# Effect of transcutaneous electrical stimulation on the hydroxyproline content in severed superficial digital flexor tendon in horses

Sharifi, D.[1*], Kazemi, D.[2], Rassouli, A.[3], Shams,G.R.[3]

[1]Department of Surgery and Radiology, 2Department of Clinical Sciences Islamic Azad University Tabriz, Tabriz-Iran. [2]Department of Physiology, Pharmacology and Toxicology, Faculty of Veterinary Medicine,University of Tehran, Tehran- Iran.

***Abstract:*** Transcutaneous electrical stimulation (TES) has recently been used in resolving experimentally severed superficial flexor tendon which leads to acute tendonitis. However, mechanism by which (TES) promote tendon repair is not fully understood. In this study 4 right superficial flexor tendon of hind limb of horses were subjected to daily therapeutic regimen of TES for 10 minutes for 15 days using 100 Hz frequency and 80 s intensity ( 2.8 – 0.6 mA) after being severed in full length thickness in total length of 10 cm in the mid- tendon area and similarly in another 4 right tendon in control horses no treatment was applied. Tendons were subjected to ultrasonographical, histological and, tensile strength evaluation which has been reported separately for publication and hydroxyproline content for 60 days after treatment. There were remarkable differences between hyroxyproline contents of severed tendon with those of normal ones in control group, whereas there was not significant differences in treated tendons with those of normal ones in the same horses. This study suggests that using hydroxyproline content as a direct marker of the effect of TES on collagen content in injured tendon. *J.Vet.Res. 62,2:43-47,2007.*

**Key words:** tendon,TES,tensile strength, horse.

## Introduction

The strength of superficial flexor tendon (SDF) is quite essential and even a slight change in the tissue may lead to severe lameness and make a risk factor for rupture during exercise ( 7,22). In the tendon, type I collagen is considered to be responsible for the mechanical strength of the tendon tissue and type III collagen for healing process (15,16). Degenerative changes and excessive mechanical forces may be considered as additional uncontrolled reasons for unnoticed rupture of this tendon (12, 13, 14). Even so ultrasonography has shown micro tears in the middle part of the tendon in association with tendon disorders (8, 10). The animals in our study were compared to the hydroxyproline content in untreated tendon with a treated one along with TES to have final correlation between functional activity and clinical signs of severed tendon for early exercise planning.

## Materials and Methods

The experiment complied with the University of Tehran,and Law on Animal Experiments and was approved by The Faculty of Veterinary Medicine and University of Tehran Research Council. The study was conducted on eight castrated horses of having 350 to 500 Kg BW with 5 years of age. The SDF of the right hind limb was exposed under deep general anesthesia and was splitted completely in full thickness in longitudinal fashion in about 10 cm in length in the mid-tendon area using BP blade no. 24 in each one, then the area was closed as routine. These animals were divided into two groups of control

*Corresponding author's email: dsharifi@vetmed.ut.ac.ir, Tel: 021-66920035, Fax: 021- 66933222

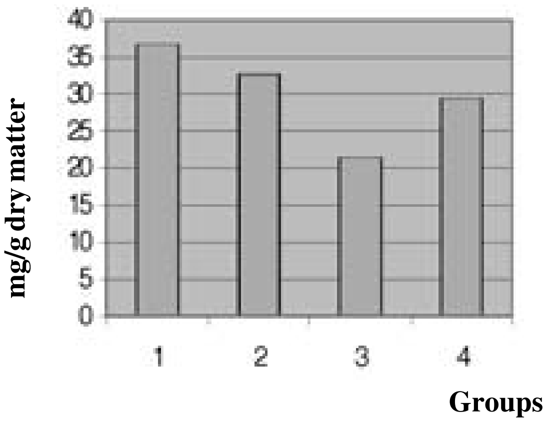


**Figure 1: Statistical differences of hydroxyproline between normal with that of control tendon.**

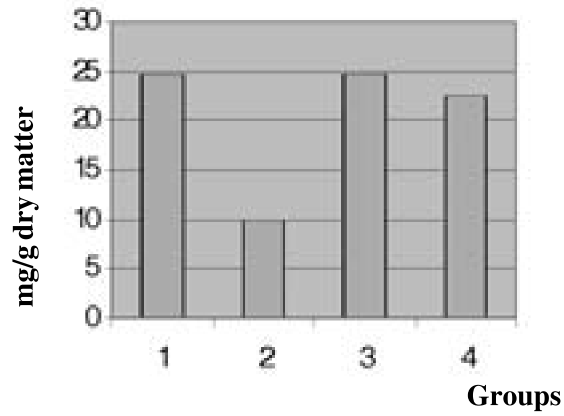


**Figure 2: Differences of hydroxyproline between normal With that of TES treated tendon.**

(untreated) and experiment (treated with TES) having 4 animals in each group. Treated group was subjected to daily therapeutic regiment of TES for 10 minutes for 15 days using 100 Hz frequency and 80 Us intensity (2.8 – 0.6 mA). The hydroxyproline concentration was measured by modified spectrophotometer method (24) by collecting samples from mid splitted area of injured and normal tendons of each animal from treated and untreated groups 60 days after surgery. The collected data were analyzed with student's T-test at $p<0.05$) as a significant level.

## Results

The SDF tendon severed completely in longitudinal fashion and splitted using B.P.blade no. 24 were in the worst shape of tendon rupture lengthwise. The degree of lameness showed by individual horse (8 horses) was almost identical due to the similarity of the lesions. Lameness was most apparent during the first 3 days post surgery and gradually improved in the treated limb until the time of euthanasia on the $60^{th}$ day. Swelling varied between individual horses but it was less severe in all the treated limbs. The hydroxyproline content was significantly different between untreated tendon and that of normal limb of the same animal in control group (Fig. 1).

Whereas this difference was quite less when treated tendon was compared with a normal limb (Fig. 2) of the same animal. In treated group, there was a marked increase in hydroxyproline content of treated tendon using TES as compared to the values of the control ones (Fig. 3).

## Discussion

The purpose of this experiment was to test the hypothesis that TES of severed tendonwould promote healing of the lesion in horses. The stimulated limb showedhigher level of clinical satisfaction and functional behavior on the second week of treatment as compared to untreated limbs. The introduction of TES for successful treatment of different disorders in the musculoskeletal system and repair of lesion have been reported (2,15,20,21,23). Most of the reports have postulated the effect of TES to be provocative of a painful level that finally achieves pain relief or analgesia through hyper stimulation and increased vascularity (21,23,26), however, due to the widespread use of conservative treatment, the effects of TES on tendon remain poorly understood (6,12). This work assessed the effect of TES experimentally induced tendopathy in a traumatic model. The stimulated limbs reached the higher level of healing as compared to the untreated ones. The differences of hydroxyproline content, between normal tendon and untreated one, were quite significant ($p<0.05$), whereas there were very narrow differences with those of treated limb. The treated limb received TES treatment resolved inflammation, increased tenocytes proliferation and restored tendon integrity, leading to restoration of biomechanical

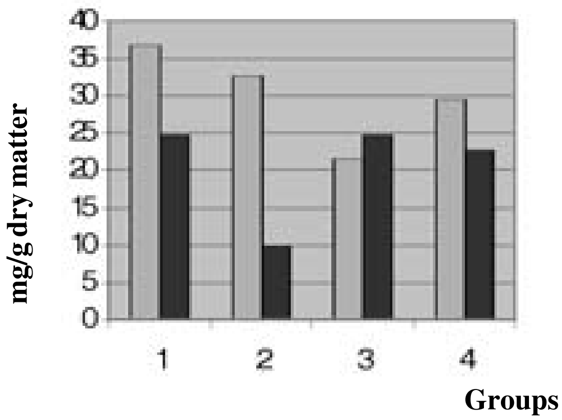


**Figure 3: Differences of hydroxyproline between control (untreated) with that of TES (treated) tendon.**
**Series 1, ( Mean±SEM) of hydroxyproline in control Tendon and series 2 ,( Mean±SEM) of hyroxyproline In treated tendon.**

properties correlated to clinical signs of having full limbs weightbearing. The findings in this study provided the first evidence that TES promotion of SDF tendonitis repairs and the similar findings were being reported by other authors to evaluate the effect of shock wave therapy (SW) on adult tendon in rats, which leads to increase in mitogenic and anabolic responses of tendon tissue that bring about the clinical success of using SW treatment in resolving tendonitis (27). The etiology of tendonitis are multifactor including avascular changes, degenerative changes and metabolic disturbances, neural factors and neovascularization (1,2,9,27,30). The acute swelling, inflammation and matrix destruction in tendon are similar to those seen in naturally occurring tendon injuries (3,4,9 28). Increasing fibroblast proliferation and biosynthesis of extra cellular matrix and collagen are crucial stages for the return of normal tendon strength (5,9,26). In the present study, TES treatment significantly reserves DNA and glycoaminoglycan and hydroxyproline content, increased tenocyte growth, and tissue regeneration indicated that some growth factors were responsible for initiating the TES induced mitogenic and morphogenic response of injured tendon (17,18,19 30). Others have shown that electrical stimulation improves the stress (21) in this study using a single modality treatment which demonstrated greater magnitudes of improvement (maximum load), stress and energy absorption. It also caused a notable increase in the collagen content around the incised tendon length (25,28,29,31) which is a similar finding reported in rats.

Application of TES shown to have beneficial effects when applied to healing tendon. There was a significant increase in collagen production with a model of an accelerated rate of collagen turn over. In the treated tendon. It had a direct effect on biochemical properties of the tissue. Early studies by Vogel (1974), Parry *et al.,* (1978)and Flint *et al.,* (1984) observed a correlation among collagen fibril organization, size and number of biochemical strength. These previously published paper studies relied on the collagen profiles of mature tendons, whereas in this study, the amount of regenerating tendons in the relationship between biomechanical strength and the absolute amount of collagen determined the biochemical assays for hydroxyproline content. The results of this study reported here, indicated that direct application of TES on severely injured or extensive lesion of tendon SDF in horses will be highly useful in enhancing hydroxyproline content in the treated tendon.

## Acknowledgments

Authors would like to thank members of the Faculty of Veterinary Medicine and university of Tehran Research Council for the approval and financial support of this extensive research work.

## References

1. Ackerman,P.W., Ahmed,M. and Kreicbergs, A. (2002) Early nerve regeneration after Achilles tendon rapture-A prerequisite for healing. A study in the rat. J.Orthop.Res. 20:pp.849-856.
2. Ackerman, P.W., Jain, L., Finn, A., Ahmed,M. and Kreicbergs,A.(2001) Autonomic innervation of tendon ligaments and joint capsules a morphologic and quantitative study in the rat. J.Orthop.Res.19: pp. 372-378.
3. An, K.N., Berglund,L., Coonery,W.P., Chao, E.Y.(1990) Kovacevic.N.Direct in vivo tendon force measurement System. J.Biomech. 23:1269-1271.
4. Chan, B.P., Fu, Sc., Oin, L., Rolf, C. and Chan,

K.M.(1998) Pyridinoline in relation to ultimate stress of the patellar tendon during healing an animal study.J.Orthop.Res. 16: pp. 597-603.

5. DesRosiers,E.A., Yahia,L. and Rivard, C.H. (1996) Proliferative and matrix synthesis response of canine anterior cruciate ligament fibroblast submitted to combined growth factors. J.Orthop.Res. 14: pp. 200-208.
6. Davison, C.J., Ganion, L.R., Gehlsen, G.M., Verhoestra, B., Roepke, J.E. and Sevier, T.L.(1997) Rat tendon morphologic and functional change resulting from soft tissue mobilization. Med.Sci. Sports. Exerc. 29: pp. 313-319.
7. Enwemeka, C.S., Rodriguez, O. and Mendosa, S.(1990) The biomechanical effects of low - intensity ultrasound on healing tendons.Ulltrasound Med.Biol. 16:pp.801-807.
8. Enwemeka, C.S.(1989) The effects of therapeutic ultrasound on tendon healing. Am. J. Phys. Med. Rehabil. 68: pp.283-287.
9. Enwemeka, C.S.(1992) Functional loading augments the initial tensile strength and energy absorption capacity of regenerating rabbit Achilles tendons. Am. J. Phys. Med. Rehabil. 71: pp.31-38
10. Farkas, L.G., Herbert, M.A. and James, J.S.(1980) Peritendinous healing after early movement of repaired flexor tendon; Anatomical study; Ann. Plast. Surg. 5: pp.298-304.
11. Flint, M.H., Craig, A.S., Reilly, H.C.(1984) Collagen fibril diameter and glycosaminoglycan content of skin: indices of tissue maturity and function. Connect Tissues Res. 13:pp.69-81.
12. Gelberman, R., Goldberg,V., An, K.N. and Banes, A.J.(1988) Tendon in: S.L-Y.Wob and J.A. Buckwalter.Editors, Injury and repair of the musculoskeletal soft tissue. American Academy of the Orthopaedic Surgeons, Park Ridge. pp.5-40.
13. Gigante,A., Specchia,N., Rapali,S., Ventura,A. and de Palma, L.(1996) Fibrillogesis in tendon healing; An experimental study. Boll. Soc. Ital. Biol. Sper. 72: pp. 203-211.
14. Goodship, A.E., Birch, H.L. and Wilson, A.M.(1994) The pathobiology and repair of tendon and ligament injury.Vet.Clin. North. Am. (Equine Pract). 10: pp. 323-349.
15. Gum, S. L., Reddy,G., Kesava,S., Britel, L.E., Emwemeka,C.S., Chukuka, S.(1997) Combined ultrasound, electrical stimulation, and laser promote collagen synthesis with moderate change in tendon biomechanics.Am.J.Phys.Med. Rehabil. 76: pp. 288-296.
16. Heidia, E., Ari, P., Leppilahti, J. R. (2002) Increased content of type III collagen at the rupture site of human Achilles tendon. J. Orthop. Res. 20: pp. 1352-1357.
17. Linda, A., Dahlgreni, A., Nixon, J. and Brent D. (2001) Brower-Toland. Effects of B-aminopropionitrile on equine tendon metabolism in vitro and on effects of insulin -like growth factor-I on matrix production by equine tenocytes. AJVR. 62: pp.1557-1562.
18. Linda, A., Dahlgren, M., John, E.A., Bertram, G. and Alan, J.N.(2002) Insulin-like growth factor -I improves cellular and molecular aspects of healing in a collagenase-induced of flexor tendonitis. J. Orthop. Res. 20: pp.910-919.
19. Murphy, D.J. and Nixon, A.J.(1997) Biochemical and site -specific effects of insulin -like growth factor -I on intrinsic tenocyte activity in equine flexor tendons. Am.J.Vet.Res. 58: pp.103-109.
20. Nessler, J.P. and Mass, D.P.(1987)Direct - current electrical stimulation of tendon healing in vitro.Clin.Orthop. 217: pp.303-312.
21. Peter, B., Ramses, F., Adelheid, S., Edwin, V.O., Marleen, P. and Rene, V.(2003) Influence of burst TENS stimulation on the healing of Achilles tendon suture in man.Acta Orthopaedic Belgica. 69: pp.528-531.
22. Parry,D.A.D., Barnes, G.R.G., Graig,A.S.A.(1987) comparison of the size distribution of collagen fibrils in connective tissue as a function of age and the possible relation between fibril size distribution and mechanical properties. Proc. R. Soc. Lond (Biol) 203: 305-321.
23. Peter, B., Ramses, F., Adelheid, S., Edwin, V.O., Marleen, P. and Rene, V.(2005) Influence of burst TENS stimulation on collagen formation after Achilles tendon suture in man. A histological

evaluation with Movat,s Pentachrome stain. Acta Orthopaedic Belgica.71: pp. 342-346.

24. Podenphant, J., Larsen, N.E. and Christiansen, I. (1984) An easy and reliable method for determination of urinary hydroxyproline. Clinica.Acta. 142: pp. 145-148.
25. Reddy, G.K. and Enwemeka, C.S.(1996) A simplified method for the analysis of hydroxyproline in biological tissue.Clin Biochem. 29: pp. 225-229.
26. Reddy, G., Kesava, G. S., Stehno,B. L., Enwemeka, C.S.(1998) Biochemistry and biomechanics of healing tendon: Part II. effects of combined laser therapy and electrical stimulation. Med. Sci. Sports. Exerc. 30: pp.794-800.
27. Robert, W. H., Wei-H, H., Ching-L, T. and Kam-F. L.(2004) Effect of shock -wave therapy on patellar tendinopathy in a rabbit model. J. orthop. Res. 22: pp. 221-227.
28. Saperia, D., Glassberg, E., Lyons, R.F.(1984) Demonstration of elevated type I and type III procollagen mRNA levels in cutaneous wounds treated with helium-neon laser, Biochemical. Biophys. Res. Commun. 138: pp.1123-1128.
29. Vogel, H.G.(1974) Correlation between tensile strength and collagen content in rat skin Effect of age and cortisol treatment. Connect Tissue Res. 2: pp.177.
30. Woessner, J.F., Jr.(1961) The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Archives of Biochemistry and Biophysics. 93:pp. 440-447.
31. Yeung-J, C., Ching-J, W., Kuender, D.Y.,Yur-R, K., Hui-C, H., Yu-T, H., Yi-C, S. and Feng-S, W. (2004) Extracorporeal shock waves promote healing of collagenase- induced Achilles tendonitis and increase TGF-B1 and IGF-I expression. J. Orthop. Res. 22: pp.854-861.