Division of flexor tendons causes progressive degradation of tendon matrix in rabbits

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Deep flexor tendons of 30 rabbits were divided at the ankle level. The effects of unloading on the synthesis and content of matrix components, the synthesis of DNA, and dry weight were investigated. The reaction of the fibrocartilaginous and non-fibrocartilaginous segments were separately analyzed.

The ability of the tendons to synthesize collagen during short-term culture and the contents of matrix components decreased inversely to the time of unloading. 12 weeks following division, the fibrocartilaginous segments had lost 2/5 of their dry weight, 2/3 of proteoglycan and 1/3 of collagen and non-collagen protein content. Less pronounced losses were observed in the non-fibrocartilaginous segments. A transient increase in cell proliferation in both types of segments was found. These findings indicate that divided flexor tendons undergo a progressive degradation, which may have implications for delayed suture of deep flexor tendon injuries.

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The effects of a delay between flexor tendon injury and repair are controversial (Ejeskär 1980, Matev et al. 1980, Gelberman et al. 1991). A number of conditions, including adhesion formation between tendon and surrounding tissues and soft tissue remodeling, affect the decision when to perform surgery and the outcome. However, the possibility of fundamental cellular and metabolic changes in the distal, unloaded part of divided tendons has not been investigated.

The cellular activities and matrix metabolism in tendons are continuously regulated by interactions of a number of factors, including loading conditions (Koob and Vogel 1987, Okuda et al. 1987, Abrahamsson 1991). At the level of the metacarpophalangeal joint, where compressive and tensional forces act on the intrasynovial deep flexor tendon, true tendon tissue transforms into an area of fibrocartilaginous-like tissue (proximal segment). At the level of the proximal phalanx, where tensional forces act on the intrasynovial deep flexor tendon, true tendon tissue is present (intermediate segment; Okuda et al. 1987, Abrahamsson et al. 1989). Disturbances of the normal interaction of forces—e.g., in dislocated, denervated or immobilized limbs—may to some extent affect collagen turnover and content of glycosaminoglycans (Akeson and LaViolette 1964, Gillard et al. 1979, Amiel et al. 1982).

We have investigated the cellular and biochemical effects of unloading in fibrocartilaginous and non-fibrocartilaginous segments of the intrasynovial region of divided deep flexor tendons.

Animals and methods

Tissue preparation

All procedures were performed under sterile conditions in an animal-operating facility. 30 New Zealand White rabbits, weighing 2.5–3.5 kg, were used. The rabbits were anesthetized with an intramuscular dose of Hypnorm® and were given 1 prophylactic intravenous dose against infections: Zinacef®. The deep flexor tendons (FDP) of both hindpaws of each animal were divided at the ankle level. Following skin suture and soft dressing, all animals were mobilized without restrictions.

On the day of surgery and at 10 days, 3, 6 and 12 weeks postoperatively, two 7 mm tendon sheath-covered segments, 1 proximal (volar to the metatarsophalangeal joint) and 1 intermediate (volar to the proximal phalanx), were excised from 2 deep flexor tendons of each of 6 rabbits (Figure 1; Abrahamsson et al. 1989). Thus, tendon segments of 6 animals were represented in each experimental group. The segments were collected from unexplored, intrasynovial parts of the flexor tendons at the level of the phalanges, approximately 12 cm distal to the level of the division. All segments were rinsed in physiological saline solution before culture in vitro.

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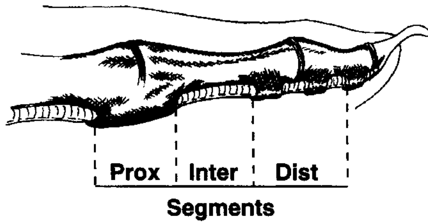

Figure 1. The intrasynovial deep flexor tendon in the region of the tendon sheath of rabbit illustrating 3 distinct segments: the proximal is volar the metatarsophalangeal joint, the intermediate is volar the proximal phalanx and the distal segment is volar the middle phalanx. The proximal and intermediate segments were used in this study.

Tendon culture and labeling protocols

The tendon segments were placed in 24 multi-dish plates, 1 segment per well (A/S Nunc, Roskilde, Denmark) and 1 mL of medium MCDB 105 (Sigma Chemical CO; McKeehan et al. 1978), supplemented with gentamicin (50 μg/mL), ascorbic acid (50 μg/mL) and bovine serum albumin (1 mg/mL), was added to each well. Tendon segments were preincubated for 24 h at 37 °C in a water-saturated atmosphere of 2% CO_2.

When matrix synthesis was studied, on the second day, the medium was replaced with fresh medium containing fetal calf serum (10%). On the third day, the procedure was repeated and tendon segments were labeled for 24 h with 3H-proline (10 μCi/mL) and 35S-sulfate (40 μCi/mL; Radiochemical Centre, Amersham, U.K.). Following labeling, the segments were rinsed once and chase-incubated twice for 30 minutes in medium supplemented with L-proline (50 μg/mL).

When hexosamine content was studied, a protocol was used like that above, but the tendon segments were labeled only with 35S-sulfate (40 μCi/mL).

When cell proliferation was examined, on the second day, the supplemented medium was replaced with fresh medium containing fetal calf serum (10%) and non-radioactive thymidine (50 μg/mL; Puzas and Brand 1986). On the third day, the procedure was repeated and tendon segments were labeled for 24h with 3H-thymidine (10 μCi/mL; Radiochemical Centre, Amersham, U.K.). Following labeling, the segments were rinsed once and chase-incubated twice for 30 minutes in medium supplemented with nonradioactive thymidine (50 μg/mL).

All tendon segments were then stored at –20 °C for later analysis. Dry weight was determined after lyophilization.

Determination of incorporation rates

Dried tendons labeled with 3H-proline and 35S-sulfate were hydrolyzed and then separated on a column of Aminex A6 eluted with citrate buffer (BioRad; Abrahamsson 1991). Radioactivity in peaks corresponding to 35S-sulfate, 3H-hydroxyproline and 3H-proline were measured with a radioactivity flow detector (Radiomatic Instruments and Chemical Co; Lohmander et al. 1976). Results of incorporation were expressed as disintegrations per minute per milligram of dry weight tendon tissue (dpm/mg dwt). The macromolecular content of 35S-sulfate and 3H-hydroxyproline were used as measures of the new synthesis of proteoglycan and collagen, respectively. The incorporation of 3H-proline, corrected for the known ratio of proline for hydroxyproline in collagen, represents the de novo synthesis of non-collagen protein (Diegelmann and Peterkofsky 1972). Percentage collagen represents the proportion of collagen synthesized from total protein synthesis.

Tendons labeled with 3H-thymidine were dissolved in potassium hydroxide and nucleic acids were precipitated by adding trichloroacetic acid (Abrahamsson 1991). The amount of 3H-thymidine was counted in a scintillation counter and the content was used as a measure of the new synthesis of DNA (cell proliferation). The results were expressed as dpm/mg dwt.

Quantification of hexosamines, hydroxyproline and proline

Hexosamine levels, indicative of proteoglycan content, were determined as total amounts of glucosamine and galactosamine. Following extraction and hydrolyzation, the glycosaminoglycans were separated on an Aminex A9 column (BioRad), eluted with phosphate buffer (Abrahamsson 1991). The hexosamine content was evaluated by the 2-cyanoacetamide procedure, using standards of glucosamine and galactosamine, and the results were expressed as μg/mg dwt (Lohmander 1986).

Hydroxyproline levels, representing collagen content, were determined colorimetrically after hydrolysis of samples and expressed as μg/mg dwt (Stegemann and Stalder 1967).

The α-amino acid content, reflecting protein levels, was determined by the Ninhydrin procedure after hydrolysis of the samples and was expressed as μg/mg dwt (Moore and Stein 1948).

Statistical analysis

Linear regression was calculated by the method of least squares. Unless stated otherwise, the results are presented as mean (standard deviation). In order to be able to express the magnitude of changes in each type of tendon segment, the rates of day 10–week 12 of

proximal and intermediate segments, respectively, were expressed in relation to those of day 0. Statistical significance of differences between the 2 groups of rates (proximal and intermediate), expressed in relation to those of day 0, was then tested by the Student t-test. Statistical significance of differences between a control group (day 0) and multiple groups (day 10–week 12 groups) was tested by Dunnet's test (ANOVA). A value of $p < 0.05$ was considered significant.

Results

Synthesis of proteoglycan, collagen and non-collagen protein and cell proliferation (Figures 2–4)

The mean rates of proteoglycan synthesis (per dry weight tendon) at each interval in proximal and intermediate segments, respectively, showed no significant differences from those of day 0 ($p > 0.05$). The mean of the rates, expressed in relation to those of day 0, did not differ significantly between the 2 types of tendon segments ($p = 0.2$).

The rates of collagen synthesis in proximal and intermediate segments at 12 weeks decreased to 43% and 13% of those of the controls, respectively ($p < 0.05$, $p < 0.05$). The mean of the rates of collagen synthesis, expressed in relation to those of day 0, did not differ statistically between the 2 types of segments ($p = 0.2$).

The rates of non-collagen protein synthesis in proximal segments did not differ significantly from that of day 0, but in intermediate segments a transient increase to 125% of that of the controls was found ($p > 0.05$, $p < 0.05$). The rates of non-collagen protein synthesis, expressed in relation to those of day 0, also did not differ significantly between the 2 types of segments ($p = 0.3$).

The percentage collagen synthesized (as a proportion of the total protein) in proximal tendon segments decreased from 4.8 (2.8) to 1.3 (0.9)% at 12 weeks ($p < 0.05$) and in intermediate segments from 4.6 (3.4) to 0.8 (0.5)% at 12 weeks ($p < 0.05$).

The rates of cell proliferation (per tendon dry weight) increased transiently up to 8 and 6 times of those of the controls in proximal and intermediate segments, respectively ($p < 0.05$). The mean rates of cell proliferation, expressed in relation to those of day 0, did not differ significantly between the 2 types of segments ($p = 0.06$).

Hexosamines

The mean content of galactosamine (per tendon dry weight) in proximal segments at 12 weeks decreased to 55% of that of the controls, but the mean content in intermediate segments did not differ significantly from that of day 0 ($p < 0.05$, $p > 0.05$). The relative rates of galactosamine content of all intervals did not differ between the 2 types of tendons ($p = 0.09$). The mean contents of glucosamine in each type of segment did not differ statistically from that of day 0

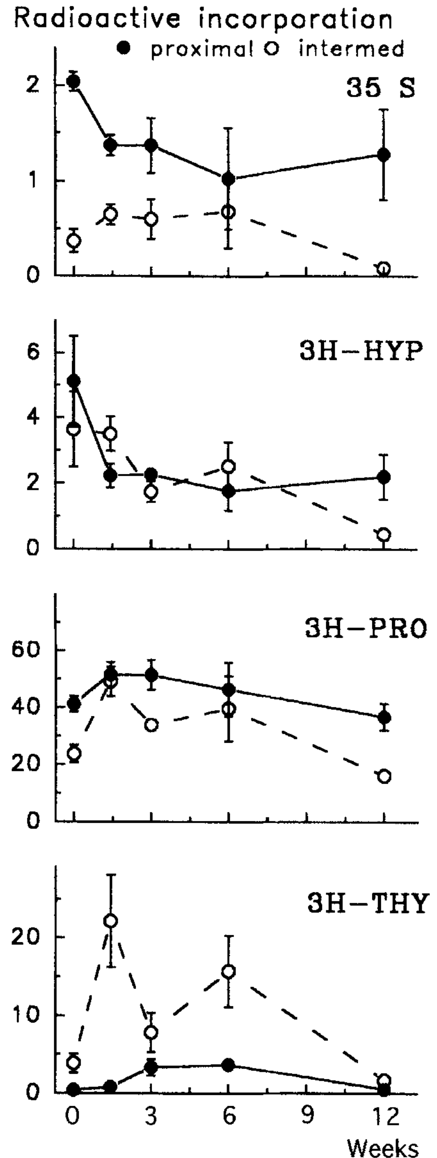

Figure 2. Synthesis of proteoglycan (A; 35S), collagen (B; 3H-Hyp), non-collagen protein (C; 3H-Pro), and DNA (D; 3H-Thy) in proximal (●)and intermediate (O) intrasynovial deep flexor tendons at various intervals following tendon transection. Values are presented as mean radioactive uptake per milligram dry weight tendon (10^3 dpm/mg dwt) ± SEM (n 6).

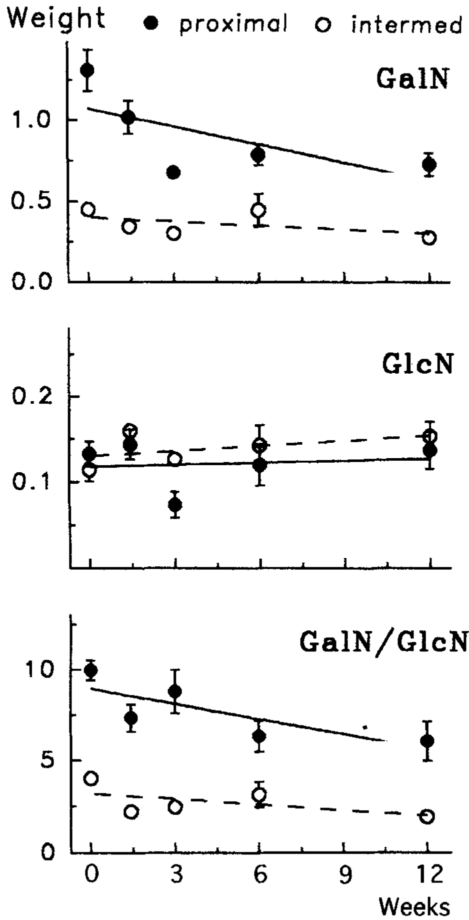

Figure 3. Total amounts of galactosamines (A; GalN) and glucosamines (B; GlcN) and ratio of GalN/GlcN (C; GalN/GlcN) in proximal (●)and intermediate (O) segments of intrasynovial deep flexor tendons of rabbits at various intervals following proximal tendon transection. Hexosamine values are given as microgram per milligram dry weight tendon (μg/mg dwt), mean ± SEM (n 6).

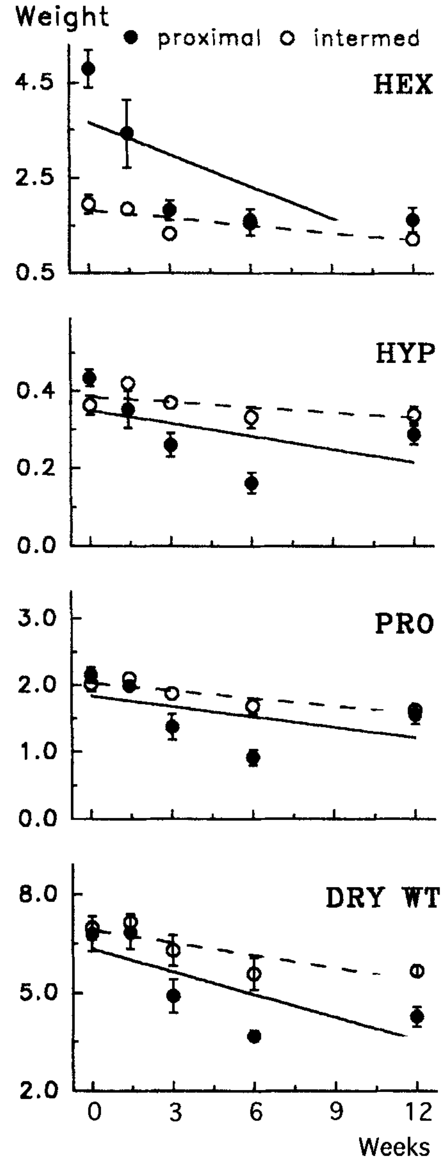

Figure 4. Total amounts of hexosamines (glucosamine and galactosamine; A; HEX), hydroxyproline (B; HYP), α-amino nitrogen protein (C; PRO) and tendon dry weight (D; DRY WT) in proximal (●)and intermediate (O) segments of intrasynovial deep flexor tendons of rabbits at various intervals following proximal tendon transection. Hexosamine values are given as microgram per tendon segment (μg/tendon), hydroxyproline, α-amino nitrogen protein and dry weight as milligram per tendon segment (mg/tendon), mean ± SEM (n 6).

($p > 0.05$, $p > 0.05$) and the mean rates of contents, expressed in relation to those of day 0, did not differ between the 2 types of segments ($p = 0.100$). The ratio between galactosamine and glucosamine (GalN/GlcN) in proximal and intermediate segments at 12 weeks decreased to 60% and 40% of those of the controls, respectively ($p < 0.05$, $p < 0.05$), but the mean rates of GalN/GlcN ratios, expressed in relation to those of day 0, did not differ significantly between the two types of segments ($p = 0.2$).

While the mean content of total hexosamines (galactosamine and glucosamine per tendon segment) in proximal and intermediate segments at 12 weeks decreased to 34% and 75% of those of the controls, respectively ($p < 0.05$, $p < 0.05$), the mean rates of total hexosamines, expressed in relation to those of day 0, differed significantly between the 2 types of segments ($p = 0.001$). Expressed per milligram of dry weight of tendon, the mean content in both types of tendon segments decreased significantly with time ($p < 0.05$, $p < 0.05$).

Hydroxyproline, proline and tendon dry weight

The mean collagen content (hydroxyproline per tendon segment) in proximal segments at 12 weeks decreased to 66% of that of the controls, but in intermediate segments did not differ from that of controls ($p < 0.05$, $p > 0.05$). Thus the mean rates of collagen content, expressed in relation to those of day 0, differed between the 2 types of tendons ($p = 0.001$). Expressed per mg/dwt, however, the mean content in both types of segments did not differ from those of day 0 ($p > 0.05$, $p > 0.05$).

As the mean content of protein (α-amino nitrogen per tendon segment) in proximal and intermediate segments at 12 weeks decreased to 71% and 81% of controls, respectively ($p < 0.05$, $p < 0.05$), the mean rates of protein content, expressed in relation to those of day 0, differed significantly between the 2 types of segments ($p = 0.001$). Expressed per milligram dry weight tendon, however, the mean content in proximal and intermediate tendon segments, respectively, did not differ significantly from those of day 0 ($p > 0.05$, $p > 0.05$).

The mean tendon dry weight (per tendon segment) in proximal and intermediate segments at 12 weeks decreased to 62% and 81% of controls, respectively ($p < 0.05$, $p < 0.05$). Thus the mean tendon dry weight in proximal tendon segments, expressed in relation to those of day 0, differed from that of intermediate tendon segments ($p = 0.02$).

Discussion

Following division, fibrocartilaginous and non-fibrocartilaginous segments in the unloaded, distal part of the deep flexor tendons sustain significant quantitative and qualitative chemical modifications. We observed a transient increase in cell proliferation, progressive changes in content and composition of matrix components and a decrease in weight. These results indicate that divided tendons undergo a progressive degradation, comparable to that of unloaded bone (Smith et al. 1992). Amiel and collaborators (1982), who studied immobilized patellar tendons of younger animals, however, observed an increased collagen turnover without any reduction in tendon mass.

We found that the collagen synthesis (as a proportion of total protein) and the galactosamine/glucosamine ratio decreased with time in unloaded tendon segments. These changes in matrix composition were caused by a combined decrease in collagen synthesis and a maintenance of non-collagen protein synthesis and a combined decrease in galactosamine content and maintenance of glucosamine content, respectively. Similar modifications in the synthesis of matrix components have also been observed during tendon culture in vitro and may reflect the early stages of tendon development and healing in vivo (for ref. see Abrahamsson 1991). Although the various types of collagen and proteoglycan have not been identified in this study, our results may indicate that parts of the tendon matrix reorganize and are replaced by "immature" tissue following division and subsequent unloading.

The intrasynovial deep flexor tendon contains fibrocartilaginous-like areas, the location and characteristic morphology and chemistry of which are caused by compressive forces acting on them (Okuda et al. 1987, Abrahamsson et al. 1989). In this study, we observed that proximal segments, containing fibrocartilaginous areas, were more sensitive to unloading and showed a more pronounced reduction in matrix constituents and dry weight than that of non-fibrocartilaginous intermediate segments. Gillard and co-workers (1979), who studied contents of glycosaminoglycans in various regions of translocated rabbit tendons, also observed a more pronounced reduction in fibrocartilaginous than in non-fibrocartilaginous regions. The rate of cell proliferation, however, did not differ between the 2 segments of intrasynovial flexor tendons.

Compared to those of controls, the rate of DNA synthesis increased 6–8 times in proximal and intermediate segments, indicating a similar relative capacity of cell proliferation in both types of segments. The absolute levels of DNA synthesis, however, differed significantly between the two types of segments, with proximal tendon segments having 5–10 times lower rates of 3H-thymidine uptake than those of intermediate segments. As we did not measure DNA content, these differences could be attributed to differences in the number of cells as well as activity of each cell or a combination of both. In the dog, however, DNA content and number of cells vary only slightly between various regions and types of tendons (Okuda et al. 1987, Ark et al. 1994).

The increased rate of DNA synthesis in both types of segments correlated with the increased rates of metabolism and "immature" reorganization that we observed in the tendon matrix. A comparable transient increase in DNA synthesis has also been observed in rat tail tendons in vitro and in rabbit cartilage of immobilized knee joints in vivo (Slack et al. 1986, Smith et al. 1992). The increased cellular activity thus may be secondary to the division and unloading of tendons.

Since the formation of adhesions between the tendon and surrounding tissues is related to the cellular activity of the tendon as well as that of the surrounding tissues, tendons expressing higher rates of cell proliferation in vitro theoretically would be more prone to form adhesions in vivo. This likelihood has been illustrated in 2 recent studies, where the cellular activities of extrasynovially-derived tendon grafts were compared with those of intrasynovial grafts (Ark et al. 1994, Abrahamsson et al. 1995). Extrapolated from our present results, which showed an increased rate of cell proliferation, divided flexor tendons may show an increased tendency to form adhesions. This has also been found in an experimental study by Gelberman and collaborators (1991).

In addition to an altered load, altered vascular supply and tissue response to laceration may affect the metabolism and cell proliferation in intrasynovial regions of divided flexor tendons. Division of proximal intratendinous vessels, however, may be of less significance since the intrasynovial and distal parts of deep flexor tendons are supported via intact vincular and distal intratendinous vessels. Within the tendon sheath region, tendon nutrition by diffusion is far more important than by vessels (Hooper et al. 1984, Manske and Lesker 1985). In order to avoid possible side-effects of the laceration, the division was performed at the level of the ankle and explants were harvested at the level of the toes. The incorporation of 3H-proline during healing is inversely proportional to the distance from the repair site (Birdsell et al. 1966). Partial unloading of tendons—e.g., following immobilization, dislocation or limb suspension—results in minor changes in tendon matrix metabolism (Akeson and LaViolette 1964, Gillard et al. 1979, Amiel et al. 1982).

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References

Abrahamsson S-O. Matrix metabolism and healing in flexor tendon. Experimental studies on rabbit tendon. Scand J Plast Reconstr Surg Hand Surg (Suppl 23) 1991.

Abrahamsson S-O, Lundborg G, Lohmander L S. Segmental variation in microstructure, matrix synthesis and cell proliferation in rabbit flexor tendon. Scand J Plast Reconstr Surg Hand Surg 1989; 23 (3): 191-8.

Abrahamsson S-O, Gelberman R H, Amiel D, Winterton P, Harwood F. Autogeneous flexor tendon grafts. Fibroblast activity and matrix remodeling in dogs. J Orthop Res 1995; 13 (1): 58-66.

Akeson W H, LaViolette D F. The connective tissue response to immobility. Total mucopolysaccharide changes in dog tendon. J Surg Res 1964; 4 (11): 523-8.

Amiel D, Woo SL-Y, Harwood F L, Akeson W H. The effect of immobilization on collagen turnover in connective tissue: A biochemical-biomechanical correlation. Acta Orthop Scand 1982; 53: 325-32.

Ark J, Gelberman R H, Abrahamsson S-O, Seiler J G III, Amiel D. Cellular survival and proliferation in autogenous flexor tendon grafts. J Hand Surg 1994; 19A: 249-58.

Birdsell D C, Tustanoff E R, Lindsay W K. Collagen production in regenerating tendon. Plast Reconstr Surg 1966; 37 (6): 616-9.

Diegelmann R F, Peterkofsky B. Collagen biosynthesis during connective tissue development in chick embryo. Dev Biol 1972; 28: 443-53.

Ejeskär A. Flexor tendon repair in No Man's Land. II. Early versus late secondary tendon repair ad modum Kleinert. Scand J Plast Reconstr Surg Hand Surg 1980; 14: 279-83.

Gelberman R H, Siegel D H, Woo SL-Y, Amiel D, Takai S, Lee D. Healing of digital flexor tendons: Importance of the interval from injury to repair. A biomechanical, biochemical and morphological study in dogs. J Bone Joint Surg (Am) 1991; 73 (1): 66-75.

Gillard G C, Reilly H C, Bell-Booth P G, Flint M H. The influence of mechanical forces on the glucosaminoglycan content of the rabbit flexor digitorum profundus tendon. Conn Tissue Res 1979; 7: 37-46.

Hooper G, Davies R, Tothill P. Blood flow and clearance in tendons. Studies with dogs. J Bone Joint Surg (Br) 1984; 66 (3): 441-3.

Koob T J, Vogel K G. Proteoglycan synthesis in organ cultures from regions of bovine tendon subjected to different mechanical forces. Biochem J 1987; 246: 589-98.

Lohmander L S. Analysis by high-performance liquid chromatography of radioactively-labeled carbohydrate components of proteoglycans. Anal Biochem 1986; 154: 75-84.

Lohmander L S, Moskalewski S, Madsen K, Thyberg J, Friberg U. Influence of colchicine on the synthesis and secretion of proteoglycans and collagen by fetal guinea pig chondrocytes. Exp Cell Res 1976; 99: 333-45.

Manske P R, Lesker P A. Flexor tendon nutrition. Hand Clin 1985; 1B (1): 13-24.

Matev I, Karagancheva S, Trichkova P, Tsekov P. Delayed primary suture of flexor tendon cut in the digital theca. Hand 1980; 12: 158-62.

McKeehan W L, Genereux D P, Ham R G. Assay and partial purification of factors from serum that control multiplication of human diploid fibroblasts. Biochem Biophys Res Commun 1978; 80 (4): 1013-21.

Moore S, Stein W H. Photometric Ninhydrin method for use in the chromatography of amino acids. J Biol Chem 1948; 176: 367-88.

Okuda Y, Gorski J P, An K-N, Amadio P C. Biochemical, histological and biomechanical analyses of canine tendon. J Orthop Res 1987; 5: 60-8.

Puzas J E, Brand J S. The effect of bone cell stimulatory factors can be measured with thymidine incorporation only under specific conditions. Calcif Tissue 1986; 39: 104-8.

Slack C, Bradley G, Beaumont B, Poole A, Flint M H. Changes in the morphology and synthesis of cultured rat tail tendon. Cell Tissue Res 1986; 245: 359-68.

Smith R L, Thomas K D, Schurman D J, Carter D R, Wong M, van der Meulen M C. Rabbit knee immobilization: Bone remodeling precedes cartilage degradation. J Orthop Res 1992; 10: 88-95.

Stegemann H, Stalder K H. Determination of hydroxyproline. Clin Chim Acta 1967; 18: 267-73.