartilage. The hip joint has been studied radiologically (Coke 1967, Arora 1968, Allen and Murray 1971, Milner 1973, Foss Hauge 1975, Murray 1976, Dinley 1978, Rönningen and Langeland 1979). Robinson et al. (1975) reported that rheumatoid synovium produced and releases large amounts of prostaglandins which are destructive to the juxta-articular bone. However, indomethacin reduced the level of prostaglandin E drastically (90%) and consequently decreased bone destruction.

Blackham et al. (1974) reported an almost complete inhibition of increase in prostaglandin levels in rabbit monoarticular arthritis by indomethacin.

Purpose of this presentation

Stimulated by numerous reports of the inhibitory effect of prostaglandin synthetase inhibitors (indomethacin) on fracture healing and on formation of ectopic bone, the fact that indomethacin causes increased fibrogenesis in association with fractures, and that the organic matrix of bone and tendon consists mainly of collagen, the following studies were undertaken. Studies IV and V deal with the biomechanical and biochemical effects of indomethacin on developing tendons and on healing tendons (Carlstedt et al. 1986b, c, 1987).

Inspired by several reports on conservative treatment of Achilles tendon ruptures (see above), the following studies were undertaken. Studies II and III deal with the elongation of the plantaris longus tendon after surgical repair and with the biomechanical and biochemical effects of conservative and surgical treatment, respectively (Carlstedt et al. 1986a, 1987).

To facilitate the biomechanical analysis of the above-mentioned studies, the following study was performed: Study I, presenting a model for computer-aided analysis of the plantaris longus tendon in the rabbit (Carlstedt and Skagervall 1986.

This presentation summarizes the findings in these studies.

Materials and methods

Animals

Male New Zealand White rabbits weighing approximately 2.6 kg (about 3 months old) were used throughout the studies. They were given an ordinary laboratory maintenance diet at 200 g/day (EWOS AB, Södertälje, Sweden) and water ad libitum. The animals were individually housed in metal cages (62 × 52 × 30 cm) at 18 °C and 55 % relative humidity with a 12-hour light/12-hour dark cycle. The animals were allocated randomly to the experimental groups and within them for different treatment periods before being sacrificed. The left leg was consistently operated upon and the right leg was left untreated.

Choice of tendon specimen

The plantaris longus tendon was chosen for several reasons:

1. The tendon must have an appropriate length in order to measure length changes, perform surgery and to be fastened into the clamps at each end (40 mm total length, with a "free" length between the clamps of 19 mm).
2. The width (diameter) of the tendon should be appropriate to perform surgery.
3. The tendon should be easily accessible from a surgical point of view and it should be easily immobilized after surgery (by external immobilization, i.e. splint of Hexalite).

The plantaris longus muscle in the rabbit inserts on the lateral condyle of the femur and the tendon passes around the heel to the plantar surface, dividing to reach the second phalanges of the four digits.

Allocation of animals

One hundred twenty-six animals were used. The distribution in groups was as follows:

1. Sixty-eight animals were tenotomized, sutured and immobilized (for four weeks) in their left hind limb. The right leg was left untreated. Half the animals were given indomethacin, the other half placebo. The animals were killed after 4, 8 and 16

weeks. The left legs were used in study V and the right legs were used in study IV.
2. Thirty-four animals were tenotomized and immobilized (for 4 weeks) (conservative treatment) in the left leg. The right leg was left untreated. They were given placebo and killed after 4, 8 and 16 weeks. In study III, the left legs of these animals were compared with the left legs of the animals in the suture plus placebo group (group 1 above).
3. Twenty-four animals were used in study II.
4. Study I was performed by using biomechanical test results (188 load-strain curves) from studies III, IV and V.

The experimental model

Study I: The biomechanical tests in study III, IV and V resulted in a large number of load/strain curves. A total of 188 plantarus longus tendons with different biomechanical properties (P_{lin} ranging from 8.73–306 N) achieved by different treatments were used. In order to simplify the statistical analysis of biomechanical properties (the curves), a model for computer-aided analysis was developed.

Study II: The purpose of this study was to investigate the elongation (tendon end separation) of the plantaris longus tendon after surgical repair. Twenty-four animals were used. The left plantaris longus tendon was tenotomized, sutured, immobilized and the elongation (tendon end separation) was studied for 33 days. Two animals were killed on each of days 2, 4, 6, 11, 16, 21, 23, 25, 27, 29, 31 and 33.

Study III: In this study the biomechanical and biochemical properties of tendons (plantaris longus tendon) treated surgically and conservatively after transversal tenotomy were studied. Sixty-eight animals were used and received placebo (Table 1). The animals were killed after 4, 8 and 16 weeks.

Table 1. Distribution of animals among the different groups.

Healing time (weeks)	Tenotomy, suture, immobilization n34	Tenotomy, immobilization n34
4	10	10
8	12	12
16	12	12

Study IV: The purpose of this study was to investigate the influence of indomethacin on biomechanical and biochemical properties in normal developing tendons. Sixty-eight animals were used (Table 2). Half of the animals were given indomethacin and half placebo. The animals were killed after 4, 8 and 16 weeks and the biomechanical and biochemical variables determined.

Study V: In this study the influence of indomethacin on tendon healing was investigated. Sixty-eight animals were used (Table 2). The left plantaris longus tendon was tenotomized, sutured and immobilized. Half of the animals were given indomethacin and the other half placebo. The animals were killed after 4, 8 and 16 weeks and biomechanical and biochemical variables determined.

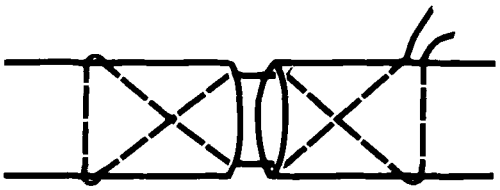
Table 2. Distribution of animals among the different groups.

Healing time (weeks)	Indomethacin n34	Placebo n34
4	10	10
8	12	12
16	12	12

Surgery (II, III, V) (Tenotomy, suture, immobilization)

A modern operating room and a sterile technique including sterile dressings and a sterilized set of instruments for each rabbit were used. The operative area was depilated with an electric razor and disinfected by soaking the extremity with chlorhexidin-alcohol.

Under general anesthesia with 0.4 ml Hypnorm Vet^R kg body weight and 0.12 mg Diazepam/kg body weight (two intramuscular injections), a transversal tenotomy of the plantaris longus tendon in the left hind limb was performed, 10 mm proximal to the calcaneal bone. This was performed through a lateral incision of the hind limb. Surgical repair was carried out using a criss-cross 4.0 silk suture (Eticon) (Fig. 8). The tendon was protected from scarification against the Achilles tendon with a 0.1 mm thick silastic membrane which was sutured to the latter tendon. The skin was closed with polyglycolic acid sutures (Dexon^R). The leg was kept immobilized for 4 weeks in a long-leg splint of Hexalite^R from above the toes to above the knee joint with the ankle joint immobilized at 15 degrees of plantar flexion and the knee joint at 75 degrees of flexion.



Figur 8. Criss-cross suture.

Conservative treatment (III)

Exactly the same procedure as described above (surgery) was performed except that the plantaris longus tendon was not sutured; only tenotomy and immobilization was performed.

Indomethacin treatment (IV, V)

An indomethacin suspension (indomethacin 10 mg/ml in carboxymethyl cellulose suspension with syrup niger) was squirted into the rabbit's mouth twice a day 5 days a week for the whole experimental period. The daily dose of indomethacin was 10 mg/kg body

weight, giving a serum concentration higher than 1 μg/ml (Sudmann 1975; Sudmann & Bang 1979). The level of indomethacin serum concentration was confirmed by a pilot study of the authors. The vehicle was administered in the same way (carboxymethl cellulose suspension with syrup niger). The rabbits were weighed weekly and the dosage was adjusted accordingly. Equivalent volumes of indomethacin and placebo suspensions were given to the animals.

Biomechanical test

The animals were killed with an intravenous overdose of barbiturate (Membumal^{R}). Immediately after the animals were killed the plantaris longus tendons of both hind limbs were dissected free and 40 mm of the tendons proximal to the calcaneal bones was resected. In the tenotomized tendons the distance between the two former tendon ends (tendon end separation or elongation) was measured with a sliding micrometer. The tendons were mounted in a special box of plexiglass for photography in two perpendicular projections. (In study II the tendon end separation (elongation) was measured on the photograph. Correction for the photographic magnification was made by a factor of 0.5.) The specimen was mounted between two metal clamps with waterproof abrasive paper glued to the inner surfaces. The distance between the clamps was always 19 mm, thus resulting in approximately 10 mm symmetrical clamping on each side.

The biomechanical tests were performed in an electrohydraulic testing machine (MTS) with a load cell (Hottinger Baldwin, Mess Technique type UI) and an amplifier (MTS type 440,21) with a total accuracy of 0.25 %. By measuring the axial motion of the superior clamp the deformation was monitored with an accuracy of 0.25 %. An XY-recorder (Hewlett Packard 7004B + 1417A) with an accuracy of 0.2 % was used for recording. A recorder gain of 20X was used for deformation. Thus, one mm on the recorder corresponded to 0.05 mm deformation.

The tendons were subjected to tensile deformation at a constant strain rate of 20 mm/min, yielding load-deformation curves. The test was carried out in a climate box with a constant temperature of 25 °C and a relative humidity of 99 %. Tendon failure most often appeared in the midsubstance or close to the upper insertion (upper clamp), or in the tendon "anastomosis" (scar tissue) (if operated). Immediately after the biomechanical test, samples of the tendon or tendon "anastomosis" were frozen in hexane-dry ice and stored at −20 °C pending biochemical analysis.

The cross-sectional areas of the tendons and the tendon anastomosis were considered to be randomly distributed in the experimental groups, since the rabbits had been chosen at random.

Analysis of biomechanical parameters

The load deformation curves were digitized using a Tektronix 4012 graphic terminal and stored in a computer (Nord 100). The digitizing of the load deformation curves was made at each millimeter (according to the deformation axis). The original length of a tendon specimen was defined at a certain low XY-recorder indication (pre-load). As the

strength of the operated tendons increased considerably, the load gain had to be changed and thus the absolute value of the pre-load increased (Table 3).

Table 3. Preload of the tendons.

Healing time (weeks)	Preload (Newton)
4	0.75
8	1.5
16	3.75
No surgery	3.75

The registrations were made from the pre-load to the load value (P_{lin}) where the load-deformation curve levels off towards the deformation axis (Viidik 1968c).

In a few cases a dip (partial rupture) occurred instead of a smooth levelling off towards the deformation axis. A dip larger than 4 mm was adopted as P_{lin} (Butler et al. 1978). Load (P) was expressed in Newton (N) and deformation as strain (ε) in percent of the original length (in mm) of the tested specimen

$$\varepsilon = \frac{l - l_0}{l_0} \times 100$$

where

ε = strain
l = length of specimen
l_0 = initial length of specimen (at the preload).

The following biomechanical parameters were calculated:

P_{lin}.

$W_{P_{lin}}$, the corresponding energy, i.e. area under the curve.

$\varepsilon_{P_{lin}}$, the strain at P_{lin}.

$\tan_{\alpha_{2.5}}$, $\tan_{\alpha_5}$ and $\tan_{\alpha_{7.5}}$. The coefficient of inclination for the tangent at a strain value of 2.5 %, 5 % and 7.5 % respectively, corresponding to the stiffness at those strain values (study IV only).

W_{65N}. The energy consumption up to load value of 65N (i.e. the area under the curve up to a load value of 65N). (study IV only).

a_1, initial stiffness and a_2, rate of stiffness increase according to the formula $P = a_1\varepsilon + a_2\varepsilon^3$, p = load, ε = strain (I) which was fitted to the curve up to a strain value of 7.5 %. (A computer program as described by McIntosh and McIntosh (1980) for non-linear curve-fitting was used.)

Biochemical analysis (III, IV, V)

Extraction Procedure. Frozen tendon samples were weighed on an analytical balance before (wet weight) and after (dry weight) freeze drying. The dried tendons were minced into small fragments and subsequently extracted with 0.5 M acetic acid (5 ml/sample) for 24 h at 4 °C. After removal of this extract, which represents relatively immature collagen (E1), the remnants were digested for 24 h at 4 °C with pepsin (1 mg/ml, EC 3.4.23.1, porcine gastric mucosa, 1:10,000 Sigma) in the same solvent adjusted to pH 2 with HCl. After digestion the pepsin was inactivated at pH 8, and the extract (E2) was dialyzed against water and acetic acid. Finally the remnants were autoclaved in water at 120 °C for 60 min, giving a third extract (E3) and an insoluble residue (R) for each sample. The extracts were then freeze-dried and stored at −20 °C pending analysis.

Analytical methods

The dried extracts and residues were first hydrolyzed in 2 ml of 6 M HCl (Aristar^R, BDH Chemicals Ltd., England) for 16 h at 110 °C, and the hydrolysates were then evaporated to dryness in a vacuum evaporator. The samples were subsequently dissolved in 0.5 ml of water and clarified by filtration through a microfilter (Bioanalytical Systems Inc., USA). Hydroxyproline was determined by the method of Stegemann and Stalder (1967).

Statistical methods (III, IV, V)

The statistical significance of differences between the groups was tested by analysis of variance and analysis of covariance (logarithmically transformed data). Two-way analysis of variance was used according to the model:

$$X_{IJK} = \mu + B_I + V_J + K_{K(IJ)} + BV_{IJ}$$

B = fix effect of treatments, I = 1–3;
V_J = fix effect of weeks, J = 4, 8 or 16;
$K_{K(IJ)}$ = random effect of animals within treatment and week;
BV_{IJ} = interactions terms. The analysis of covariance was according to the same model with the addition of a covariate. The analyses were performed at the Department of Medical Information Processing of the Karolinska Institute.

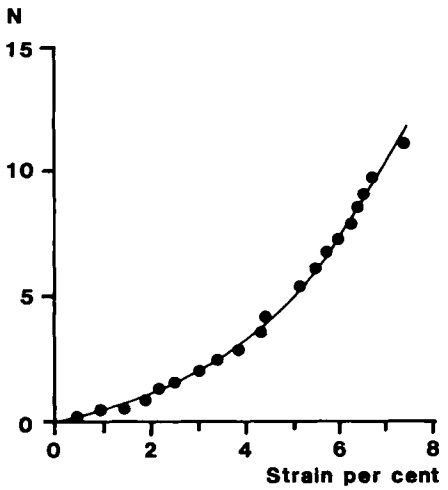
Results

Mathematical model (I)

In order to describe the characteristics of the total load-strain curve for all the biomechanically different tendons (188 tendons, P_{lin} ranging from 8.73–306.2 N), the function $P = a_1\varepsilon + a_2\varepsilon^3$ was used, where P = load, ε = strain, a_1 = linear parameter, and a_2 = cubic parameter.

The load-strain data were analyzed using a statistical program package (Statpac) in the computer (Nord 100). The function was fitted to the curves up to a strain of 7.5 %, which is somewhat above the upper limit for "physiological" strain (2–5 %; Fung 1981) by using the Marquardh-Lavenberg algorithm which uses the method of least squares. The program for non-linear curve fitting described by McIntosh and McIntosh (1980) was employed.

The function $P = a_1\varepsilon + a_2\varepsilon^3$ has a mean fraction of explained variance (FEV) of 0.996 (SD 0.002) (Fig. 9), a condition number less than 1,000 and a dependency parameter



Figur 9. Example of curve fitting (using the function $P = a_1\varepsilon + a_2\varepsilon^3$) to registration points of a load-strain curve for the plantaris longus tendon (data from IV).

range between 0.81 and 0.89 (the dependency parameter is not a linear parameter). The FEV, condition number and dependency parameters in two experimental groups with a great difference in strength (P_{lin}) are shown in Table 4. Curve fitting was performed up to a strain of 7.5 %. At strain values higher than 7.5 %, FEV decreased in some cases, although in a preliminary study with a few tendons, curve fitting up to a strain value of 15 % was performed. In most cases this resulted in an acceptable FEV of 0.8–0.9 ($\varepsilon_{P_{lin}}$ for the 188 tendons was 12.59 ± 1.90 %). The punctual recording (every millimeter) mentioned previously resulted in an error which was estimated at 2 % of the parameters a_1 and a_2, which we found acceptable.

Table 4. Applicability of the function $P = a_1\varepsilon + a_2\varepsilon^3$. Two groups with considerably varying biomechanical properties were chosen as an illustration. The load-strain curves were fitted up to a strain value of 7.5 %. P_{lin} = end of "linear phase" (secondary region). FEV = fraction of explained variance. Min and Max refer to the minimum and maximum value in respective groups.

Group		P_{lin} (N)	FEV	Condition no.	Dependency
"Weak"	$\bar{x}$	22	0.993	725	0.86
n9	SD	8	0.002	105	0.01
	Min	8.7	0.992	686	0.86
"Strong"	$\bar{x}$	237	0.996	730	0.88
n11	SD	57	0.001	20	0.01
	Max	306	0.997	755	0.88

Tendon elongation (II)

Mean body weight of the rabbits at the beginning of the experiment was 2.614 g. All animals gained weight during the experiment and showed no signs of concomitant disease. No insufficiency of the suture knot or scarification against the Achilles tendon was observed in any animal.

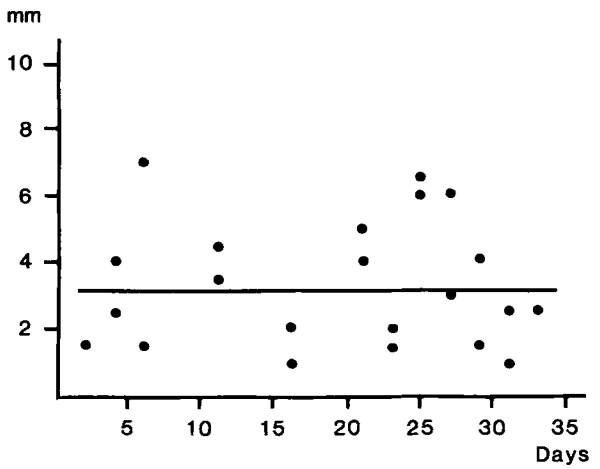


Figure 10. The elongation of the plantaris longus tendon plotted for each animal during the observation period. The regression line was calculated, the r-value was 0.03.

No biphasic separation was found (Fig. 10). The mean distance between the two former tendon ends (tendon elongation) was small and varied between 1.5–6 mm during the observation period. The regression line for the whole observation period (2–33 days) was almost horizontal (r-value 0.03) and at a level of about 3 mm.

Conservative and surgical treatment (III)

Sixty-six of the animals appeared healthy and gained weight throughout the experiment. Two animals (one from each group) developed paraplegia and were excluded. Owing to technical problems, two tendons were excluded from the non-suture group – one because of incorrect mounting of the specimen in the testing machine at 4 weeks, and one because of slipping in the metal clamps at 8 weeks. Furthermore, the biochemical results for one tendon at 4 weeks in the suture group were missing because of a laboratory error and one elongation recording at 16 weeks in the non-suture group was also missing.

There was no significant difference between the two groups concerning the weight at death or the weight increase, but between the two 8-week groups there was a significant difference in initial weight (weight at surgery) (data not shown). Thus, weight was considered as a covariate in the statistical analysis.

The results of the biomechanical analyses are presented in Table 5 (Appendix 1). Four weeks after the tenotomy there was a significant decrease ($p < 0.01$) in parameter a_1 in the suture group, but after 8 weeks this parameter was significantly increased ($p < 0.01$) in the same group. These are two isolated observations; however, the biolog-

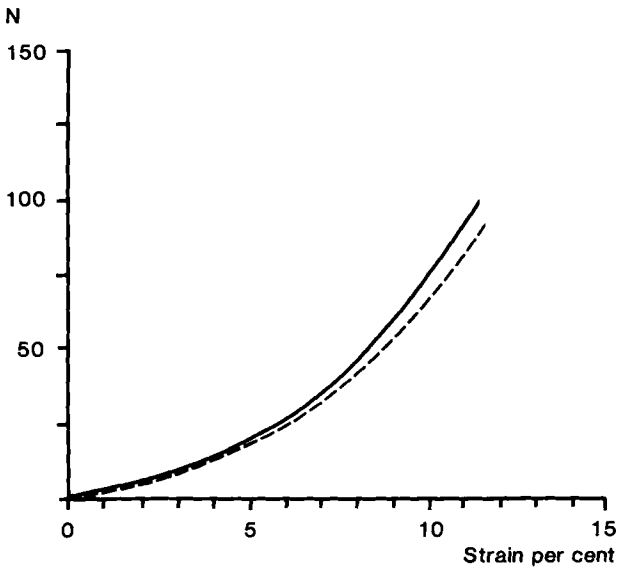


Figure 11. Load-strain curves based on the mean values of the parameters a_1 and a_2 in the formula $P = a_1\varepsilon + a_2\varepsilon^3$ for the group treated conservatively (– – –) and surgically (——) after a healing period of 16 weeks. The curves are cut at the mean value of P_{lin}.

ical relevance is uncertain. After a healing time of 16 weeks there were no differences in biomechanical parameters between the groups (Fig. 11). At this time the mean strength of the tendons measured by P_{lin} had approached 57 % of the strength for normal tendons. (Normal strength in healing cutaneous wounds in the rabbit is regained after 25 weeks (Mason and Allen 1941).)

The results of the biochemical analyses are presented in Table 6 (Appendix 1). Four and eight weeks after the tenotomy there were no differences between the two groups. After 16 weeks there was a slight but significant ($p < 0.05$) decrease in E2 + E3 in the suture group, but no difference between the groups E1 or the total hydroxyproline content.

The results concerning tendon elongation are presented in Table 7 (Appendix 1). After 4, 8 and 16 weeks the mean elongations were 14.1 mm, 10.3 mm and 16.8 mm respectively. There was no significant difference between the groups at 4, 8 or 16 weeks.

Analysis of covariance regarding the biomechanical factor a_2, with weight parameters and biochemical parameters as covariates, was performed. This did not alter the results.

The influence of indomethacin on developing tendon (IV)

All but three of the animals appeared healthy and gained weight throughout the experiment. One animal from each group developed paraplegia, and one animal in the indomethacin group died of gastric ulceration after 5 weeks of medication. These animals were excluded from the results. Furthermore, one tendon from each group at 8 weeks was excluded because of technical problems during biomechanical testing (temporary XY-recorder dysfunction and incorrect mounting of the specimen, respectively). There was no significant difference in weight increase, weight at sacrifice or initial weight between the groups except for the initial weight for the 16-week subgroups (data not shown). Thus weight was considered as a covariant in the statistical analysis.

After 4 and 8 weeks of observation there were no significant differences between the two groups in any of the biomechanical variables studied. After 16 weeks in the indomethacin group there was a significant increase in $W_{P_{lin}}$, $\tan\alpha_5$, $\tan\alpha_{7.5}$, a_2 and a significant decrease of W_{65N}, which is inversely related to the stiffness, indicating an increase in tensile strength (Fig. 12; Table 8, Appendix 1).

As shown by Table 9 (Appendix 1), the biochemical parameters did not differ significantly between the experimental and the placebo group after 16 weeks of indomethacin treatment. However after 16 weeks of indomethacin treatment, the collagen appeared to be less soluble (not significant), presumably due to a higher content of cross-links. After 4 and 8 weeks there were no differences between the groups (not shown). Furthermore, analysis of covariance regarding the biomechanical factor a_2 with the weight variables and biochemical parameters as covariates was performed. This did not alter the results. The parameter a_2 was chosen because of its high representativeness of the curves.

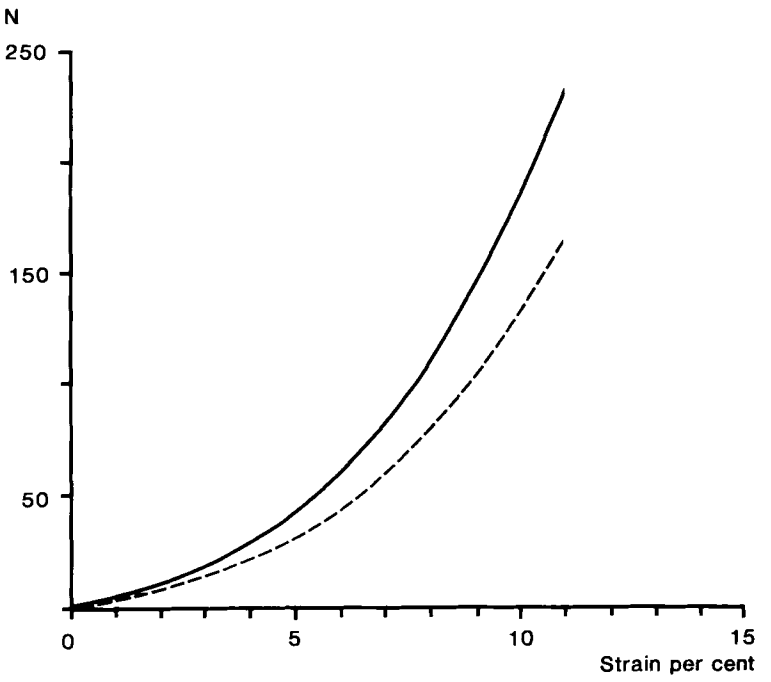


Figure 12. Load-strain curves according to the mean values of the parameters a_1 and a_2 in the formula $p = a_1\varepsilon + a_2\varepsilon^3$ for the indomethacin group (——) and the placebo group (– – –) after 16 weeks of treatment. The curves are cut at the mean value of P_{lin}, respectively.

The influence of indomethacin on tendon healing (V)

All the animals except three appeared healthy and gained weight throughout the experiment. One animal in the indomethacin group died of gastric ulceration after 5 weeks of medication and one animal from each group developed paraplegia. These animals were excluded from the results.

Furthermore, three tendons were excluded from the indomethacin group on account of technical problems at the biomechanical testing, two at 4 weeks because of slipping in the metal clamps and one at 8 weeks because of incorrect mounting of the specimen.

The weight of the animals when killed and the weight increase did not differ significantly between the two groups. The initial weight of the 16-week subgroups differed slightly but significantly (data not shown). However, weight did not have any influence on the biomechanical results in analysis of covariance.

The biomechanical parameters are presented in Table 10 (Appendix 1). After four weeks there was no significant difference between the two groups. After eight weeks P_{lin} was decreased in the indomethacin group ($P < 0.05$). The biological implication of this finding is uncertain. After 16 weeks P_{lin} was increased in the indomethacin group ($P < 0.01$). This change was furthermore accompanied by an increase of $W_{P_{lin}}$ ($P < 0.05$) (Fig. 13), indicating an increased tensile strength in the indomethacin group.

There was no significant difference in tendon elongation ($\varepsilon_{P_{lin}}$), nor were there any

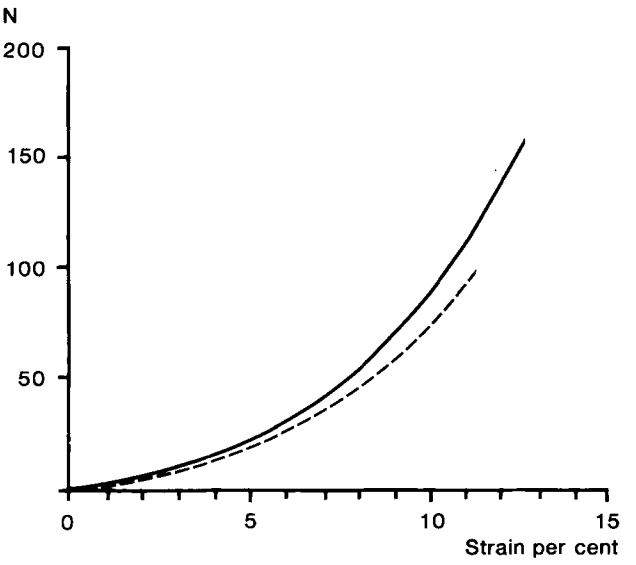


Figure 13. Load-strain curves according to the mean value of the parameters a_1 and a_2 in the formula $p = a_1\varepsilon + a_2\varepsilon^3$ for the indomethacin group (——) and the placebo group (– – –) after a healing period of 16 weeks. The curves are cut at the mean values of P_{lin} respectively.

differences in initial stiffness (a_1) or rate of stiffness increase (a_2) between the groups.

There were only minor differences in the biochemical parameters between the groups. However, there was a slight but significant decrease in E1 (relatively immature collagen) in the indomethacin group (Table 11, Appendix 1).

Discussion

Mathematical model (I)

Derivation of the function $P = a_1\varepsilon + a_2\varepsilon^3$

$$\text{gives} \quad \frac{dp(\varepsilon)}{d\varepsilon} = a_1 + 3a_2\varepsilon^2$$

$$\text{if} \quad \varepsilon = 0$$

$$\text{then} \quad \frac{dp(0)}{d\varepsilon} = a_1$$

The parameter a_1 corresponds to the tangent at the starting point (preload) of the curve. Furthermore, a_1 is a sensitive indicator of changes in the toe region. Such changes may be due to (1) actual differences in biomechanical properties, or (2) an excessively high setting of the preload (i.e. the curves have already diverged at the preload). In either case a significant difference in the a_1 parameter between two groups should suggest a more careful investigation of the toe-region.

The initial length of the tendons was defined at a certain preload (Viidik 1980b). This preload was set as low as was technically possible (3 mm above the calibrated zero-load line on the XY-recorder) and thus the absolute value of the preload varied with different gains. By using a fixed recorder indication rather than a specific force value, we have attempted to compensate for different tendon strengths as the gain differed for strong and weak tendon groups. (The registration was made from the preload to P_{lin}.)

The preload for all tendons was $2.60 \pm 0.97\%$ of the P_{lin}. The preload was set low in order to obtain as much information as possible for the graph. If it had been set even lower, a significant inaccuracy in the length definition of the tendon would have occurred (a zero-point definition fault), as the graph reaches the deformation axis asymptotically (Fig. 14). Thus, another potentially troublesome source of error may occur during the recording of load-deformation curves with the digitizer. This fault is aggravated by a too-low preload. However, the integral-to-specific-load value (W_n) is very insensitive to zero-point definition faults (Fig. 15).

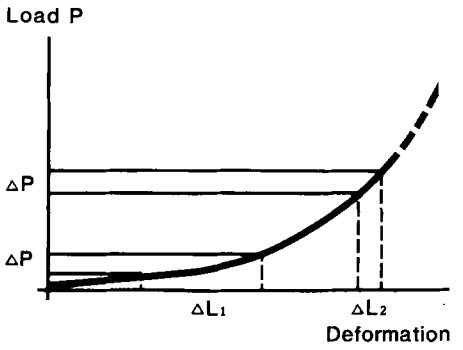


Figure 14. The effect of different preloads of the error in length determination (Δ1). At a given error in load determination, (ΔP)Δ1 becomes greater when the preload decreases.

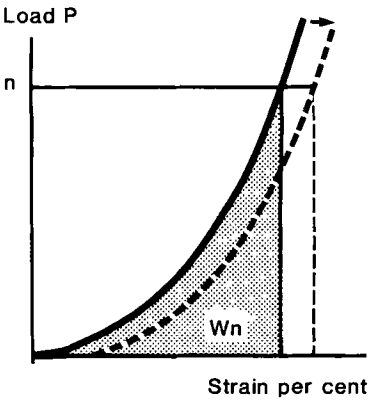


Figure 15. Effect of zero-point definition fault on the W_n parameter. A zero-point definition fault results in a parallel displacement of the curve which has very little influence on the integral of the curve to a specific load value (W_n).

Thus, a true biomechanical difference between two curves reflected in, for example, the a_2 parameter should be accompanied by a difference in W_n. Otherwise a zero-point definition fault should be suspected.

Statistical analysis of biomechanical properties and comparison between different curves or groups of curves may be performed using the parameters a_1 and a_2 or their mean values. In the statistical analysis care should be taken not to compare groups with widely diverging preloads. This may alter the shape of the load-strain curve and thereby introduce false differences in the biomechanical parameters. Other biomechanical parameters, such as load, stiffness (the derivative) and energy consumption (the integral) at different chosen strain points are easily calculated using the parameters a_1 and a_2.

The function of $P = a_1\varepsilon + a_2\varepsilon^3$ (Carlstedt and Skagervall 1986) is an empirically derived mathematical model which gives several benefits compared to previous mathematical models. The empirically derived and earliest presented mathematical model for tendons $\varepsilon^2 = c_1\sigma^2 + c_2\sigma$ by Wertheim (1847) was not tested, mainly because it is difficult to translate its constants into mechanical equivalents. Morgan (1960) proposed the function $\varepsilon = c_3\sigma^{0.812}$ for isolated tendon fibers. Elden (1968) proposed the function $\sigma = c_4\varepsilon^2$. The latter function followed experimental data from rat tail tendons for $\varepsilon = 0$–0.02 very closely. In a preliminary investigation a function was tested which is a general form of the two latter formulas ($\sigma = c_1\varepsilon^{c_2}$) (see Appendix 2, page 58). When applied to our material for a strain range of 0–7.5 %, it resulted in unsatisfactory curve fitting indicated by a low value of fraction of explained variance (FEV). The two empirically derived formulas

$$E = c_5 + c_6 \log L$$

for the initial phase (toe-region) and

$$E = c_7 + c_8 L^{c_9}$$

for the second phase ("linear region") presented by Ridge and Wright (1965) were also considered. The equations may only be used in separate strain regions. The statistical comparisons may only be used in separate strain regions. The statistical comparison between whole stress/load-strain sequences becomes complicated. Furthermore, the equations use altogheter five different constants. The function $P = a_1\varepsilon + a_2\varepsilon^3$ offers a simpler alternative and a possibility for direct statistical comparison of the whole load-strain curve. Fung's (1967) "tangent modulus" as a function of stress $dT/d\lambda$ vs. T ($dP/d\varepsilon = k \times P$) (see Appendix 2, page 58) was also tested (Table 12). This gives less good curve fitting, indicated by a significant lower value of FEV. However, the "tangent modulus" was developed for analysis of the toe-region of stress-strain curves from rabbit mesentery, and rabbit mesentery has a very pronounced toe-region (which is not the case in our material of extremity tendons). Recent investigators (Danielsen 1982) have used Fung's "tangent modulus" as a function of strain $dP/d\varepsilon = c_{10} \times \varepsilon$. However, an integration (see Appendix 2, page 59) gives

$$P = c_{10}\frac{\varepsilon^2}{2} + c_{11}$$

and this function is analogous to the function $\sigma = c_4\varepsilon^2$ presented by Elden (1968) which has already been tested on our material (see above). Some of the functions tested on our material are presented in Appendix 2, page 59).

A disadvantage of the function

$$P = a_1\varepsilon + a_2\varepsilon^3 \text{ (P)}$$

is the absence of theoretical framework. However, the parameters a_1 and a_2 can be identified as the initial stiffness and the rate of stiffness increase respectively as the initial stiffness and the rate of stiffness increase respectively. Furthermore, the function P gives very good curve fitting and has a high flexibility as shown by the FEV value 0.996 (SD 0.002) for all curves within a large range of strength (P_{lin} 8.73–306.2 N) shown in

Table 4. By using the a_1 parameter the function P also makes it possible to determine when an excessively high preload level has been chosen and the consequent dislocation of the strain scale to a higher range, thus preventing the loss of interesting information in the low range. A significant difference in a_1 should be taken as an indication for a more careful investigation of the toe-region. In such a study we recommend the use of a lower preload (by using a larger gain) and that the mathematical analysis be complemented by Fung's "tangent modulus".

Table 12. Fraction of explained variance (FEV) for 10 randomly selected load-strain curves to a strain value of 2.5 % (within the toe-regon) and to strain value of 7.5 % when using Fung's "tangent modulus" as a function of stress $dT/d\lambda$ vs. T ($dP/d\varepsilon = k \times P$) and the function $P = a_1\varepsilon + a_2\varepsilon^3$ (P).

No	$\varepsilon = 2.5\,\%$		$\varepsilon = 7.5\,\%$	
	"Tan. mod."	P	"Tan. mod."	P
1	0.900	0.998	0.891	0.995
2	0.986	0.991	0.906	0.996
3[1]	0.472	0.991	0.502	0.997
4	0.924	0.993	0.900	0.997
5	0.886	0.994	0.833	0.995
6	0.923	0.994	0.904	0.996
7	0.895	0.998	0.921	0.998
8	0.912	0.986	0.868	0.994
9	0.790	0.985	0.893	0.993
10	0.992	0.981	0.953	0.997
Group/median	0.906	0.992**[2]	0.897	0.996***[2]
Common median	0.986		0.973	

[1] The load-strain curve for this tendon was of a different shape which explains the low FEV value.
[2] Statistical significance was tested by median test; ** = $P < 0.01$, *** = $P < 0.001$.

Compared to using all the coordinates for the stress/load-strain curve in statistical analysis, the use of only two parameters (a_1 and a_2) gives a great data reduction and thus a considerable simplification of the statistical analysis. The parameters a_1 and a_2 also reflect the whole stress/load-strain curve, not isolated observations.

However, other biomechanical parameters, such as load, stiffness (the derivative) and energy consumption (the integral) at specific strain values are easily calculated using the parameters a_1 and a_2. The parameters a_1 and a_2 may, of course, also be correlated to biochemical and morphometrical factors such as collagen content, body weight, etc.

In this investigation the tendons represented a wide range of biomechanical properties (strength), thus indicating that the function P may be used for biomechanical analysis of other tendons besides the plantaris longus tendon of the rabbit.

Tendon elongation (II)

Previous reports on tendon elongation after surgical repair are contradictory. Nyström and Holmlund (1983) found a larger and typical biphasic separation with a plateau du-

ring weeks 2 and 3. They explained this with muscular paralysis secondary to surgery and therefore discussed the possibility of the very short fixation time, e.g. 1 week, with plaster of Paris. Ejeskär (1982) found a gradual but small separation of flexor tendons after surigical repair which is in accordance with our findings (Carlstedt and Wredmark 1984).

The tendon elongation after surigical repair is a result of tension in the tendon despite immobilization of the joints affecting the tendon. This elongation may be due to inadequate immobilization, muscular tonus, resorption of the tissue on the pressure side of the suture, suture insufficiency (type of suture material and suture technique) or a combination of these factors. Nyström and Holmlund (1983) found that immobilization for 7, 16 and 35 days resulted in a very small early separation and similar separation patterns for the whole observation period (35 days). It has also been suggested by Nyström and Holmlund (1983) that large early separation results in impaired tendon healing. Consequently they suggested a very short immobilization time (7 days) as adequate for a good result for tendon healing. However, this suggestion was not tested biomechanically.

The present investigation (Carlstedt and Wredmark 1984) does not demonstrate any biphasic separation pattern and consequently the idea of a very short immobilization period is not supported. However, only two animals were sacrificed and measured at each observation time, and as the individual variation in this experimental model is considerable, the result must be considered with care. However, Mason and Allen (1941) found that healing occurs despite a progressive tendon separation and that the holding power of the immobilized tendon for the suture at first diminishes. At two days the strength is only about one-fifth of that present immediately after suture, and in 14 days the strength is equal. This does not either support a very short immobilization time (7 days).

Conservative and surgical treatment (III)

No consistent differences in biomechanical properties (i.e. strength, stiffness, energy uptake) or in tendon elongation were found between the group treated with both suturing and immobilization (surgical treatment) and that treated with immobilization alone (conservative treatment). Nor was there any consistent difference in total hydroxyprolin content, the amount of mature and of immature collagen or water content. More mature (less soluble) collagen results in increased tensile strength on account of more cross-linkage (Piez 1968). As we found no biomechanical or biochemical differences (i.e. in collagen solubility) between the two groups, results are compatible.

In an experimental study on sutured dog tendons, Mason and Allen (1941) found that the tensile strength exhibited three phases parallel with the phases of healing: (1) A phase of exsudation and fibrinous union. In this phase tensile strength diminishes as a result of wound edema, which lasts about five days. The strength is primarily due to the suture. (2) A phase of fibroplasia. The tensile strength now increases, reaching a plateau on about the 16th day. (3) A phase of maturation, organization and differentiation. This phase probably begins between the 19th and 21st days, after which tensile strength continues to increase for an undertermined period of time. Mason and Allen (1941) also reported that the suture mostly prevented tendon elongation in the initial

phase (0–5 days). This is in accordance with Nyström and Holmlund (1983) who reported a large early elongation with conservative treatment (plaster cast only, no suture). They also suggested that conservative treatment (yielding large early elongation) resulted in impaired tendon healing. Our study does not corroborate this suggestion, as we did not find any biomechanical or biochemical differences between the conservatively and the surgically treated groups. However, our results are in accordance with Nyström and Holmlund (1983) in the respect that no difference in final tendon elongation between the groups was registered (Table 7, Appendix 1). In our material the tendon elongation appeared to be less than the values reported by Nyström and Holmlund (1983) for Achilles tendons in the rabbit (about 35 mm after 35 days). Our results are also in accordance with the good clinical results of conservative treatment of Achilles tendon ruptures in homo (Gillies and Chalmers 1970; Lea and Smith 1972; Nistor 1981).

This study (Carlstedt et al. 1986a) does not indicate any advantages (improved tendon healing) with surgical treatment, since conservative and surgical treatment resulted in equal biomechanical properties. This suggests interesting clinical possibilities of conservative treatment of tendon ruptures. However, in the experimental model used, the effectiveness of unloading for tensile forces of the tendon healing region is uncertain. In this respect the experimental mode is analogous to the Achilles tendon in homo but not to tendons where effective unloading by immobilization is obtained.

The influence of indomethacin on developing tendon (IV)

The increase in tensile strength in the plantaris longus tendons after indomethacin treatment is not in accordance with the inhibitory effect of indomethacin on bone formation (Almåsbakk and Røysland 1977; Rø et al. 1978b; Sudmann 1975; Sudmann and Bang 1979; Sudmann et al. 1979; Sudmann and Hagen 1976). However, our samples were not subjected to any post-traumatic state. Interesting observations are those of Rø et al. (1978) and Elves et al. (1982) who found after indomethacin treatment of rats an increased hydroxyproline synthesis and increased fibrogenesis respectively in association with fractures.

Furthermore, our results (Carlstedt et al. 1987) are in accordance with Vogel (1977) who found an increased tensile strength in rat tail tendons after indomethacin treatment. He also observed an increase in the proportion of insoluble collagen and in total collagen content. Our results are also in accordance with Ohkawa (1982) who investigated the effect of indomethacin on the mechanical strength of the periodontum in the rat and found an increase in ultimate loads following indomethacin treatment.

The physical properties of collagen are highly dependent on cross-links within and between the collagen molecules. During maturation the number and quality of the cross-links increase resulting in increased tensile strength and decreased collagen solubility (Piez 1968, Vogel 1978, Viidik et al. 1982). A possible mechanism for the indomethacin effect could be by accelerating the collagen maturation. Indomethacin is a well-known inhibitor of prostaglandin synthesis (Samuelsson 1974), but at the present moment it is not clear whether indomethacin exerts its actions on connective tissue through this pathway.

An increase in cross-sectional area in the indomethacin group would naturally explain the increased strength. However, the tendons were photographed in two perpendicular projections prior to the biomechanical test and the cross-sectional area in the middle of the sample was measured. The diameters *a* and *b* of the tendons were measured with a magnifier using the photographs. The cross-sectional area was calculated by using the formula for area determination of an ellipse. After 16 weeks, the cross-sectional area of the plantaris longus tendons in the placebo group was 8.81 ± 0.93 mm^2 and in the indomethacin group 8.15 ± 1.88 mm^2. Thus the increase in strength in the indomethacin group must be explained by the material properties (i.e., increased strength of the connective tissue).

The influence of indomethacin on tendon healing (V)

The increased tensile strength after indomethacin treatment in healing plantaris longus tendons contrasts with the effect of indomethacin on bone healing, where indomethacin exerts an inhibitory effect (Sudmann 1975, Rø et al. 1976, Sudmann and Hagen 1976, Almåsbakk and Røysland 1977, Sudmann and Bang 1979, Sudmann et al. 1979, Törnkvist et al. 1984). However, Rø et al. (1978a) in an *in vitro* study of callus tissue from closed femoral fractures in rats treated with indomethacin found a significantly increased hydroxyprloine synthesis. They also reported a significantly increased incorporation of hydroxyproline into collagen.

Furthermore, our results (Carlstedt et al. 1986b) are in accordance with Vogel (1977), who found an increased tensile strength in rat tail tendons after indomethacin treatment. He also observed an increase in the proportion of insoluble collagen and in the total collagen content, but no significant change in the glycosaminoglycan concentration. The latter finding is in accordance with a report of chondrocyte cell culture experiments that indomethacin did not influence the synthesis of proteoglycans (Palmoski and Brandt 1980).

The exact mechanism through which indomethacin acts on connective tissue is unclear. Indomethacin is a well-known inhibitor of prostaglandin synthesis (Samuelsson 1974). Investigations of prostaglandin effects on fibroblast collagen synthesis have given conflicting results (Raisz and Koolemans-Beynen 1974, Parnham et al. 1982). Studies of the effect of indomethacin on fibroblast collagen synthesis are also conflicting (Winter 1965, Kulonen & Potila 1975). The reason for this apparent conflict may the be different indomethacin concentrations used by these investigators.

In a study by Carlstedt et al. (1986c) the influence of indomethacin on collagen synthesis in intact and healing plantaris longus tendons in the rabbit was investigated by using ^{3}H-proline. Indomethacin affected the collagen metabolism differently depending on whether or not the tendons were involved in wound healing. In intact tendons the drug caused a slight general inhibition of collagen synthesis. In the healing tendons there was a shift towards synthesis of more insoluble collagen following indomethacin treatment with little effect on the total synthesis. After 4 weeks there was also a slight but significant decrease in the amount of hydroxyproline in the most soluble collagen fraction from the latter group. The mode of action is unclear, however. Indomethacin is a well-known inhibitor of cyclooxygenase. It is possible that the drug affects collagen metabolism by

this mechanism. Another possibility is that indomethacin interferes with the synthesis or activity of lysyl oxidase, influencing the rate of cross-link formation.

The effects of indomethacin on healing tendon thus appears to be similar to those changes produced by maturation. A possible mechanism for the action of indomethacin on tendon healing may be an accelerated maturation.

Appendix 1

Tables

Table 5. Results of the biomechanical analysis.

Healing time	4 weeks				8 weeks				16 weeks			
	Sut./immobil.		Immobil.		Sut./immobil.		Immobil.		Sut./immobil.		Immobil.	
	n10		n9		n12		n10		n11		n12	
Parameters	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
P_{lin}	33.6	16.1	21.9	8.0	81.3	21.3	94.5	23.7	101.3	34.9	94.3	48.4
Wp_{lin}	2.07	1.4	1.07	0.56	4.2	1.4	4.9	1.6	5.2	2.0	4.8	3.4
εp_{lin}	15.2	4.1	12.0	3.5	13.9	2.3	14.5	2.3	13.5	2.1	13.0	3.3
a_1	58.8**	16.5	83.3	19.4	207.4**	56.6	156.6	35.7	270.5	93.9	257.	80.7
a_2	13129	5606	10490	5233	31370	10630	38840	11720	46610	16520	41070	7320

Statistical comparisons of the two groups were made after 4, 8 and 16 weeks by analysis of variance; logarithmically transformed data (*=$p<0.05$, **=$p<0.01$).

Table 6. Results of the biochemical analysis.

Healing time	4 weeks				8 weeks				16 weeks			
	Sut./immobil.		Immobil.		Sut./immobil.		Immobil.		Sut./immobil.		Immobil.	
	n9		n9		n12		n10		n11		n12	
Parameters	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
E1 (µg/mg dw)	4.2	3.7	4.0	2.9	3.6	3.3	3.1	2.4	3.4	4.0	3.5	1.6
E2 (µg/mg dw) + E3 (µg/mg dw)	74.4	32.8	66.0	28.8	82.5	9.7	82.3	10.7	80.4*	26.0	102.9	19.2
R (µg/mg dw)	11.8	23.4	5.6	13.6	2.7	3.2	3.3	3.3	12.8	25.6	2.1	4.7
T_{hyp} (µg/mg dw)	90.5	42.4	75.7	33.8	89.1	8.5	88.8	10.0	95.1	15.4	109.6	19.4
aq (%)	71.8	7.5	69.9	10.0	74.4	2.7	62.1	19.4	63.6	8.7	61.9	16.1

For legend see Table 5.

Table 7. The tendon elongation in the experimental groups after healing times of 4, 8 and 16 weeks.

Healing time (weeks)	Suture/immobilization (mm)			Immobilization (mm)		
		$\bar{x}$	SD		$\bar{x}$	SD
4	n10	12.90	6.02	n9	15.67	4.66
8	n12	10.58	3.60	n10	10.00	2.58
16	n11	14.82	4.83	n11	18.73	5.53

Statistical comparisons of the groups were made by analysis of variance; logarithmically transformed data.

Table 8. Results of the biomechanical analysis after 16 weeks of indomethacin treatment (10 mg/kg/day).

Parameter		Indomethacin n11		Placebo n11	
		$\bar{x}$	SD	$\bar{x}$	SD
P_{lin}	(N)	237.1	56.67	169.9	44.02
Wp_{lin}	(N%)	12.31*	3.799	7.334	2.669
εp_{lin}	(%)	13.41	2.2	12.05	1.46
$\tan\alpha_{2.5}$	(N/%)	769.1	272.9	572.8	137.8
$\tan\alpha_5$	(N/%)	1522*	471.9	1092	212.8
$\tan\alpha_{7.5}$	(N/%)	2776*	835.7	1958	368.3
a_1		518.4	219.2	399.7	122.2
a_2		133800*	40350	92320	18230
W_{65N}	(N%)	1.530*	0.1777	1.716	0.1046

Statistical comparisons of the two groups were made after 4, 8 and 16 weeks by analysis of variance; logarithmically transformed data. Only after 16 weeks were there significant differences. Therefore data for 4 and 8 weeks are not included. (*=$p<0.05$).

Table 9. Results of the biochemical analysis after 16 weeks of indomethacin treatment (10 mg/kg/day).

Parameter	Indomethacin n11		Placebo n11	
	$\bar{x}$	SD	$\bar{x}$	SD
E1 (µg/mg dw)	1.21	1.24	4.569	6.945
E2 (µg/mg dw) + E3 (µg/mg dw)	99.17	19.26	91.14	12.34
R (µg/mg dw)	17.96	18.60	13.82	15.88
T_{hyp} (µg/mg dw)	118.0	17.84	109.5	11.21
aq (%)	50.34	16.18	52.44	7.271

Statistical analysis was performed as described in Table II. There were no significant differences between the groups. E_1, E_2, E_3, and R represent collagen extracts of decreasing solubility. The values represent hydroxyproline as estimated by the method of Stegeman and Stalder (1967). T_{hyp} = total hydroxyproline content.

Table 10. Results of the biomechanical analysis.

Healing time	4 weeks				8 weeks				16 weeks			
	Indomethacin n8		Placebo n10		Indomethacin n10		Placebo n12		Indomethacin n11		Placebo n11	
Parameters	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
P_{lin} (N)	27	10	33	16	58*	22	81	21	159**	50	101	34
Wp_{lin} (N%)	1.4	0.8	2.0	1.4	3.0	1.9	4.2	1.4	9.8*	4.9	5.2	2.0
εp_{lin} (%)	13	3	15	4	12	3	13	2	15	3	13	2
a_1	66	32	58	16	184	60	207	56	295	43	270	93
a_2	11660	4212	13120	5606	27290	10380	31370	10630	59180	14140	46610	16520

Statistical analysis was performed between the two groups after 4, 8 and 16 weeks by analysis of variance, logarithmically transformed data (*=$p<0.05$, **=$p<0.01$).

Table 11. Results of the biochemical analysis.

Healing time	4 weeks				8 weeks				16 weeks			
	Indomethacin n8		Placebo n10		Indomethacin n10		Placebo n12		Indomethacin n11		Placebo n11	
Parameters	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
E1 (µg/mg dw)	1.3*	1.6	4.2	3.7	3.0*	4.0	3.6	3.3	1.4*	1.7	3.4	4.0
E2 (µg/mg dw) + E3 (µg/mg dw)	76.8	67.8	74.4	32.8	79.3	7.6	82.5	9.7	80.4	16.8	80.4	26.0
R (µg/mg dw)	4.1	6.0	11.8	23.4	2.3	3.3	2.7	3.2	25.0	25.4	12.8	25.6
T_{hyp} (µg/mg dw)	82.3	68.2	90.5	42.5	84.7	8.5	89.1	8.5	106.9	19.1	95.1	15.4
aq (%)	65.9	15.4	71.8	7.5	75.1	3.8	74.4	2.7	63.3	12.3	63.3	8.7

For legend see table 10.

Appendix 2

Mathematical equations

Morgan (1960) $\varepsilon = c_3\sigma^{0.812}$ (1)

Elden (1968) $\sigma = c_4\varepsilon^2$ (2)

Tested general form $\sigma = c_1\varepsilon^{c_2}$ (3)

(2) = (3)

$\sigma = c_4\varepsilon^2$ (2)

is equal to $\sigma = c_4\varepsilon^{n_1}$

rewritten $\sigma^{\frac{1}{n_1}} = c_4^{\frac{1}{n_1}} \cdot \varepsilon^{\frac{n_1}{n_1}}$

gives $\sigma^{\frac{1}{n_1}} = c_4^{\frac{1}{n_1}} \cdot \varepsilon$

gives $\varepsilon = \frac{1}{c_4^{\frac{1}{n_1}}} \cdot \sigma^{\frac{1}{n_1}}$

gives $\varepsilon = c_5\sigma^{\frac{1}{n_1}}$

gives $\varepsilon = c_5\sigma^{c_6}$ (4)

(4) = (2)

gives (4) = (3)

gives (4) = (3) = (2)

Fung's "tangent modulus" as a function of stress

$$\frac{dp}{dc} = K \cdot P \quad (1)$$

An integration gives

$$P = C \cdot e^{K \cdot \varepsilon} + k_1 \quad (2)$$

If $k_1 = 0$

then $\ln P = \ln C + K \cdot \varepsilon$ (3)

Equation (3) was tested on our material and resulted in a significantly lower FEV (I), compared to use of the function $P = a_1\varepsilon + a_2\varepsilon^3$.

$$P = C \cdot e^{K \cdot \varepsilon} + k_1 \quad (2)$$

if $\varepsilon = -\infty$

then $k_1 = P =$ the preload

An additional analysis was performed in which to each load value (P) the preload was added (then if $\varepsilon = -\infty$, gives $k_1 = 0$). This analysis also resulted in a lower FEV value in each case (compared to use of the function $P = a_1\varepsilon + a_2\varepsilon^3$).

Danielsen (1982) $\frac{dp}{d\varepsilon} = c_{10} \cdot \varepsilon$

An integration gives $p = c_{10} \cdot \frac{\varepsilon^2}{2} + c_{11}$

if $c^{11} = 0$

then $p = c_{10} \cdot \frac{\varepsilon^2}{2}$

gives $p = c_{12} \cdot \varepsilon^2$ (1)

is equivalent to Elden (1968) $\sigma = c^4\varepsilon^2$ (2)

$(1) = (2)$

Some of the functions tested:

$$P = a_1\varepsilon + a_2\varepsilon^2 \quad (1)$$

High dependency between the parameters.

$$P = a_1\varepsilon^2 \quad (2)$$

Low value of FEV.

$$P = a_1\varepsilon^3 \quad (3)$$

Low value of FEV.

$$P = a_1\varepsilon + a_2\varepsilon^2 + a_3\varepsilon^3 \quad (4)$$

Many parameters.

$$P = a_1\varepsilon^{1.5} \quad (5)$$

Low value of FEV.

$$P = a_1 e^{a_2\varepsilon} \quad (6)$$

Low value of FEV.

$$P = a_1\varepsilon^{a_2} \quad (7)$$

Low value of FEV.

Conclusions

1. The equation $P = a_1\varepsilon + a_2\varepsilon^3$ (P = load, ε = strain, a_1 = linear parameter, a_2 = cubic parameter) is a useful function for biomechanical analysis of the plantaris longus tendon in the rabbit. The function has a very good fit and a high degree of flexibility, indicating its usefulness for analysis of other tendon specimens.
2. Indomethacin treatment increases the tensile strength in developing and healing plantaris longus tendons in the rabbit.
3. The mechanism through which indomethacin increases the tensile strength in the plantaris longus tendon in the rabbit is probably through an increased cross-linkage in the collagen.
4. Tendon healing as measured by biomechanical and biochemical parameters is not improved by surgical treatment compared to non-surgical (conservative) treatment.

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