WILEY InterScience® DISCOVER SOMETHING GREAT

VEGF, VEGFR-1, and CTGF Cell Densities in Tendon Are Increased with Cyclical Loading: An In Vivo Tendinopathy Model

Leena H. Nakama,[1] Karen B. King,[2] Sven Abrahamsson,[3] David M. Rempel[1,2]

[1]University of California, Berkeley and San Francisco, Joint Graduate Group in Bioengineering, 1301 South 46th Street, Bldg 163, Richmond, California 94804

[2]University of California, San Francisco, Department of Medicine and University of California, Berkeley and San Francisco, Joint Graduate Group in Bioengineering

[3]Malmo University Hospital, Malmo, Sweden, Department of Hand Surgery

Received 7 June 2005; accepted 5 August 2005

Published online 6 January 2006 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jor.20053

ABSTRACT: Tendon injuries can occur in athletes and workers whose tasks involve repetitive, high-force hand activities, but the early pathophysiologic processes of tendinopathy are not well known. The purpose of this animal study was to evaluate the effects of cyclical tendon loading on the densities of cells producing growth factors such as vascular endothelial growth factor (VEGF), its receptor, vascular endothelial growth factor receptor 1 (VEGFR-1), and connective tissue growth factor (CTGF) in the Flexor Digitorum Profundus (FDP) tendon at the epicondyle. The FDP muscles of nine New Zealand rabbits were electrically stimulated to contract repetitively for 80 h of cumulative loading over 14 weeks. The contralateral limbs served as controls. The tendons at the medial epicondyle insertion sites were harvested, and sections were immunostained with antibodies directed against VEGF, VEGFR-1, or CTGF. Positive-staining cells were counted in six regions of interest: three along the enthesis, and three corresponding regions 1500 microns distal to the enthesis. VEGF ($p = 0.0001$), VEGFR-1 ($p = 0.046$), and CTGF ($p = 0.0001$) cell densities were increased in the tendon of the loaded limb compared to the nonloaded limb. In addition, regional differences in VEGF, VEGFR-1, and CTGF cell densities were found. VEGF, VEGFR-1, and CTGF are increased in tendon experiencing cyclical loading and may play a role in the early vascular changes in the progression to tendinosis. © 2006 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 24:393–400, 2006

Keywords: VEGF; CTGF; in vivo loaded tendon

INTRODUCTION

Tendon injuries due to overuse are a common problem in athletes and workers, and account for 30 to 50% of all sports-related injuries[1,2] and almost half of the occupational illnesses in the United States.[3] Epicondylitis, a tendinopathy at the elbow, is a common disorder in adults; the incidence in general practice is approximately 4 to 7 per 1000 patients per year, with an annual incidence of 1 to 3% in the general population.[4,5] Although epicondylitis is related to forceful and repetitive hand activities, little is known about the early mechanisms of injury that ultimately lead to tendinopathy. Identifying the initial biological changes in tendons exposed to cyclical loading may ultimately improve prevention and treatment options and further expand our understanding of the etiology of tendinosis and its pathogenesis.

Epicondylitis presents as localized pain, tenderness, and occasionally swelling.[6] Biopsies of the tendon and surrounding scar tissue in patients with epicondylitis reveal fibrovascular and cellular proliferation, intratendinous calcification and

Correspondence to: David Rempel (Telephone: 510 665-3403; Fax: 510-665-3423 E-mail: drempel@itsa.ucsf.edu)

© 2006 Orthopaedic Research Society. Published by Wiley Periodicals, Inc.

cartilage formation, loss of parallel tendon fibers, fibrofatty degeneration, partial tendon rupture, and the formation of capillary buds.[7–12] The absence of inflammatory cells has led some authors to propose the term tendinosis instead of tendonitis.[8,13,14]

Vascular Endothelial Growth Factor (VEGF), also known as Vascular Permeability Factor for example, from ruptured Achilles Tendons (VPF), is one of the most important angiogenic components of tissue healing. VEGF has been found in human biopsies of degenerated tendons, for example, from ruptured Achilles tendons[15–17] and in cyclically strained fibroblast cell cultures,[18] indicating that it may play a role in overuse injuries leading to tendon degeneration. VEGF stimulates the proliferation of microvascular endothelial cells, inducing angiogenesis and rendering the microvasculature hyperpermeable.[19,20] In the tendon, expression of VEGF can be upregulated by both mechanical, for example, cyclic strain,[18] and biochemical stimuli, for example, hypoxia,[21] and the presence of other growth factors.[21–23] Recent studies have shown that in an acutely injured tendon, the highest concentrations of VEGF occur after inflammation when it acts as a potent stimulator of angiogenesis.[24] The growth of new blood vessels towards the repair site from within the healing tendon appears necessary for healing to occur.

Several receptors for VEGF play important roles in pathological conditions involving angiogenesis. VEGFR-1, also known as Flt-1, and VEGFR-2, also known as Flk-1/KDR, are tyrosine kinase receptors for VEGF. VEGFR-1 has the highest affinity for VEGF165, one of the several isoforms of VEGF, with a dissociation constant (K_d) of approximately 10–20 pM.[25] VEGFR-2 has a lower affinity for VEGF, with a K_d of approximately 75–125 pM.[26] VEGFR-1 and VEGFR-2 have been observed in ruptured human Achilles tendons, but not in healthy adult tendons.[16] VEGFR-1 expression is upregulated during angiogenesis and hypoxic conditions, while VEGFR-2 is not.[27]

Connective Tissue Growth Factor (CTGF) has recently been investigated in wound healing and scar formation studies. CTGF is increased in the synovial sheaths of rats trained to do repetitive reaching,[28] but its role in tendon pathophysiology has not been well characterized. CTGF is a cysteine-rich secretory protein and belongs to the CCN family, which consists of six distinct members, CYR61, CTGF, and NOV ("CCN") and the Wnt-induced secreted proteins-1, -2, and -3.[29] The members of this group are known to be involved in many fundamental biological processes, including cell proliferation,[30] attachment,[31] migration,[29] differentiation,[32] wound healing,[33–35] matrix production,[30] and angiogenesis,[22,36] as well as in the development of several pathologic conditions, including fibrosis and tumorigenesis.[37] The role CTGF plays in tendon repair or degeneration is not yet known, but may involve stimulating angiogenesis and matrix production.[30,34] Its interaction with VEGF has not been investigated in the tendon; however, like VEGF, CTGF is known to increase in fibroblasts with mechanical loading.[38,39] Identifying the role or roles CTGF plays in tendon overuse injuries is important in understanding the underlying mechanisms involved in tendinopathy.

Clarifying the cellular and molecular pathways that occur during early periods of cyclical loading may lead to a better understanding of mechanisms associated with tendon injury and remodeling. A rabbit model of epicondylosis was used in which the Flexor Digitorum Profundus (FDP) muscle was repeatedly stimulated against a load.[40] The purpose of this study was to evaluate the regional variation of cells producing VEGF, VEGFR-1, and CTGF in the FDP tendon at the epicondyle in response to cyclical loading. We hypothesized that our in vivo loading model would increase the number of cells producing the aforementioned growth factors in the loaded limbs compared to nonloaded limbs of the same animal. The presence of these growth factors may play a significant role in the beginning phases of tendinosis.

MATERIALS AND METHODS

The animal loading model was described previously to establish microtear formation in a cyclically loaded tendon.[40] To summarize, nine female, young adult, New Zealand White rabbits weighing 3.49 kg (±0.30) were used. Under general anesthesia, the FDP muscle of one forelimb was electrically stimulated (Fig. 1) to contract repetitively for 2 h per day, 3 days a week, for 80 h of cumulative loading. The stimulation train was adjusted to maintain a mean peak digit flexion force of 0.42 N (15% of peak tetanic force). The contralateral limb, although supported in the same posture as the loaded limb during loading, did not receive a stimulus and served as the control. This study was approved by the University of California, Berkeley's Committee on Animal Research. Weekly examinations of the paw, forearm, and elbow revealed no tenderness, limping,

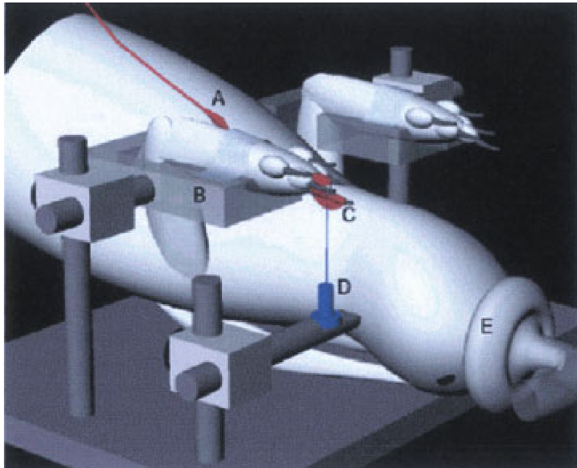

Figure 1. Cartoon of loading apparatus with rabbit in a supine position with head to the right and forearms supported. (A) Stimulation needle, (B) forearm support, (C) third digit with metal glove, (D) load cell, (E) anesthesia mask.

nodules, swelling, limitation in range of motion, reduction in gross claw flexion strength, or skin breaks.

After 80 h of cumulative loading, animals were weighed (3.89 ± 0.19 kg) and euthanized. Evaluation of the subcutaneous area at the stimulation needle insertion site revealed minimal scar tissue localized within 5 mm of the insertion site; the scar tissue did not extend to the FDP tendon. Both medial epicondyles were dissected with the FDP tendon and muscle attached, fixed in 10% formalin for 24 h, decalcified in EDTA for 3 weeks, paraffin embedded, and sectioned 7 μm longitudinally.

Nine serial sections from the center of the tendon block were deparaffinized and rehydrated. Sections to be stained for VEGF and VEGFR-1 were pretreated with hyaluronidase (600 units/mL, Sigma-Aldrich) and sections to be stained for CTGF were pretreated with trypsin (No. 00-3008, Zymed Laboratories), for 10 min at 37°C. Sections were then treated with 1% H_2O_2 in a phosphate-buffered solution (pH = 7.4) for 15 min to block endogenous peroxidase activity. Tissue sections were blocked with normal horse serum for 45 min at room temperature then incubated for 1 h with a mouse monoclonal antibody directed against VEGF (2 μg/mL) (No 350-P0, NeoMarkers, Fremont, CA), VEGFR-1 (15 μg/mL) (No. MAB321, R&D Systems, Minneapolis, MN), or CTGF (15 μg/mL) (No. MAB660, R&D Systems). Sections were then incubated with a biotinylated horse antimouse 2° antibody (Vector Laboratories) at room temperature for 30 min. Sections were stained with the Vectastain ABC system and developed with 3,3′-diaminobenzidine (DAB), then dehydrated and coverslipped.

Six regions of interest (ROI) were digitally photographed at 200× magnification using an Axiocam digital camera and Axiovision software v3.1 (Carl Zeiss, Germany). Prior to image acquisition, the camera was white balanced to ensure a uniform background color. The microscope's light intensity was maintained at a constant level to ensure the background mean gray values of the images were similar throughout the acquisition process. The six ROIs (Fig. 2) include the three areas along the enthesis distinguished by a tidemark (classified as inner, center, and outer) and three corresponding areas 1500 μm distal to the enthesis. The inner area was that part nearest the bone. Positive staining cells were manually counted in each region (200×400 μm^2) and normalized by the area observed to calculate density. Tissue and histological preparation and cell counting were completed at the same time for tissues from both limbs and were performed blinded to limb loading status.

A mixed model repeated-measures ANOVA was used to analyze differences in cell density by region (six regions) and by limb loading status (loaded or unloaded). Post hoc analysis was performed using the Tukey method for multiple comparisons.

RESULTS

Across the six ROIs, the density of VEGF (Fig. 3A and B) labeled cells ranged from 372 to 774 cells/mm^2 in the unloaded tendon and from 539 to 1011 cells/mm^2 in the loaded tendon (Fig. 4). The limb × region interaction term in the repeated-measures ANOVA was not significant ($p = 0.99$). Loaded limbs had significantly greater VEGF-staining cell densities than the unloaded limbs ($p = 0.0001$) across all regions. Based on the Tukey follow-up tests, significant regional differences also existed. The outer regions of the

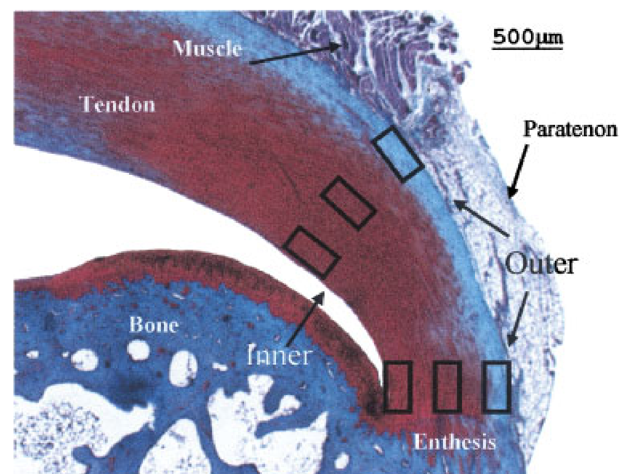

Figure 2. Safranin O and Fast Green stained epicondyle with bone, tendon, paratenon, and muscle. Six regions of interest are highlighted: three along the enthesis, and three 1500 μm distal to the enthesis. The regions of interest are 200×400 μm.

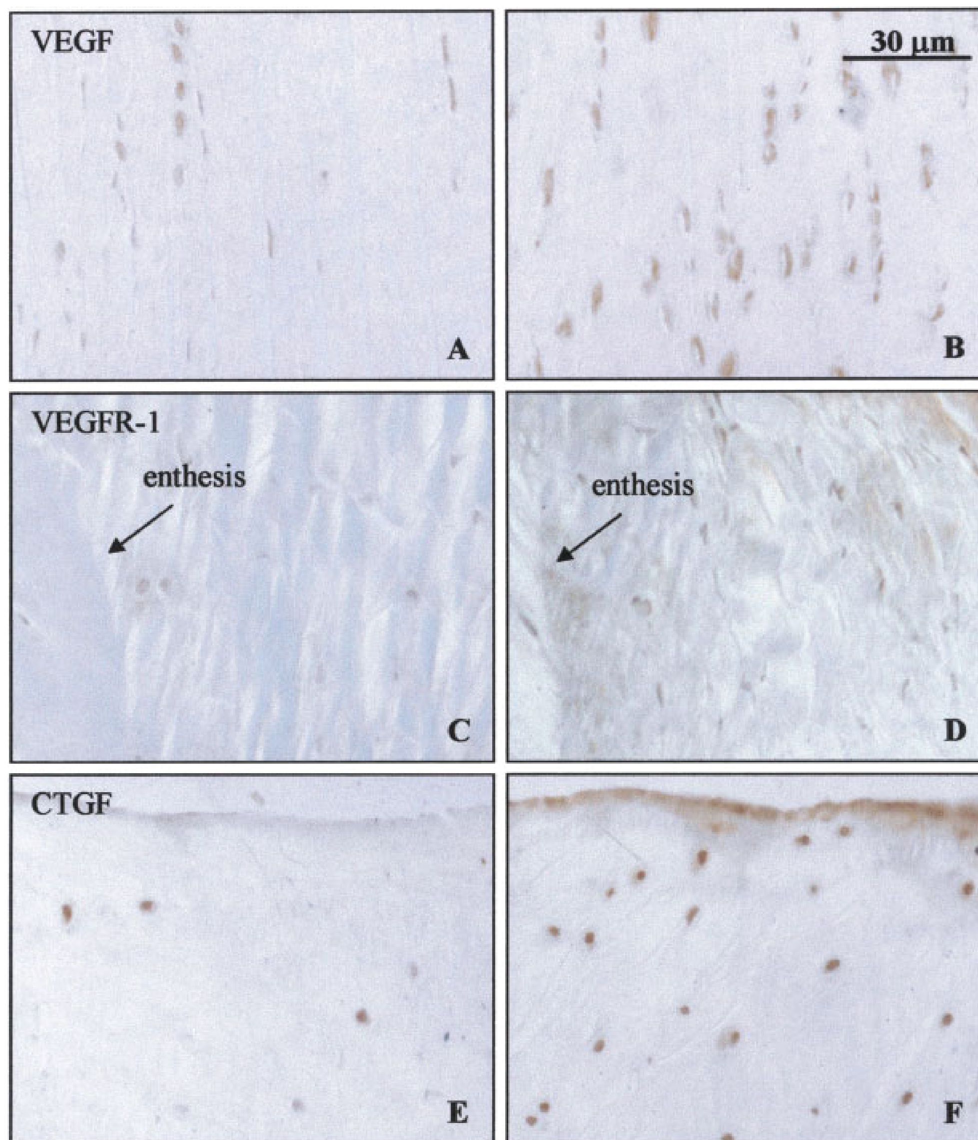

Figure 3. VEGF stained cells in the unloaded (A) and loaded (B) center distal region. VEGFR-1-stained cells in the unloaded (C) and loaded (D) outer enthesis region. CTGF-stained cells in unloaded (E) and loaded (F) inner distal region of the tendon. 400× (original) magnification.

tendon, both at the enthesis and distal to the enthesis, had significantly higher VEGF cell densities than the other four regions (Fig. 4).

Across the six ROIs, the density of VEGFR-1 (Fig. 3C and D) staining cells ranged from 440 to 611 cells/mm^2 in the unloaded tendon and 514 to 744 cells/mm^2 in the loaded tendon (Fig. 4). The limb × region interaction term was not significant ($p = 0.87$). Loaded limbs had significantly greater VEGFR-1 staining cell densities than the unloaded limbs ($p = 0.046$) across all regions. Based on the Tukey follow-up tests, significant regional differences existed. The outer region of the tendon at the enthesis had a significantly greater VEGFR-1 cell density than the inner ($p = 0.019$) region distal to the enthesis (Fig. 4).

Across the six ROIs, the density of CTGF (Fig. 3E and F) staining cells varied less by region (Fig. 4) and ranged from 397 to 570 cells/mm^2 in the unloaded tendon and from 584 to 778 cells/mm^2 in the loaded tendon. The limb by region interaction term was not significant ($p = 0.48$). The density of CTGF-staining cells was significantly greater in the loaded tendon than the unloaded

 DOI 10.1002/jor

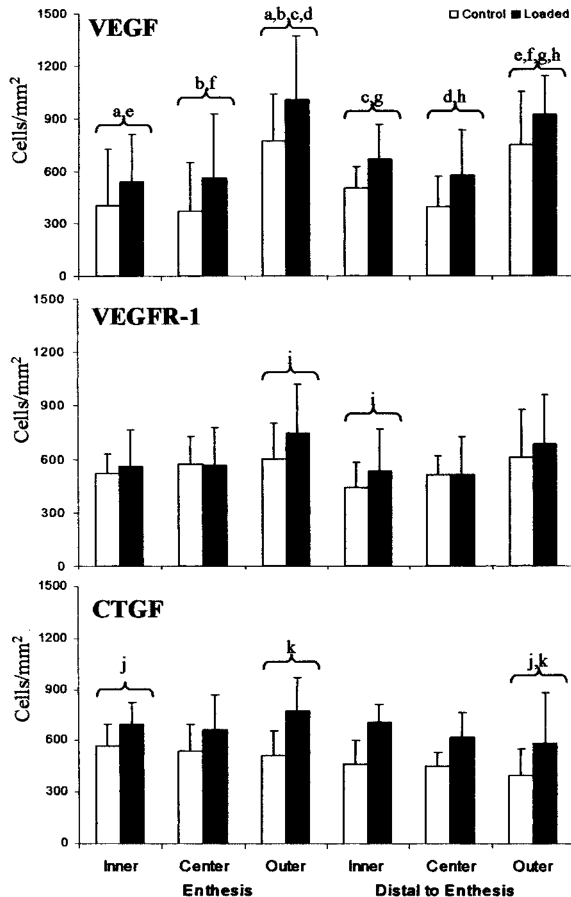

Figure 4. Densities of VEGF, VEGFR-1, and CTGF staining cells (mean ± SD) for loaded and unloaded tendon at the epicondyle. Across all regions, cell densities were significantly increased in the loaded tendon compared to unloaded tendon. The limb × region interaction terms were not significant for all three. Regions marked with the same lower case letter are significantly different based on the Tukey follow-up test ($n = 9$).

tendon ($p < 0.0001$) across all regions. Based on the Tukey follow-up tests, regional differences existed. Both the inner ($p = 0.02$) and outer ($p = 0.008$) regions of the tendon at the enthesis had significantly greater CTGF cell densities than the outer region of the tendon distal to the enthesis (Fig. 4).

DISCUSSION

Studies have shown that VEGF is involved in the tendon's healing response in acute tendon injuries.[41,42] This is the first study to regionally quantify VEGF, VEGFR-1, and CTGF-staining cells using an in vivo overuse animal model. The densities of VEGF, VEGFR-1, and CTGF staining cells were increased in the rabbit flexor tendon at the epicondyle as cyclical loads were applied in vivo for a total of 80 h over a period of 14 weeks. Regional variations were also present, and mainly occurred between the outer region at the enthesis and other regions in the tendon. The highest cell densities occurred at the outer region at the enthesis in the loaded tendon for all three proteins.

The cell densities of VEGF and VEGFR-1 were inhomogeneously distributed, having a tendency to be lower at the inner and center regions, both at the enthesis and distal to the enthesis, and higher at the outer regions. This distribution was similar in both the loaded and unloaded tendon. A differential stress or strain distribution exists throughout the loaded tendon at this type of tendon bone junction.[43] Cyclic strains upregulate VEGF synthesis in tendon fibroblast cell cultures[18] while hydrostatic pressure inhibits VEGF production in cultured tendon cells.[44] The greater compressive forces experienced in the inner region of the tendon may have inhibited VEGF production, while the higher strains experienced in the outer region may have lead to increased VEGF production.

The densities of CTGF staining cells were also increased (25 to 70%) in the loaded tendon regardless of region. However, the regional distribution of CTGF staining cells varied less than that of VEGF staining cells. The highest concentration of CTGF cells was in the outer region of the loaded tendon at the enthesis, while the lowest concentrations were along the outer region distal to the enthesis. Recent studies demonstrated a pronounced upregulation of CTGF expression in fibroblasts by contractile mechanical stresses,[38,39] which may partially explain the elevated number of CTGF staining cells at the inner regions, where compressive loads dominated.[43] No studies have examined the effect of cyclical loading on CTGF expression in tendons where both compressive and tensile loads are present. The current results may indicate that both compressive and tensile stresses play a role in CTGF upregulation in tendons exposed to cyclical loads.

These changes in VEGF, VEGFR-1, and CTGF are similar to changes in microtear density that we previously reported in this model.[40] We found increased microtear densities with loading, plus the microtear density was greater at the outer region at the enthesis compared to other regions. We have not examined earlier time points, but the

regional overlap in findings suggests that either the microtears alter local tissue stress patterns and signal cells to express these growth factors or that the growth factors lead to regional alterations in tendon structure, and these regions become more susceptible to structural damage. VEGF is increased in response to an acute injury and plays an important role in healing.[41,42] The time course and levels of expression of VEGF are probably different for the acute tendon injury than the injury due to overuse. The prolonged elevation of VEGF with overuse may be involved in a process leading to degeneration.

Previous in vivo cyclical tendon loading studies offer varying findings. Backman et al.[45] loaded rabbit Achilles tendon with repetitive eccentric exercise (30 to 36 h of cumulative loading) and found fibrillation and an increased number of inflammatory cells and blood vessels in the tendon and paratenon. The semiquantitative results showed changes to the entire tendon and paratenon, but did not focus on specific areas within the tendon, such as near the tendon–bone junction or the tendon–muscle interface. Archambault et al.[46] also used a rabbit to model Achilles tendinosis ($n = 4$, 66 h of cumulative loading). They found no changes in degeneration or density of inflammatory cells, but some suggestion of an increase in mRNA expression of collagen III and IL-1β and a decrease in expression of IGF-II.

Other overuse injury animal models have demonstrated an increase of VEGF[17,47] and CTGF[28] with loading. In the rat supraspinatus tendon, Perry et al.[47] reported elevated VEGF mRNA expression after 3 days of treadmill running. These levels dropped at 1 week, only to increase at later time points. The regional variation of VEGF was not examined in this study. The same model demonstrated a decreased maximum tensile load in tendons after 20 h of cumulative loading[48] and after longer loading periods, larger cross-sectional tendon areas, decreased moduli, smaller allowable maximum stresses, increased cellularity, collagen disorganization, and changes in cell morphology were seen in a loaded tendon compared to nonexercised cage control rats. Barbe et al.[49] reported tendon fibrillation and an infiltration of macrophages in their rat tendinosis model after 18 h of cumulative repetitive loading that involved rats reaching for food. Although the animals had a preferential limb to use for the task, these results were not compared to the nonloaded limbs of the same animals, but rather to cage controls. Some weaknesses of the aforementioned studies include a lack of characterization and control of the biomechanical loads.

The biological activity of VEGF is mediated by binding to and being activated by its receptors. Activated VEGF in human umbilical vein endothelial cells (HUVEC) can lead to an induction of interstitial collagenase.[50] If the mechanism in tenocytes is similar, this pathway may lead to the modification of the tendon's mechanical properties.

Expression of VEGF and its receptors, VEGFR-1 and VEGFR-2, were recently shown to be present in degenerative Achilles and fetal tendons, but not in normal adult tendon.[16,17,44,51] Petersen et al.[9] found mRNA and protein expression of the VEGF receptors in injured Achilles tendons at the site of rupture; other sites were not investigated. In our model, the highest cell densities for VEGF and VEGFR-1 were found at the outer region at the enthesis, which may indicate that this region is more susceptible to damage.

Other investigators have reported increased numbers of capillaries, infiltrates of inflammatory cells, and edema[45,49,52] after just 15 h of repetitive loading (5 weeks) in the rabbit and rat. These changes were not observed in our model, but our loading pattern differs from that used by other researchers and may be inadequate to cause changes at earlier time points as in the other studies. In our model, the force and loading frequency were selected to be within a range that may be experienced by workers and athletes.[53] Backman et al.,[45] for example, used a loading frequency of 2.5 Hz, which is considered a fast hopping rate for rabbits. Archambault et al.[46] used a loading frequency that was half that used in the Backman study. The loading frequency was decreased in the Archambault study because it was considered a slow hopping rate for rabbits and within physiological limits.

Mechanical stimulation is important for cell survival and growth as well as various tissue-specific functions.[54,55] However, excessive mechanical loading and overuse likely triggers a repair response that may eventually contribute to the degenerative changes observed in tendinopathies. This study demonstrates that prolonged, repetitive tendon loading leads to an increased production of the growth factors VEGF, VEGFR-1, and CTGF by tendon cells. Furthermore, the highest VEGF, VEGFR-1, and CTGF cell densities occurred along the outer regions of the loaded tendon. These locations may be the at-risk regions in the tendon that will ultimately demonstrate the

changes typical of tendinosis, such as degenerative changes with new capillary formation.

ACKNOWLEDGMENTS

This work was supported by a grant from the Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health (R01-0H07359).

REFERENCES

1. Jozsa L, Kannus P. 1997. Histopathological findings in spontaneous tendon ruptures. Scand J Med Sci Sports 7:113–118.
2. Kannus P. 1997. Tendons—a source of major concern in competitive and recreational athletes. Scand J Med Sci Sports 7:53–54.
3. United States Bureau of Labor Statistics. 1995. Occupational injuries and illnesses—counts, rates, and characteristics. Washington, DC: The Bureau: For sale by the U.S. G.P.O. Supt. of Docs, p v.
4. Allander E. 1974. Prevalence, incidence, and remission rates of some common rheumatic diseases or syndromes. Scand J Rheumatol 3:145–153.
5. Chard MD, Hazleman BL. 1989. Tennis elbow—a reappraisal. Br J Rheumatol 28:186–190.
6. Wetzel BJ, Nindl G, Swez JA, et al. 2002. Quantitative characterization of rat tendinitis to evaluate the efficacy of therapeutic interventions. Biomed Sci Instrum 38:157–162.
7. Chard MD, Cawston TE, Riley GP, et al. 1994. Rotator cuff degeneration and lateral epicondylitis: a comparative histological study. Ann Rheum Dis 53:30–34.
8. Kraushaar BS, Nirschl RP. 1999. Tendinosis of the elbow (tennis elbow). Clinical features and findings of histological, immunohistochemical, and electron microscopy studies. J Bone Joint Surg Am 81:259–278.
9. Pfahler M, Jessel C, Steinborn M, et al. 1998. Magnetic resonance imaging in lateral epicondylitis of the elbow. Arch Orthop Trauma Surg 118: 121–125.
10. Potter HG, Hannafin JA, Morwessel RM, et al. 1995. Lateral epicondylitis: correlation of MR imaging, surgical, and histopathologic findings. Radiology 196:43–46.
11. Regan W, Wold LE, Coonrad R, et al. 1992. Microscopic histopathology of chronic refractory lateral epicondylitis. Am J Sports Med 20:746–749.
12. Steinborn M, Heuck A, Jessel C, et al. 1999. Magnetic resonance imaging of lateral epicondylitis of the elbow with a 0.2-T dedicated system. Eur Radiol 9:1376–1380.
13. Maffulli N, Khan KM, Puddu G. 1998. Overuse tendon conditions: time to change a confusing terminology. Arthroscopy 14:840–843.
14. Soslowsky LJ, Thomopoulos S, Esmail A, et al. 2002. Rotator cuff tendinosis in an animal model: role of extrinsic and overuse factors. Ann Biomed Eng 30:1057–1063.
15. Alfredson H, Lorentzon M, Backman S, et al. 2003. cDNA-arrays and real-time quantitative PCR techniques in the investigation of chronic Achilles tendinosis. J Orthop Res 21:970–975.
16. Petersen W, Pufe T, Zantop T, et al. 2004. Expression of VEGFR-1 and VEGFR-2 in degenerative Achilles tendons. Clin Orthop 420:286–291.
17. Pufe T, Petersen W, Tillmann B, et al. 2001. The angiogenic peptide vascular endothelial growth factor is expressed in foetal and ruptured tendons. Virchows Arch 439:579–585.
18. Petersen W, Varoga D, Zantop T, et al. 2004. Cyclic strain influences the expression of the vascular endothelial growth factor (VEGF) and the hypoxia inducible factor 1 alpha (HIF-1alpha) in tendon fibroblasts. J Orthop Res 22:847–853.
19. Dvorak HF, Brown LF, Detmar M, et al. 1995. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 146:1029–1039.
20. Shalaby F, Rossant J, Yamaguchi TP, et al. 1995. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 376:62–66.
21. Petersen W, Pufe T, Zantop T, et al. 2003. Hypoxia and PDGF have a synergistic effect that increases the expression of the angiogenetic peptide vascular endothelial growth factor in Achilles tendon fibroblasts. Arch Orthop Trauma Surg 123:485–488.
22. Brigstock DR. 2002. Regulation of angiogenesis and endothelial cell function by connective tissue growth factor (CTGF) and cysteine-rich 61 (CYR61). Angiogenesis 5:153–165.
23. Deroanne CF, Hajitou A, Calberg-Bacq CM, et al. 1997. Angiogenesis by fibroblast growth factor 4 is mediated through an autocrine up-regulation of vascular endothelial growth factor expression. Cancer Res 57:5590–5997.
24. Boyer MI, Watson JT, Lou J, et al. 2001. Quantitative variation in vascular endothelial growth factor mRNA expression during early flexor tendon healing: an investigation in a canine model. J Orthop Res 19:869–872.
25. de Vries C, Escobedo JA, Ueno H, et al. 1992. The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science 255:989–991.
26. Terman BI, Dougher-Vermazen M, Carrion ME, et al. 1992. Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem Biophys Res Commun 187:1579–1586.
27. Gerber HP, Condorelli F, Park J, et al. 1997. Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. J Biol Chem 272:23659–23667.

28. Barr AE, Barbe MF. 2004. Inflammation reduces physiological tissue tolerance in the development of work-related musculoskeletal disorders. J Electromyogr Kinesiol 14:77–85.
29. Bradham DM, Igarashi A, Potter RL, et al. 1991. Connective tissue growth factor: a cysteine-rich mitogen secreted by human vascular endothelial cells is related to the SRC-induced immediate early gene product CEF-10. J Cell Biol 114:1285–1294.
30. Frazier K, Williams S, Kothapalli D, et al. 1996. Stimulation of fibroblast cell growth, matrix production, and granulation tissue formation by connective tissue growth factor. J Invest Dermatol 107: 404–411.
31. Yang GP, Lau LF. 1991. Cyr61, product of a growth factor-inducible immediate early gene, is associated with the extracellular matrix and the cell surface. Cell Growth Differ 2:351–357.
32. Nishida T, Nakanishi T, Asano M, et al. 2000. Effects of CTGF/Hcs24, a hypertrophic chondrocyte-specific gene product, on the proliferation and differentiation of osteoblastic cells in vitro. J Cell Physiol 184:197–206.
33. Blalock TD, Yuan R, Lewin AS, et al. 2004. Hammerhead ribozyme targeting connective tissue growth factor mRNA blocks transforming growth factor-beta mediated cell proliferation. Exp Eye Res 78:1127–1136.
34. Leask A, Abraham DJ. 2003. The role of connective tissue growth factor, a multifunctional matricellular protein, in fibroblast biology. Biochem Cell Biol 81:355–363.
35. Leask A, Abraham DJ. 2004. TGF-beta signaling and the fibrotic response. FASEB J 18:816–827.
36. Babic AM, Chen CC, Lau LF. 1999. Fisp12/mouse connective tissue growth factor mediates endothelial cell adhesion and migration through integrin alphavbeta3, promotes endothelial cell survival, and induces angiogenesis in vivo. Mol Cell Biol 19:2958–2966.
37. Lau LF, Lam SC. 1999. The CCN family of angiogenic regulators: the integrin connection. Exp Cell Res 248:44–57.
38. Schild C, Trueb B. 2002. Mechanical stress is required for high-level expression of connective tissue growth factor. Exp Cell Res 274:83–91.
39. Schild C, Trueb B. 2004. Three members of the connective tissue growth factor family CCN are differentially regulated by mechanical stress. Biochim Biophys Acta 1691:33–40.
40. Nakama L, King K, Abrahamsson S, et al. Evidence of tendon microtears due to cyclical loading in an in vivo tendinopathy model. J Orthop Res (in press).
41. Bidder M, Towler DA, Gelberman RH, et al. 2000. Expression of mRNA for vascular endothelial growth factor at the repair site of healing canine flexor tendon. J Orthop Res 18:247–252.
42. Tsubone T, Moran SL, Amadio PC, et al. 2004. Expression of growth factors in canine flexor tendon after laceration in vivo. Ann Plast Surg 53:393–397.
43. Wakabayashi I, Itoi E, Sano H, et al. 2003. Mechanical environment of the supraspinatus tendon: a two-dimensional finite element model analysis. J Shoulder Elbow Surg 12:612–617.
44. Petersen W, Pufe T, Kurz B, et al. 2002. Angiogenesis in fetal tendon development: spatial and temporal expression of the angiogenic peptide vascular endothelial cell growth factor. Anat Embryol (Berl) 205:263–270.
45. Backman C, Boquist L, Friden J, et al. 1990. Chronic achilles paratenonitis with tendinosis: an experimental model in the rabbit. J Orthop Res 8: 541–547.
46. Archambault JM, Hart DA, Herzog W. 2001. Response of rabbit Achilles tendon to chronic repetitive loading. Connect Tissue Res 42:13–23.
47. Perry SM, McIlhenny SE, Hoffman MC, et al. 2005. Inflammatory and angiogenic mRNA levels are altered in a supraspinatus tendon overuse animal model. J Shoulder Elbow Surg 14:79S–83S.
48. Soslowsky LJ, Thomopoulos S, Tun S, et al. 2000. Neer Award 1999. Overuse activity injures the supraspinatus tendon in an animal model: a histologic and biomechanical study. J Shoulder Elbow Surg 9:79–84.
49. Barbe MF, Barr AE, Gorzelany I, et al. 2003. Chronic repetitive reaching and grasping results in decreased motor performance and widespread tissue responses in a rat model of MSD. J Orthop Res 21:167–176.
50. Unemori EN, Ferrara N, Bauer EA, et al. 1992. Vascular endothelial growth factor induces interstitial collagenase expression in human endothelial cells. J Cell Physiol 153:557—562.
51. Petersen W, Pufe T, Unterhauser F, et al. 2003. The splice variants 120 and 164 of the angiogenic peptide vascular endothelial cell growth factor (VEGF) are expressed during Achilles tendon healing. Arch Orthop Trauma Surg 123:475–480.
52. Messner K, Wei Y, Andersson B, et al. 1999. Rat model of Achilles tendon disorder. A pilot study. Cells Tissues Organs 165:30–39.
53. National Research Council (U.S.). 2001. Panel on Musculoskeletal Disorders and the Workplace, Institute of Medicine (U.S.): Musculoskeletal disorders and the workplace: low back and upper extremities. Washington, DC: National Academy Press, p xv, 492.
54. Kjaer M. 2004. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. Physiol Rev 84:649–698.
55. See EK, Ng GY, Ng CO, et al. 2004. Running exercises improve the strength of a partially ruptured Achilles tendon. Br J Sports Med 38:597–600.