Eur J Appl Physiol (2008) 102:265–270
DOI 10.1007/s00421-007-0582-9

ORIGINAL ARTICLE

Evaluation of gene expression through qRT-PCR in cyclically loaded tendons: an in vivo model

Krishna R. Asundi · Karen B. King · David M. Rempel

Accepted: 18 September 2007 / Published online: 6 October 2007

Abstract An in vivo rabbit animal model for the tendinopathy, epicondylitis, was used to examine the effects of repetitive load on the expression of various genes associated with matrix remodeling. Following 80 h of cumulative load, tissue from the distal and proximal regions of the flexor digitorum profundus tendon was collected. Quantitative RT-PCR was used to asses mRNA levels of collagenase-1 (MMP-1), stromelysin (MMP-3), vascular endothelial growth factor (VEGF), connective tissue growth factor (CTGF), cyclooxygenase-2 (COX-2), interleukin-1β (IL-1β), type III collagen (COL-III) and fibronectin (FBRN). No significant differences in expression levels were found between loaded and unloaded limbs at either region of the tendon. The findings were unexpected as the same model has already demonstrated an increase in the density of cells staining for VEGF and CTGF. Different regulatory mechanisms between mRNA and protein expression or localized changes missed due to homogenization of the tissue samples, may explain the discrepancy in findings.

D. M. Rempel (✉)
Ergonomics Program, 1301 South 46th Street, Building 163,
Richmond, CA 94804, USA
e-mail: david.rempel@ucsf.edu

K. R. Asundi · D. M. Rempel
Joint Graduate Group in Bioengineering,
University of California, Berkeley/San Francisco, CA, USA

D. M. Rempel
Department of Medicine,
University of California, San Francisco, CA, USA

K. B. King
Department of Orthopaedics, Division of Bioengineering,
University of Colorado at Denver and Health Sciences Center,
Aurora, CO, USA

Keywords Overuse injury · Quantitative RT-PCR · Vascular endothelial growth factor · Connective tissue growth factor

Introduction

Workers and athletes who perform forceful repetitive hand activities are at increased risk for developing tendon overuse injuries (NRC 2001). Employees performing hand intensive jobs such as in the industrial, textile and food industries, exhibit a higher prevalence of tendinopathies compared to less strenuous jobs such as shop assistants and construction foremen (Roto and Kivi 1984; Viikari-Juntura et al. 1991; McCormack et al. 1990). In athletes, those who participate in sports such as tennis and volleyball present greater incidents of overuse tendon injuries (Jarvinen 1992).

Such injuries are characterized by soreness, pain and reduced range of motion (Jozsa and Kannus 1997a). Tendon biopsies provide clues as to the changes in tendon tissues related to tendinosis. Findings from histological studies of injured tendons are consistent with degenerative changes and include collagen disorientation, disorganization and fiber separation, regions of increased cellularity, neovascularization and focal necrosis (Astrom and Rausing 1995; Jarvinen et al. 1997; Jozsa and Kannus 1997b; Kraushaar and Nirschl 1999). Biochemical analyses have shown several matrix proteins including matrix metalloproteinases (Ireland et al. 2001; Riley et al. 2002), type III collagen (Maffulli et al. 2000) and vascular endothelial growth factor (Pufe et al. 2005) to be differentially regulated in such tendons as well. These findings suggest that some aspect of the degenerative process is cell mediated.

A useful method for examining cellular activity is gene expression analysis. Changes in gene expression in tendons

and ligaments have been measured to study the effects of acute injury (Boykiw et al. 1998; Reno et al. 1998), stress deprivation (Arnoczky et al. 2004; Majima et al. 2000) and repetitive loading (Perry et al. 2005). While various mechanisms regulate protein levels and enzyme activity within the cell and the extracelluar matrix, modulation of gene transcription is often a critical step in this process. In addition, mRNA quantification through real time RT-PCR has become relatively quick and easy compared to other methods of examining cellular behavior such as immunostaining, enzyme-linked immunosorbant assays (ELISA) and Western blot analysis.

In order to examine the initial response of tendons to repetitive loads, our laboratory has developed an in vivo model for the tendinopathy, medial epicondylitis. This model has demonstrated increased size and density of microtears (Nakama et al. 2005) as well as greater density of cells staining for VEGF and CTGF (Nakama et al. 2006) in repetitively loaded tendons compared with the unloaded control limb. These results provide evidence of early microstructural changes along with a cellular response to cyclical loading.

This study uses the same animal model but evaluates changes in mRNA expression levels of several matrix molecules in both the proximal and distal FDP tendons. The expression of VEGF, CTGF, MMP-1, MMP-3, IL-1β, COX-2, COL-3 and FBRN are measured. These genes are involved in tendon development, remodeling or wound healing (Jozsa and Kannus 1997; Matrisian 1990; Archambault et al. 2002; Yang et al. 2005). Many of them have also been found in tendinopathic tissues (Maffuli et al. 2000; Ireland et al. 2001; Riley et al. 2002) indicating they may be involved in the degenerative process as well. Because our model has already demonstrated an increase in the number of micro-tears in the loaded tendons, we hypothesize that expression of these genes, which are associated with both repair and remodeling of tendon matrix will also be up-regulated. Determining whether their expression is modulated with long-term repetitive loading may help identify the proteins that play a role in the development of tendinopathies.

Methods

Loading model

The protocol of the animal loading model was described previously (Nakama et al. 2005) and was approved by the institutional IACUC. Briefly, under general anesthesia, the flexor digitorum profundus (FDP) muscle in the left forepaw of 12 female New Zealand White rabbits was electrically stimulated by needle electrodes. A brass glove was placed over digit three of the stimulated limb and connected to a load cell in order to measure digit flexion force. The stimulation train was adjusted to maintain a peak flexion force of 0.42 N. Animals were subjected to 1 Hz repetitive loading for 2 h per day, 3 days a week for 80 h of cumulative loading. The right forepaw was not stimulated and was used as the control limb. The repetition rate and load were within the range of that experienced by workers who perform repetitive tasks (NRC 2001).

After 80 cumulative hours of loading over 14 weeks, the animals were weighed (3.76 ± 0.29 kg) and euthanized. Examinations of the paw, forearm and elbow revealed no tenderness, limping, swelling, limitations in range of motion or reduction in flexion strength. Evaluation of the subcutaneous area at the stimulation needle insertion site revealed minimal scar tissue that did not extend to the distal or proximal FDP tendon. A 5 mm section of the distal FDP tendon approximately 8 mm from the myotendinous junction was harvested from the right and left forepaws of each animal. A 4 mm section of the proximal FDP tendon, cut directly at the origin was harvested from eight animals. Samples were immediately snap frozen in liquid nitrogen and stored at −70°C until processed.

mRNA extraction and quantification

Frozen tissue was powdered using a Braun Dismembrator (B. Braun Biotech Inc., Allentown, PA) and total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA) following the manufacturers protocol. After extraction, RNA was quantified using the Ribogreen assay (Invitrogen, Carlsbad, CA) and treated with amplification grade DNase (Invitrogen, Carlsbad, CA). Treated RNA (500 ng) was reverse transcribed (Taqman Reverse Transcription Reagents, Applied Biosystems, Foster City, CA) to cDNA for PCR analysis. Relative quantification was done by real time PCR (ABI-Prism 7000 Sequence Detection System) using Applied Biosystem's SYBR Green master mix. Oligonucleotide sequences for the genes of interest were obtained from the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/). Primer sets were designed from the sequences using Applied Biosystem's Primer Express software V2 (Table 1). PCR reactions were carried out on a 96-well plate in a 25 µl total volume. Each well contained 12.5 µl SYBR green master mix, 5 µl of template cDNA, 6.5 ul H_2O, 200 nM forward primer and 200 nM reverse primer. After an initial incubation for 2 min at 50°C and 10 min at 90°C, 40 reaction cycles were run at 95°C for 15 s and 60°C for 1 min. Following the PCR cycles, a dissociation curve was obtained for each reaction. Reactions were run in duplicate. Expression values for each gene were measured relative to a standard curve of four ten-fold serial dilutions. 18s was used as the internal control (Corps et al. 2006).

Table 1 Primer sequences used for real-time PCR analysis

Genes	Gene name	Primer sequences (forward/reverse)	Accession
GAPDH	Glyseraldehyde-3-phosphate dehydrogenase	5′-GGATTTGGCCGCATTGG-3′	L23961
		5′-CAACATCCACTTTGCCAGAGTTAA-3′	
18s	18s rRNA	5′-AGTGCGGGTCATAAGCTTGC-3′	X00640
		5′-GGTGTGTACAAAGGGCAGGG-3′	
VEGF	Vascular Endothelial Growth Factor	5′-TGCCCACCGAGGAGTTCA-3′	AF022179
		5′-TGGCCCTGGTGAGGTTTG-3′	
CTGF	Connective Tissue Growth Factor	5′-TCCCGAGGAGGGTCAAACT-3′	AB217855
		5′-GTCCTTGGGCTCATCACACA-3′	
MMP-I	Matrix Metalloproteinase-1	5′-AGGAGCCTTCCCAAGAGGAA-3′	M25663
		5′-CTTGTCTCTTGCATATCAGGATGATG-3′	
MMP-3	Matrix Metalloproteinase-3	5′-AGCCAATGGAAATGAAAACTCTTC-3′	M25664
		5′-CCAGTGGATAGGCTGAGCAAA-3′	
COL-3	Type III Collagen	5′-CCTGAAGCCCCAGCAGAAA-3′	S83371
		5′-AACAGAAATTTAGTTGGTCACTTGTACTG-3′	
FBRN	Fibronectin	5′-AAGACCGGACCAATGAAAGAAA-3′	AF135404
		5′-CACCATGAGTCCTGACACAACA-3′	
IL-1β	Interlukin-1β	5′-TCCAGACGAGGGCATCCA-3′	D21835
		5′-CTGCCGGAAGCTCTTGTTG-3′	
COX-2	Cyclooxygenase-2	5′-CACGCAGGTGGAGATGATCTAC-3′	U97696
		5′-TTCCTGGCCCACAGCAAA-3′	

Abbreviations and gene names, primer sequences and Genebank accession number are presented

In vitro cyclic strain

As a positive control, expression levels were also examined in flexor tendons exposed to cyclic strain in culture. Twelve flexor profundus tendons, approximately 45 mm in length, were harvested from the hind paws of six New Zealand White rabbits under sterile conditions. The tendons were immediately placed in Dulbecco's Modified Eagle's Media supplemented with 10% FBS, 1% antibiotics and ascorbic acid (57 μg/ml). A custom built loading system, described elsewhere (Asundi et al. 2007) was used to apply cyclic loads to each tendon. Tendons were loaded to a peak strain of 1% ($n = 6$) or 5% ($n = 6$) strain. Loading was applied using a saw-tooth wave form at 1 Hz with a 50% duty cycle and 20% per second strain rate for 24 h. Throughout loading, tendons were maintained in supplemented DMEM at 37°C. After loading, a 4 mm section of tendon, cut from the midsubstance of each tendon, was snap frozen in liquid nitrogen and stored at −70°C until processed. Expression levels of GAPDH, Type III collagen, MMP-1, VEGF, and IL-1β were quantified following the same procedure described above. GAPDH was used as the internal control.

Statistics

For in vivo loaded samples, gene expression ratios between loaded and contra lateral control samples were calculated for each gene. For the in vitro loaded samples, gene expression ratios between samples loaded to 5 and 1% peak strain were calculated. For 18s and GAPDH comparisons, raw values were used, for the other genes of interest, values were first normalized to either 18s, for samples loaded in vivo, or GAPDH, for samples loaded in vitro. Ratios were log transformed in order to normalize the distribution. Means were compared to zero using a one sample t test to determine if loading had a significant effect on expression. Values are presented as the exponent of the mean log transformed ratios. Error bars represent the coefficient of variability in the expression ratios.

Results

Expression levels of 18s were not significantly different between loaded and unloaded controls of distal (Ratio: 0.95 ± 0.28) or proximal (Ratio: 0.69 ± 0.42) tendon sections. Expression levels of GAPDH were not significantly different in tendons loaded to 5 or 1% peak strain (Ratio: 1.36 ± 0.31).

Expression levels for each of the eight genes of interest examined in the distal FDP tendon were not significantly different between loaded and control limbs (Fig. 1). MMP-1 mRNA was undetectable in 3 of the 12 control limb samples. In these cases, threshold was set at 40 cycles, the total number of thermal cycles run.

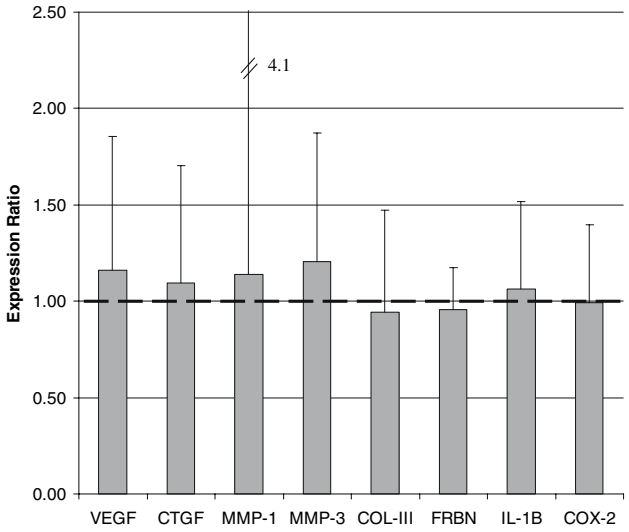

Fig. 1 Mean (±COV) gene expression ratio between loaded and contra lateral control tendon samples harvested from the *distal* FDP. A value of 1 (*dashed line*) indicates no difference between loading groups. No significant differences were found in expression levels between control and loaded limbs ($P > 0.05$, $n = 12$, $n = 8$ for IL-1β and COX-2)

Expression levels of the six genes of interest examined in the proximal FDP tendon were not significantly different between loaded and control limbs (Fig. 2). MMP-1 mRNA was undetectable in one of the loaded tendon samples. Again, the threshold for this sample was set at 40 cycles.

For tendons cultured in vitro, the COL-III expression ratio between tendons loaded to 5% peak strain and 1% peak strain was 0.56(±0.18) a significant decrease (Fig. 3). Similar decreases in expression of MMP-1 [0.42(±0.26)] and IL-1β [0.53(±0.27)] were also observed. No differences were observed in the expression ratio of VEGF [1.05(±0.27)].

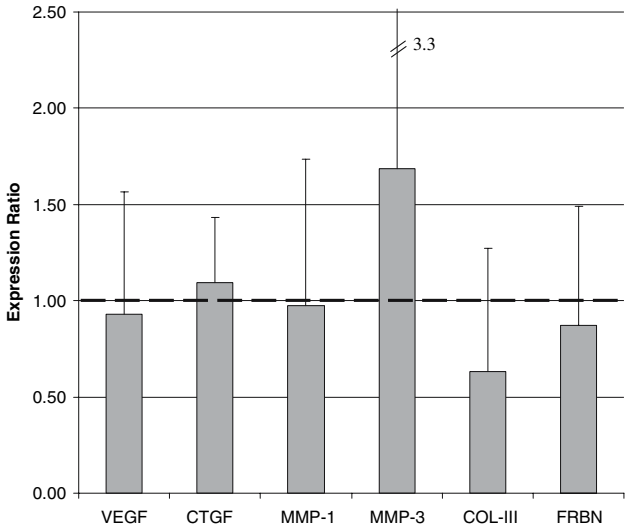

Fig. 2 Mean (±COV) gene expression ratio between loaded and contra lateral control tendon samples harvested from the *proximal* FDP. A value of 1 (*dashed line*) indicates no difference between loading groups. No significant differences were found in expression levels between control and loaded limbs ($P > 0.05$, $n = 8$)

Discussion

We examined the effect of repetitive loading, using an in vivo tendinopathy model, on the expression of various genes associated with tendon degradation. Contrary to our hypothesis, 80 cumulative hours of repetitive loading did not lead to significant changes in mRNA expression levels of any of the evaluated genes in the distal or proximal FDP tendon compared to the contralateral unloaded limb.

The results were unexpected since our model already demonstrated an increase in the density of cells staining for VEGF and CTGF (Nakama et al. 2006). The proximal FDP tendon tissue sampled was the same as that evaluated in the Nakama et al. study (2006). A possible reason for the different findings may have to do with the differences in the regions of the tendons examined. In our prior study (Nakama et al. 2006), using immunohistochemistry, differences in VEGF and CTGF staining cell densities were measured in six regions of the proximal FDP tendon, no more than 1.5 mm distal to the enthesis, with each region measuring 0.8 mm^2 in size. Changes were primarily observed in the

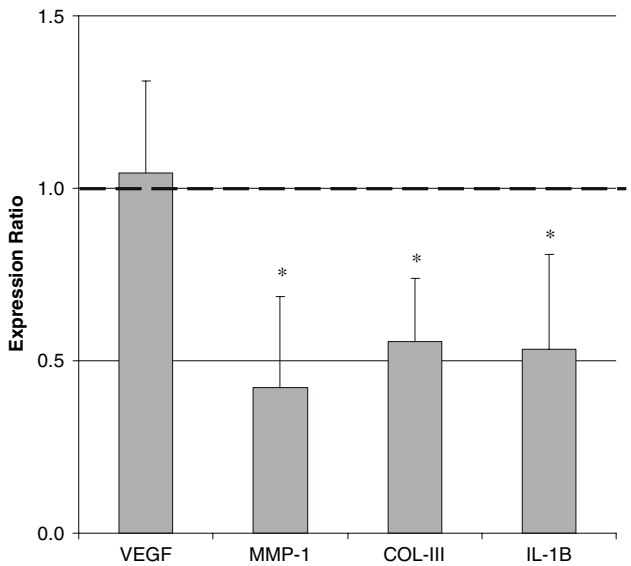

Fig. 3 Mean (±COV) gene expression ratio between tendons exposed to 5% cyclic strain and 1% cyclic strain in vitro. A value of 1 (*dashed line*) indicates no difference between loading groups. Cyclic loading to a peak strain of 5% produced a significant decrease in expression of MMP-1, COL-III and IL-1β. No significant changes were seen in VEGF expression (*$P < 0.05$, $n = 6$)

outer region of the tendon. In the present study, mRNA was extracted from the whole tendon tissue, which was approximately 4 mm long. Repetitive loading may have caused changes in small regions of the tendon that, when evaluating the whole sample, were diluted and therefore not detectable. If so, in situ hybridization may be the more appropriate method of identifying local changes in gene expression.

The data from the in vitro experiment was presented as a positive control in order to demonstrate that mechanical loading can lead to modulation of mRNA expression at levels that can be detected through our RT-PCR methods. One of the limitations of the in vivo model is that we are unable to directly compare the loads to the in vitro study. However, the much longer duration of the in vivo study (15 weeks) and the availability of a vascular system with proteins and leukocytes would be expected to turn on different repair processes than the 1 day in vitro study.

Another possible reason for the discrepancy between changes in mRNA and protein levels may be due to different protein regulatory mechanisms. Such discrepancy has been documented for VEGF in rat (Birot et al. 2004) and fetal sheep (Gilbert et al. 2005) hearts. Gene transcription is often a critical step in regulating protein levels; however, several other mechanisms exist for regulating proteins including translational efficiency, protein turnover rates, post-translational activation and inhibitory proteins. Differences in translational efficiencies and turnover rates could account for the discord between mRNA and protein levels.

Finally, a recent study by Heinemeier et al. 2007, suggested that some housekeeping genes, commonly assumed to be constitutively expressed, may be affected by load. They examined the expression of the large ribosomal protein P0 (RPLP0) and GAPDH in muscle and tendon in response to different types of muscle contractions. They found the RPLPO-to-GAPDH ratio was increased in both muscle and tendon in response to loading. The RPLP0 mRNA/mg tendon tissue weight and RPLP0/μg total RNA ratios were also affected by loading. These findings challenge the assumption that 18s levels are unaffected by load. While our study found no difference in 18s levels in 500 ng of RNA between loaded and unloaded controls, we did not examine total RNA synthesis or tissue cellularity. These changes could affect overall levels of target mRNA, while maintaining mRNA/18s ratios. Future studies should assess tissue cellularity and total RNA production in addition to levels of constitutively expressed genes in order to fully understand how loading may affect target mRNA levels.

This model found that changes due to loading occurred at the epicondyle on the microscopic (Nakama et al. 2005) and cellular (Nakama et al. 2006) level; however, clinical findings (e.g., tenderness, limited joint range of motion or swelling) were not found in the loaded limb. In a rabbit model somewhat similar to ours, Archambault et al. (2001) repetitively loaded the Achilles tendon 2 h per day, three times a week for 11 weeks. She found no changes in the gross morphology of the tissue, its water content or cellularity due to loading. They suggested the loading protocol did not exceed the tendons capacity for repair, which may have also been the case in our study.

Previous in vivo models have demonstrated that repetitive loading over prolonged periods can lead to changes in mRNA expression. In a rat model, altered levels of COX-2, VEGF (Perry et al. 2005) and various NOS isoform (Szomor et al. 2006) mRNA levels were found in the supraspinatus tendon of rats exposed to treadmill running for 16 weeks. Exposure to treadmill running however is likely to result in greater loads to the supraspinatus tendon compared to the loads used in our model. The difference in exposure as well as other differences such as species, tendon and genes examined may account for the different findings.

While exposure to cyclic loading of tendons in vivo did not lead to significant changes in expression of any of the genes examined, cyclic loading in vitro did. Tendons exposed to 5% cyclic strain expressed lower levels of MMP-1, IL-1β and COL-III compared to tendons exposed to a 1% cyclic strain. VEGF expression however, was unaffected by load.

Comparisons between changes in mRNA expression in tendons loaded in vitro and tendons loaded in vivo should be made cautiously. There are several significant differences between the models, which may explain why differences were found in one and not the other. These include applied loading pattern, time of harvest after load, location of the tissue section examined and culture media. However, the results from the in vitro experiments demonstrate that changes in mRNA expression due to mechanical loading can be detected by our quantitative RT-PCR techniques.

Conclusions

This study found no significant changes in mRNA expression levels, as measured by quantitative RT-PCR, for matrix inflammatory and degradative genes in tendons subjected to 80 cumulative hours of repetitive loading compared to contra lateral controls. The applied loading protocol may not lead to up-regulation at the transcriptional level of these eight genes. However, because the same model has already demonstrated an increase in the density of cells staining for VEGF and CTGF we cannot rule out that regional or localized changes may have been missed due to homogenization of the tissue samples.

Acknowledgments This work was supported by the National Institute for Occupational Safety and Health (R01-AR0H07359). The authors would like to acknowledge Yuka Nakamura, DVM for her assistance.

References

Archambault JM, Hart DA, Herzog W (2001) Response of rabbit Achilles tendon to chronic repetitive loading. Connect Tissue Res 42(1):13–23

Archambault J, Tsuzaki M, Herzog W, Banes A (2002) Stretch and interleukin-1beta induce matrix metalloproteinases in rabbit tendon cells in vitro. J Orthop Res 20(1):36–39

Arnoczky S, Tian T, Lavagnino M, Gardner K (2004) Ex vivo static tensile loading inhibits MMP-1 expression in rat tail tendon cells through a cytoskeletally based mechanotransduction mechanism. J Orthop Res 22(2):328–333

Astrom M, Rausing A (1995) Chronic Achilles tendinopathy. A survey of surgical and histopathologic findings. Clin Orthop Relat Res 316:151–164

Asundi K, Kursa K, Lotz J, Rempel D (2007) In vitro system for applying cyclic loads to connective tissues under force or displacement control. Ann Biomed Eng 35(7):1188–1195

Birot O, Peinnequin A, Simler N, van Cuyck-Gandre H, Hamel R, Bigard XA (2004) Vascular endothelial growth factor expression in heart of rats exposed to hypobaric hypoxia: differential response between mRNA and protein. J Cell Physiol 200(1):107–115

Boykiw R, Sciore P, Reno C, Marchuk L, Frank CB, Hart DA (1998) Altered levels of extracellular matrix molecule mRNA in healing rabbit ligaments. Matrix Biol 17(5):371–378

Corps A, Robinson A, Movin T, Costa M, Hazelman B, Riley G (2006) Increased expression of aggrecan and biglycan mRNA in Achilles tendinopathy. Rheumatology (Oxford) 45(3):291–294

Gilbert JS, Lang AL, Nijland MJ (2005) Maternal nutrient restriction and the fetal left ventricle: decreased angiotensin receptor expression. Reprod Biol Endocrinol 14:3–27

Heinemeier KM, Olesen JL, Schjerling P,Haddad F, Langberg H, Baldwin KM, Kjaer M (2007) Short-term strength training and the expression of myostatin and IGF-I isoforms in rat muscle and tendon: differential effects of specific contraction types. J Appl Physiol 102:573–581

Ireland D, Harral R, Curry V, Holloway G, Hackney R, Hazleman B, Riley G (2001) Multiple changes in gene expression in chronic human Achilles tendinopathy. Matrix Biol 20:159–169

Jarvinen M (1992) Epidimiology of tendon injuries in sports. Clin Sports Med 11(3):493–504

Jarvinen M, Jozsa L, Kannus P, Jarvinen TL, Kvist M, Leadbetter W (1997) Histopathological findings in chronic tendon disorders. Scand J Med Sci Sports 7:86–95

Jozsa L, Kannus P (1997a) In: Human tendons: anatomy, physiology and pathology. Human Kinetics, Champaign

Jozsa L, Kannus P (1997b) Histopathological findings in spontaneous tendon ruptures. Scand J Med Sci Sports 7:113–118

Kraushaar B, Nirschl R (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

Maffulli N, Ewen S, Waterston SW, Reaper J, Barrass V (2000) Tenocytes from ruptured and tendinopathic Achilles tendons produce greater quantities of type III collagen than tenocytes from normal Achilles tendons. Am Orthop Soc Sports Med 28(4):499–505

Majima T, Marchuk LL, Shrive NG, Frank CB, Hart DA (2000) In-vitro cyclic tensile loading of an immobilized and mobilized ligament autograft selectively inhibits mRNA levels for collagenase (MMP-1). J Orthop Sci 5(5):503–510

Matrisian LM (1990) Metalloproteinases and their inhibitors in matrix remodeling. TIG 6:121–125

McCormack R, Inman R, Wells A, Berntsen C, Imbus H (1990) Prevalence of tendinitis and related disorders of the upper extremity in a manufacturing workforce. J Rheumatol 17(7):958–964

Nakama L, King K, Abrahamsson S, Rempel D (2005) Evidence of tendon microtears due to cyclical loading in an in vivo tendinopathy model. J Orthop Res 23(5):1199–1205

Nakama L, King K, Abrahamsson S, Rempel D (2006) VEGF, VEGFR-1, and CTGF cell densities in tendon are increased with cyclical loading: an in vivo tendinopathy model. J Orthop Res 24(3):393–400

National Research Council, the Institute of Medicine (2001) Musculoskeletal disorders and the workplace: low back and upper extremities. In: Panel on Musculoskeletal Disorders and the Workplace. Commission on Behavioral and Social Sciences and Education. National Academy Press, Washington, pp 196–199

Perry S, McIlhenny S, Hoffman M, Soslowsky L (2005) Inflammatory and angiogenic mRNA levels are altered in a supraspinatus tendon overuse animal model. J Shoulder Elbow Surg 14(1 Suppl S):79S–83S

Pufe T, Petersen WJ, Mentlein R, Tillmann BN (2005) The role of vasculature and angiogenesis for the pathogenesis of degenerative tendons disease. Scand J Med Sci Sports 15(4):211–222

Reno C, Boykiw R, Martinez ML, Hart DA (1998) Temporal alterations in mRNA levels for proteinases and inhibitors and their potential regualtors in the healing medial collateral ligament. Biochem Biophys Res Commun 252(3):757–763

Riley GP, Curry V, DeGroot J, van El B, Verzijl N, Hazleman BL, Bank RA (2002) Matrix metalloproteinase activities and their relationship with collagen remodeling in tendon pathology. Matrix Biol 21:185–195

Roto P, Kivi P (1984) Prevalence of epicondylitis and tenosynovitis among meat cutters. Scand J Work Environ Health 10(3):203–205

Szomor ZL, Appleyard RC, Murrell GA (2006) Overexpression of nitric oxide synthases in tendon overuse. J Orthop Res 24(1):80–86

Viikari-Juntura E, Kurppa K, Kuosma E, Huuskonen M, Kuorinka I, Ketola R, Konni U (1991) Prevalence of epicondylitis and elbow pain in the meat-processing industry. Scand J Work Environ Health 17(1):38–45

Yang G, Im HJ, Wang JH (2005) Repetitive mechanical stretching modulates IL-1beta induced COX-2, MMP-1 expression, and PGE2 production in human patellar tendon fibroblasts. Gene 363:166–172