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THE STIMULUS AND INHIBITION OF MMP-13 IN RAT TAIL TENDONS: THE ROLE OF MECHANICAL STIMULUS AND EXOGENOUS INHIBITORS

By

Keri Lynn Gardner

A THESIS

Submitted to
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THE STIMULUS AND INHIBITION OF MMP-13 IN RAT TAIL TENDONS: THE ROLE OF MECHANICAL STIMULUS AND EXOGENOUS INHIBITORS

By

Keri Lynn Gardner

Tendinopathy, a degenerative condition of tendons associated with overuse injuries, is characterized by catabolic alterations in the extracellular matrix secondary to increases in matrix metalloproteinase (MMP) synthesis. While the precise etiology of tendinopathy is unknown, some investigators believe that changes in the mechanobiological signaling pathyways, which control the tendon cell's response to tensile loading forces, may play a significant role in regulating MMP gene expression. This research focuses on the role of tendon cell load in the expression and inhibition of a specific MMP (rat interstitial collagenase; MMP-13) mRNA in an *in vitro* rat tail tendon model. In addition, the effects of exogenous and endogenous MMP inhibitors on ameliorating the degenerative effects of MMP-13 in the model were also examined. The results of the study suggest that loss of normal tendon cell signaling due to alterations in cell matrix interactions result in an increase in MMP-13 mRNA expression and protein synthesis and a subsequent degeneration of tendon material properties. These degenerative changes can be inhibited by the application of exogenous MMP inhibitors or through an increase in endogenous tissue inhibitors of metalloproteinases (TIMPs) secondary to cyclic tensile loading. These findings may have significant implications in the development of therapeutic modalities for the prevention and treatment of tendinopathy.

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I. INTRODUCTION

Tendinopathy is a generic term used to describe a common condition affecting the tendons, which causes pain, swelling, or impaired performance. It includes tendinosis, tendonitis, paratendonitis and paratendonitis with tendinosis (Khan et al. 1999). Tendinopathy remains one of the most common injuries encountered in sports or at the workplace and describes clinical conditions in and around tendons resulting from over-load or overuse. It is a major medical problem associated with sports and physical activity accounting for 30-50% of all sports injuries and more than 48% of occupational problems (Renstrom *et al.* 1991, NIOSH 1996). Treatment of tendinopathy is difficult and problem management is often based on trial and error. The basic cell biology of tendons is still not fully understood, and the management of tendon injury poses a considerable challenge for clinicians.

Tendons connect muscle to bone and allow transmission of forces generated by muscle to bone, resulting in joint movement (Sharma 2005). They have a fibroelastic texture, which includes tenoblasts and tenocytes within the extracellular matrix network. These cell types constitute about 90-95% of the cellular element of tendons (Kannus 2000). Collagen type I accounts for 65%-80% of the dry mass of tendons (O'Brien M, 1997) and is arranged in hierarchical levels of increasing complexity. Tropocollagen (triple-helix polypeptide chain) unites into fibrils. Fibrils are considered the primary bundles with fascicles being the secondary bundles, then tertiary bundles and then the tendon itself (AnstromM 1997, Jozsa LG 1997, Movin T 1998). The ground substance of the extracellular matrix network surrounding the collagen and the tenocytes is

composed of proteoglycans, glycosaminoglycans, glycoproteins and several other small molecules (Kannus 2000).

When tendons transmit force from muscle to bone they act as a buffer by absorbing external forces to limit muscle damage (Best et al 1994). The mechanical behavior of collagen depends on the number and types of intramolecular and intermolecular bonds (Fyfe et al 1992). A stress-strain curve helps to demonstrate the behavior of tendon. Microscopic failure occurs when the strain exceeds 4% and beyond 8-10% strain macroscopic failure occurs from intrafibril damage (O'Brien 1992, Butler DL 1978, Kastelic J 1980). The tensile strength of tendons is positively related to thickness and collagen content (Oakes BW 1998, Shadwich RE 1990).

Tendon injuries can be acute or chronic and are caused by intrinsic or extrinsic factors, either alone or in combination (Pankaj Sharma et al 2005). It has been claimed that intrinsic factors such as alignment and biomechanical faults (muscle weakness and imbalance) play a causative role in two-thirds of Achilles tendon disorders in athletes (Kvist 1991, 1994). Tendon damage may occur from stresses within physiological limits, as frequent cumulative microtrauma (small tears or stress) may not allow enough time for repair (Selvannett, A et al 1997). The oxygen consumption of tendons is 7.5 times lower than that of skeletal muscle (Vailas et al 1978) and this low metabolic rate results in slow healing after injury (Williams JG, 1986). Microtrauma can also result from non-uniform stress with the tendons, producing abnormal load concentrations and frictional forces between the fibrils causing localized fiber damage (Arndt AN, et al 1998). Localized fibril damage and subsequent altered cell matrix interactions could lead

to a catabolic cascade initiated by the understimulation of the tendon cells in the damaged portion of the tendon (Arnoczky et al 2007-book).

The ability of tendon cells to sense and respond to load is central to the concept of mechanotransduction and maintenance of tissue homeostasis (Wang & Ingber 1994, Banes et al 1995, Ingber 1997). The precise level (magnitude, frequency, and duration) of mechanobiological stimulation required to maintain normal tendon homeostasis is not currently known. The tendon cells sense load through a mechanoelectrochemical sensory system(s), which detects mechanical load through the deformation of the cellular membrane and/or the cytoskeleton (Ben-Ze'Ev 1991; Watson 1991; Adams 1992; Wang et al 1993, 1994; Banes et al 1995b; Ingber 1997; Brown et al 1998; Wang 2006). Studies have demonstrated that *in situ* deformation of rat tail tendon cells occurs in response to tensile load (Arnoczky et al 2002a). The relationship between changes in cell morphology and tissue strain is thought to occur through the binding of the cell to extracellular matrix proteins such as collagen and fibronectin (Banes et al 1995b; Rosales et al 1995, Sung et al 1996).

Studies have suggested that tendon cells are capable of establishing an internal cytoskeletal tensional homeostasis through interactions with their local extracellular environment (Lavagnino 2005). Alterations in the cytoskeletal tension, secondary to changes in the extracellular matrix strain have been shown to regulate gene response in a reciprocal manner (Lavagnino 2005; Arnoczky 2004). One of the genes known to have increased expression with tendinosis is interstitial collagenase (Fu SC 2002; Riley 2002; Jones GC 2006). An increase in interstitial collagenase and the resulting degradation of the extracellular matrix collagen has been implicated in the pathogenesis of tendinopathy.

Interstitial collagenase is known as matrix metalloproteinase-1 (MMP-1) in humans and matrix metalloproteinase-13 (MMP-13) in rats. Matrix metalloproteinases (MMPs), a family of zinc and calcium dependent endopeptidases, are involved in the remodeling of extracellular matrix (ECM) through broad proteolytic capability (Parks & Mecham 1998; Woessner & Nagase 2000; Oblander 2003). Degradation of collagen in tendon ECM is initiated by MMPs (Riley 2002) and they are crucial to the development and maintenance of healthy tendon. An imbalance in the expression of MMPs and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), can lead to pathologic conditions such as rheumatoid arthritis (Green MJ), metastasis (Gomez DE 1997) and tendinopathy (Fu et al 2002). In normal tendons, it is thought that a balance between TIMPs and MMPs is required to maintain tissue homeostasis (Magra 2005) and an imbalance of TIMP/MMP has been implicated in tendinopathy (Jones et al 2006).

In processes that involve excessive proteolytic ECM breakdown, TIMPs can counteract MMP-mediated tissue destruction.

A better understanding of the interactions that occur at a cellular and molecular level during tendon degeneration may allow the development of more targeted therapeutics and result in a greater number of positive clinical outcomes. This research will focus on the effect of the loss of homeostatic tension and exercise on the ratio of TIMP/MMP mRNA expression in tendons.

References

Adams, D.S., Mechanisms of cell shape change: the cytomechanics of cellular response to chemical environment and mechanical loading. *J Cell Biol,* 117:83-93, 1992.

Arndt, A.N., Komi, P.V., Gruggemann, G.P., Lukkariniemi, J., Individual muscle contributions to the in vivo Achilles tendon force. *Clin Biomech (Bristol, Avon),* 13:532-541, 1998.

Arnoczky, S.P., Lavagnino, M., Whallon, J.H., Hoonjan, A., In situ cell nucleus deformation under tensile load: a morphologic analysis using confocal laser microscopy. *J Orthop Res,* 20:29-35, 2002a.

Arnoczky, S.P., Lavagnino, M., Egerbacher, M., The response of tendon cells to changing loads: implications in the etiopathogenesis of tendinopathy. *Tendinopathy in Athletes,* Blackwell Publishing, 46-59, 2007.

Banes, A.J., Tsuzaki, M., Hu, P., Brigman, B., Brown, T., Almekinders, L., Lawrence, W.T., Fischer, T., Cyclic mechanical load and growth factors stimulate DNA synthesis in avian tendon cells. *J Biomech,* 28:1505-1513, 1995a.

Banes, A.J., Tsuzaki, M., Yamamoto, M., et al., Mechanoreception at the cellular level: the detections interpretation, and diversity of responses to mechanical signals. *Biochem Cell Biol,* 73:349-365, 1995b.

Brown, R.A., Prajapati, R., McGrouther, D.A., Yannus, I.V., Eastwook M., Tensional homeostasis in dermal fibroblasts: mechanical responses to mechanical loading in three dimensional substrates. *J Cell Phys,* 175:323-332,1998.

Ben-Ze'ev, A., Animal cell shape changes and gene expression. *Bioessays,* 13:207-212, 1991.

Best, T.M., Garrett, W.E., Basic science of soft tissue: muscle and tendon. *In: Orthopaedic sports medicine: principles and practice.* Philidelphia: WB Saunders; 1994, p 1.

Butler, D.L., Grood, E.S., Noyes, F.R., Zernicke, R.F., Biomechanics of ligaments and tendons. *Exerc Sport Sci Rev,* 6:125-181, 1978.

Fu, S.C., Chan, B.P., Wang, W., Pau, H.M., Chan, K.M., Rolf, C.G., Increased expression of matrix metalloproteinase 1 (MMP1) in 11 patients with patellar tendinosis. *Acta Ortho Scand,* 73(6):658-662, 2002.

Gomez, D.E., Alonso, D.R., Yoshiji, H., Thorgeirsson, U.P., Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *Eur J Cell Biol,* 74:111-122, 1997.

Fyfe, I., Stanish, W.D., The use of eccentric training and stretching in the treatment and prevention of tendon injuries. *Clin Sports Med,* 11:601-624, 1992.

Green, M.J., Gough, A.K., Devlin, J., Smith, J., Astin, P., Taylor, D., Emery, P., Serum MMP-3 and MMP-1 and progression of joint damage in early rheumatoid arthritis. *Rheumatology (Oxford),* 42(1):83-88, 2003.

Ingber, D.E., Tensegrity: the architectural basis of cellular mechanotransduction. *Ann Rev Physiol,* 59:575-599, 1997.

Jones, G.C., Corps, A.N., Pennington, C.J., Clark, I.M., Edwards, D.R., Bradley, M.M., Hazleman, B.L., Riley, G.P., Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human Achilles tendon. *Arthritis and Rheum,* 54(3):832-842, 2006.

Kannus, P., Jozsa, L., Jarvinnen, M., Basic science of tendons. *In: Principles and practice of orthopaedic sports medicine.* Philadelphia: Lippincott Williams and Wilkins, 21-37, 2000.

Kastelic, J., Baer, E., Deformation in tendon collagen. *Symp Soc Exp Biol,* 34:397-435, 1980.

Khan, K.M., Cook, J.L., Bonar, F., Harcourt, P., Astrom, M., Histopathology of common tendinopathies. Update and implications for clinical management. *Sports Med,* 27(6), 393-408, 1999.

Kvist, M., Achilles tendon overuse injuries: a clinical and pathophysiological study in athletes [thesis]. Turku, Finland: University of Turku; 1991.

Kvist, M., Achilles tendon injuries in athletes. *Sports Med,* 18:173-201, 1994.

Lavagnino, M., Arnoczky, S.P., In vitro alterations in cytoskeletal tensional homeostasis control gene expression in tendon cells. *J Ortho Res,* 23:1211-1218, 2005.

Magra, M., Maffulli, N., Molecular events in tendinopathy: a role for metalloproteinases. *Foot Ankle Clin N Am,* 10:267-277, 2005.

Oakes, B.W., Singleton, C., Aut, R.C., Correlation of collagen fibril morphology and tensile modulus in the repairing and normal rabbit patella tendon. *Trans Orthop Res Soc,* 23:24, 1998.

Oblander, S.A., Somerville, R.P.T., Apte, S.S., An update on metalloproteinases in the musculoskeletal system. *Curr Opin Orthop,* 14:322-328, 2003.

O'Brien, M., Functional anatomy and physiology of tendons. *Clin Sports Med,* 11:505-520, 1992.

O'Brien, M., Structure and metabolism of tendons. *Scand J Med Sci Sports*, 7:55-61, 1997.

Parks, W.C., Mecham, R.P., *In: Matrix Metalloproteinases.* Academic Press, 1998.

Renstrom, P., Sports traumatology today. A review of common current sports injury problems. *Annales Chirurgiae et Gynaecologiae (Helsinki),* 80(2):81-93, 1991.

Riley, G.P., Curry, V., DeGroot, J., van El, B., Verzijl, N., Hazleman, B.L., Bank, R.A., Matrix metalloproteinase activities and their relationship with collagen remodeling in tendon pathology. *Matrix Biol,* 21(2):185-195, 2002.

Rosales, C., O'Brien, V., Kornberg, L, Juliano, R., Signal transduction by cell adhesion receptors. *Biochim Biophy Acta,* 1242:77-98, 1995.

Shadwich, R.E., Elastic energy storage in tendons: mechanical differences related to function and age. *J Appl Physiol,* 68:1033-1040, 1990.

Sharma, P., Maffulli, M., Basic biology of tendon injury and healing. *Surgeon*, 3(5):309-16, Oct. 2005.

Selvanetti, A., Cipolla, M., Puddu, G., Overuse tendon injuries: basic science and classification. *Oper Tech Sports Med,* 5:110-117, 1997.

Sung, K-L.P., Whittemore, D.E., Yang, L., Amiel, D., Akeson, W.H., Signal pathways and ligament cell adhesiveness. *J Orthop Res,* 14:729-735, 1996.

Wang, N., Butler, J.P., Ingber, D.E., Mechanotransduction across the cell surface and through the cytoskeleton. *Science,* 260:1124-1127, 1993.

Wang, N., Ingber, D.E., Control of cytoskeletal mechanics by extracellular matrix, cell shape, and mechanical tension. *Biophysical J,* 66:2181-2189, 1994.

Wang, J.H.-C., Iosifidis, M.I., Fu, F.H., Biomechanical basis for tendinopathy. *Clin Orthop Related Res,* 443:320-332, 2006.

Watson, P.A., Function follows form: generation of intracellular signals by cell deformation. *FASEB J,* 5:2013-2019.

Woessner, J.F., Hideaki, N., *Matrix metalloproteinases and TIMPs.* Oxford University Press, New York, 2000.

II. L ITERATURE SURVEY

Tendon Anatomy

A complete understanding of the structure and metabolism of normal tendons is required for understanding the pathogenesis of tendon injuries and diseases. Tendon is a soft connective tissue that usually experiences longitudinal/tensile forces as it transmits the contraction of muscle to bone, making joint movement possible. Each muscle has two tendons, proximal and distal. The point of union with a muscle is called the myotendinous junction and the point of union with a bone is the osteotendinous junction. Tendons vary considerably in shape and in the way they are attached to bone. They range from wide and flat to cylindrical, fan-shaped, and ribbon-shaped (Jozsa, L & Kannus, P., 1997). Tendons receive their blood supply from three main sources: the intrinsic systems at the myotendinous junction and osteotendinous junction, and the extrinsic system through the paratenon or the synovial sheath (Carr AJ, 1989; Kvist M, 1995)

The fibrous structure of a tendon begins with triple helical collagen molecules assembled into fibrils and the fibrils are assembled into fibers. Bundles of these parallel fibers are sometimes called fascicles and the whole tendon is composed of several fiber bundles enclosed by the endotenon, which enables the tendon to glide against adjacent tissue (Vogal 2003). The division of tendons into fibrils ensures that minor damage does not necessarily spread to the entire tendon, and also provides a high total structural strength (Kjaer 2004) (Figure 2.1).

Tendon Biochemistry

The major constituents of tendon are water (~ 55% of wet weight) and Type I collagen (~ 85% of dry weight). The dominant cells of the tendon are fibroblasts, which are also called tenocytes. They are very elongated and found in the spaces between the fibrils, lying along the long axis (Jozsa & Kannus 1997). Tenocytes are responsible for the production and maintenance of tendon collagen and non-collagenous constituents (O'Brien 1997; Jozsa et al 1979; Kvist et al 1987) that are in the extracellular matrix. In general the synthetic activity of these components is high during growth and diminishes with age, although the activity pattern can change drastically with pathological conditions (O'Brien 1997; Jozsa et al 1979; Kvist et al 1987).

Collagen cross-links, discovered by David Eyre (Eyre 1997), increase as the tendon ages, and can be used to estimate turnover rates. The cross-links within and between mature collagen molecules provide mechanical stiffness (Puxkandl et al 2002; Avery & Bailey 2005). They also provide resistance to breakdown by proteases, which contributes to the remarkably long half-life of collagen in tendon (>100days)(Sell 1995). The critical importance of cross-linking is demonstrated by a complete absence of mechanical strength of the collagen fiber following inhibition of the cross-linking enzyme (Puxkandl et al 2002).

The ground substance of tendon extracellular matrix network is composed of proteoglycans, glycosaminoglycans, glycoproteins, and several other small molecules (Kannus et al 2000; Vogel 2003; Merrilees & Flint 1980; Jarvinen et al 1991; Kvist et al 1991). The proteoglycans are strongly hydrophilic, enabling rapid diffusion of water-

soluble molecules and migration of cells (Sharma & Maffulli 2005; Jozsa & Kannus 1997; Wolfe 1993). Mature collagen and its associated proteoglycans provide tendon with its tensile strength and shield the intratendinous cells from injury (Scott A et al 2007). Adhesive glycoproteins, such as fibronectin, laminin and thrombospondin, participate in repair and regeneration processes in tendon and ligament (Balint BJ 1978; Lawler J. 1986; Miller RR, 1991; Jozsa et al 1991a). These macromolecules are able to bind either other macromolecules together or cell surfaces together (Jozsa & Kannus 1997). Fibronectin participates in both degenerative and repair processes of tendons (Jozsa et al 1991b). Thrombospondin, like laminin, is a multi-domain adhesive glycoprotein that can interact with fibrinogen, fibronectin, plasminogen, glycolipids and calcium (Lawler 1986) and is a good candidate for mediating cell-matrix interactions.

Tendon cells (fibroblasts) are responsible for tendon homeostasis, which includes production of the components of the extracellular matrix Jozsa & Kannus). Internal tendon cells are organized into linear arrays along the axis of the tendon, parallel to the fascicles, separated from load-bearing collagen (Ritty et al 2003). They have a distinct pericellular matrix that contains the cells within descrete multicellular units in this connective tissue (Ritty et al 2003).

In total, the complex architecture of the fibroblasts and their connection to the extracellular matrix provides a three-dimensional network. This allows for transmission of force through the extracellular matrix to the cell resulting in a cell signaling response.

Tendon Mechanical Function

To transmit force from muscle to bone, the tendon must be capable of resisting high tensile forces with limited elongation (Kannus 2000; Best & Garrett 1994). Because the

collagen fibers within tendon are aligned in a parallel orientation, they transmit force with minimal energy loss and deformation (Squier & Bausch 1984; Jozsa, L. & Kannus, P, 1997).

Tendon rotation, if present, can play an important role in tendon mechanics and function. For example, Achilles tendon twists as it descends and the forces are in two planes. These combined stresses can create unequal tensile forces on different parts of the tendon (Reynolds & Worrell, 1991) leading to torsional ischemia and possible damage (Clement, Taunton & Smart 1984; Smart et al 1980). In addition to the load-transmitting role, tendons must be flexible enough to bend at joints and they must absorb sudden shock to limit damage to muscle (Best & Garrett 1994).

The mechanical properties of tendons are usually measured by *in vitro* tensile tests. The tissue is elongated to failure at a prescribed rate while changes in force are recorded. The force is plotted against time, but a constant displacement rate is normally used. From this testing, tendon stiffness, load, strain time, strain to failure and energy to failure can be measured (Butler 1978). The graphical curve is adjusted to the tendon's cross sectional area and length (Stone 1992; Viidik 1966). The stress-strain curve obtained from such studies allows the calculation of various mechanical parameters such as stiffness or modulus and is a valuable tool for studying mechanical properties of tendon.

Tendons are remarkably strong. A tendon with a cross-sectional area of $1cm^2$ is capable of supporting a weight of 500-1000kg. The tensile strength of tendon is actually stronger than that of compact bone (Barnett et al 1961) and it has more than twice the strength of its attached muscle (Frey & Shereff 1988; Kvist ,1991). Forces that place the highest stress on the tendon occur during eccentric muscle contractions vs. concentric

contractions (Fyfe & Stanish 1992; Komi 1984; Stanish et al 1985). An example of this kind of contraction would be when stepping up a stair, the heel slides back off the edge of the stair and drops below the foot. During eccentric contraction the whole muscle-tendon unit lengthens and thus most strain injuries occur during such contraction (Zarins & Ciullo 1983; Best & Garrett 1994; Noonan & Garrett 1992).

In tendinous injuries, "overuse" implies that then tendon has been strained repeatedly until it is unable to endure further tension, then injury occurs (Curwin & Stanish 1984, Hess et al 1989, Renstrom & Johnson 1985). According to current concepts, repetitive strain (3-5-8% strain) may lead to cumulative fiber microtrauma (Kannus 1997) (Figure 2.2). The microscopic structure of the tendon is disrupted by the repetitive strain and if damage progresses, tendinosis, partial tears or complete ruptures may occur.

Tendinopathy (Epidemiology)

Since tendons allow transmission of forces generated by muscle to bone, tendon injuries produce considerable disability that may last for months, despite appropriate management (Almekinders & Almekinders 1994). Such injuries can lead to loss of normal morphologic and functional characteristics of the tendon. This could lead to altered performance and increased risk of recurrent injury (Sharma et al 2005; Jarvinen et al 2005; Jozsa et al 1994).

Tendon overuse injury normally occurs as a result of work-induced or sports-induced repetitive overloading of the muscle-tendon unit. Work-induced overuse injuries usually occur in the upper extremities of workers who perform monotonous repetitive movements (Jozsa 1997). In textile workers there was an 11.6% prevalence of overuse tendonitis and related disorders (McCormack et al 1990). Among musicians, the

prevalence of performance-impairing medical problems was 76% of which two-thirds were of musculotendinous origin (Fishbein et al 1988).

About 10% of traumatic injuries treated in the emergency room of industrialized countries are sustained in sports. Chronic problems caused by sports-related overuse of tendons probably account for 30% of all running-related injuries (James et al 1978), and the prevalence of elbow tendinopathy in tennis players can be as high as 40% (Gruchow & Pelletier 1979). The exact incidence of overuse injuries is complicated by difficult diagnosis and the population at-risk is unknown, although absolute numbers of overuse injuries has increased dramatically during the last decades due to a general increase in sporting activities (Jarvinen 1992, Kannus el al 1988, Markison 1990, 1992,). About one-third of sports injuries that are treated at outpatient clinics involve the knee and many are due to overuse (Kannus 1987,1988,1989; Kujala et al 1986; Kvist & Jarvinen 1978).

Tendinopathy (Pathogenesis)

Tendinopathy encompasses various tendon injuries including tendinosis, tendonitis, and paratenonitis (Khan et al 1999). It can be defined as a syndrome of tendon pain, localized tenderness, and swelling that impairs performance and is a major medical problem associated with sports and physical activity in active people over 25 years of age (Renstrom & Woo 2007).

Many factors contribute to the development of tendinopathy. These factors can be extrinsic factors, such as excessive distance, intensity, technique, equipment, playing surface and fatigue or intrinsic factors, such as malalignment, overweight, age and gender (Jozsa et al 1989a; Malmivaara et al 1995; Nirschl 1992; Lorentzon 1988; Stanish 1984; Renstrom & Woo 2007). Extrinsic means all factors that act externally on the body

(Nigg 1998). It is thought that the most common extrinsic factor is excessive loads on the body due to training errors. In a runner, a force of three to five times the body weight ascends the lower extremity at heel strike (Mann 1982) and in a long distance runner this heel strike could happen about three million times a year (Renstrom 1988). It is not surprising that these causal factors result in injuries.

Intrinsic factors related to tendon injuries are entirely or partly genetically determined (Jozsa et al 1989; Malmivaara et al 1995; Nirschl 1992). The most common malalignment in the foot is hyperpronation where the ankle is medially angled placing excessive stress on the Achilles tendon (Jozsa & Kannus 1997). James et al (1978) noticed that increased pronation was present in 60% of a group of injured runners.

Currently, the cause of tendinopathy is not known. The etiopathogenesis remains unclear and many causes have been theorized. Etiology can be viewed as a failure of cell matrix to adapt to a variety of stresses as a result of an imbalance between matrix degeneration and synthesis (Selvanetti et al 1997; Leadbetter et al 1992). Additionally, it has been suggested that the destructive mechanism that precedes pathological development may be the catabolic response of cells to the loss of homeostatic strain as a result of microscopic tendon damage (Arnoczky et al 2007; Jones et al 2006). Proposed etiopathologic mechanisms for tendinopathy such as: repetitive (chronic) loading (Leadbetter 1992), ischemia reperfusion (Gordon 1991), microtrauma (Curwin & Stanish 1984; Renstrom & Johnson 1985; Ker 2002), hypoxia (Kraus-Hansen et al 1992), hyperthermia (Wilson & Goodship 1994), and reactive oxygen species (Kannus 1997; Bestwick & Maffulli 2000) have been suggested.

The intensive repetitive activity, which often is eccentric by nature, may lead to cumulative microtrauma, which further weakens the collagen cross-linking, non-collagenous matrix, and vascular elements of the tendon (Stanish et al 1985). Overuse has also been speculated to cause chronic tendon problems, by disturbing the micro- and macrovasculature of the tendon and resulting in insufficiency in the local blood circulation. Decreased blood flow simultaneous with an increased activity may result in local tissue hypoxia, impaired nutrition and energy metabolism, and increase in reactive oxygen species (Clement et al 1984; Rathbun & Macnab 1970). Together these factors are likely to play an important role in the sequence of events leading to tendon degeneration (Jarvinen 1997).

It is believed that repetitive loading and microtrauma are the most rational etiopathologic mechanisms for tendinopathy (Kannus 1997; Kvist 1994, 1991; Jarvinen et al 2001). Numerous investigators have suggested that overstimulation of tendon cells, secondary to repetitive loading, results in a gene expression pattern that can lead to tendinopathy (Almekinders et al 1993; Skutek et al 2001; Archambault et al 2002; Tsuzaki et al 2003; Wang et al 2003). Although pathological changes exist in tendinopathy, the etiopathogenesis of it remains unknown (Cook & Khan 2007).

An algorithum has been theorized that overuse injuries in tendons are caused by a failure of the tissue to adapt to repetitive microtrauma and a resultant deterioration of the extracellular matrix over time (Archambault et al 1995). In this algorithum, repetitive loading of a tendon is thought to elicit a cellular matrix response. This cell matrix response can be adequate or inadequate. If this response is adequate the tissue will adapt to repetitive load. However, an inadequate cell matrix response leads to transient

weakness in the tendon and continual loading will exceed the tendon's healing capacity resulting in an overuse injury (Figure 2.3).

Tendinopathy (Pathology)

Tendon pathology can be examined using macroscopic features or molecular biology techniques. Gene expression can be measured in normal and pathologic tendons (Ireland et al 2001; Alfredson et al 2003). The extent of tendon pathology can also be described or quantified according to functional outcomes or by mechanical testing (Sano et al 1998). Tendinosis involves altered expression of matrix proteins, growth factors, and cytokines, pointing to a pathologic process involving the resident tenocytes, endothelium, perivascular cells, and neurons (Maffulli et al 1998, 2003; Khan et al 2002). Type I and III collagen synthesis is increased concomitantly with altered matrix metalloprotease (MMP) activity leading to pathologic state of remodeling that fails to restore tendon architecture and function (Maffulli et al 2000; Ireland et al 2001; Fu et al 2002a,b; Riley et al 2002; Tillander et al 2002; Alfredson et al 2003b).

Matrix metalloproteinases, such as interstitial collagenase, are a family of zinc- and calcium-dependent endopeptidases involved in the normal remodeling of the extracellular matrix of connective tissues through their broad proteolytic capabilities (Bramono et al 2004; Parks et al 1998; Magra & Maffulli 2005). The activity of endogenous MMPs is normally inhibited by endogenous tissue inhibitors of metalloproteinases (TIMPs) (Bramono et al 2004; Parks & Mecham1998). The metabolic balance between the activities of endogenous MMPs and TIMPs is responsible for regulating normal tendon remodeling (Bramono et al 2004; Dalton et al 1995; Parks et al 1998). An excess of MMP activity can lead to a progressive degeneration and weakening of the extracellular

matrix of tendons (Lavagnino et al 2005). Some researchers say that there is an increase in the expression of TIMP-1 and MMP-1 in injured tendon (Jones et al 2006). ACL injury synovial fluid samples have shown increased MMP-3 and TIMP-1 (Higuchi et al 2006), while others say there is a decrease in the expression of TIMP-1 with tendinosis (Fu et al 2002). Still others describe an increase in serum or peritendinous fluid TIMP-1 levels with exercise (Koskinen et al 2001, 2004; Mackey et al 2004; Tyebjee et al 2005).

In other tissues such as osteoarthritic cartilage, there is an increased expression of MMPs and a decreased expression of TIMP-1. The processes that occur in cartilage may be the same as those occurring in tendon. This disturbed balance, resulting in an excess of metalloproteinase over TIMP, underlies pathologic cartilage destruction (Kavorkian et al 2004). From the literature it is apparent that the local balance of metalloproteinase and TIMP activities is pivotal in regulating tissue homeostasis.

Mechanotransduction

Mechanotransduction is the process by which cells elicit a metabolic action in response to mechanically derived signals secondary to extracellular matrix strain (Kjaer 2004; Wang 2006; Swartz et al 2001). The ability of tendon cells to respond to load is central to the concept of mechanotransduction and subsequent maintenance of tissue homeostasis (Wang & Ingber 1994; Banes et al 1995b; Ingber 1997). Progressive rehabilitation or increasingly intense rehabilitation is widely used following tendon and ligament injuries and surgery (Gamble et al 1984; Noyes 1977; Woo et al 1982). However, the amount of loading necessary to improve/accelerate the healing process without causing damage to the healing tissue remains unclear (Steadman et al 1989; Zeichen et al 2000).

Tendon cells are able to detect mechanical signals from deformation of their cellular membrane and /or cytoskeleton through a mechanoelectrochemical sensory system resulting in specific cellular responses (Banes et al 1995b). Previous work in our lab demonstrated that tensile loading of rat tail tendons resulted in an alteration of cell nuclear shape in a dose dependent manner. The cells were stained with acridine orange and strains of 0, 2, 4, and 6% were used to deform the tendon cells resulting in thinner, longer cells with increasing strain. Using confocal laser microscopy we were able to demonstrate a significant, but weak, correlation between cellular (nuclear) strain and tendon strain (Arnoczky et al 2002a).

Deformation of cellular membranes can affect the control of second-messenger molecules that can, in turn, activate a wide array of cellular machinery (Sachs 1998; Banes et al 1995b). Another study from our laboratory has shown that cytosolic calcium is increased in tendon cells in response to *in situ* deformation. As the strain is increased (0%, 2%, 4%, 6%) the cells increased in brightness due to calcium influx. Also strain from 0-4% produced significant cell elongation. The increase in cytosolic calcium associated with cell deformation supports a calcium channel mediated signaling system in tendon cells that is responsive to load (Shirakura et al 1995). These second messengers can activate a wide array of cellular machinery including DNA synthesis, mitosis, cell differentiation and gene expression (Sachs 1998, Banes et al 1995).

Mechanotransduction signals are known to be mediated through the pericellular matrix to the nucleus via integrin-based cell matrix connections (Sachs 1988; Ingber 1991; Watson 1991; Wang et al 1993, 2007; Banes et al 1995b; Ritty et al 2003). Large modular extracellular matrix (ECM) proteins, such as fibronectin, tenascin, laminin and

thrombospondin, have regions that participate in protein–protein interactions, as well as sites that can be bound by cell-surface receptors. Each of these proteins contain both integrin and heparin/heparan sulphate (HS)-binding sites (Labat-Robert et al 1990; Mostafavi-Pour et al 2003; Aumailley & Krieg 1996; Aumailley & Smyth 1998; Chowdhury et al 2003; Yurchenco et al 1994).

Integrins participate in a wide-range of biological interactions including development, tissue repair, angiogenesis, inflammation and hemostasis. Fibroblasts anchor to ECM by focal adhesions that physically link with actin cytoskeleton via integrin family of ECM receptors. Stretching induced conformational changes in the ECM may alter integrin structure, which leads to activation of several secondary messenger pathways in the cells (Katsumi et al 2005). Hence, integrins are regarded as receptors capable of inducing biochemical signals that regulate gene expression (Ingber, 1991; Schwartz et al., 1995; Ross, 2004).

Besides playing the role of indirect mechanosensors, integrins can act as direct mechanosensors, physically connecting the cytoskeleton to ECM, thus transmitting mechanical loads acting on the ECM (Hynes 1992). The extracellular domain of integrins binds to ECM proteins and functions as signaling receptors for them. Besides integrins, other various potential biochemical mediators of mechanotransduction such as mechanosensitive cell surface receptors (RTKs, G proteins) have also been suggested in fibroblasts (Ruwhof & van der Laarse 2000; Wang 2006; Wang & Thampatty 2006). While the cell matrix response is important to cellular homeostasis, it does not explain the mechanisms that result in transient weakness seen in tendinopathy.

Research Models

Although the pathology is well characterized, the pathogenesis is still unclear. Research models for tendinopathy have basically been divided into two groups: *in vitro* and *in vivo*. *In vitro* cell culture monolayers have been used to examine the response of tendon cells to various stimuli outside their extracellular matrix (Banes et al 1995,1999; Tsuzaki et al 2003). *In vivo* models have utilized rabbits, rats, and horses to document changes associated with repetitive loading (Nakama et al 2005; Scott et al 2007; Dahlgren et al 2002). However, these models lack the ability to precisely control confounding variables such as the ability to control or measure load and the effects of biological variation (genes, physical shape). *In vitro* studies can be conducted using monolayer cell cultures, but in this environment the cells are not in their natural cell matrix and normal cell interactions are not duplicated. Uniform local strains are present in monolayers, but not in a normal tendon (Berenson et al 1996; Fallon et al 2002) and high cell numbers with increased intracellular communication may introduce a paracrine stimulus. Conversely, *in situ* tissue culture would allow for natural cell matrix interactions and visco-elasticity. The cell number and relationship are those dictated by nature.

While some investigators have implicated overstimulation of tendon cells as a mechanobiological stimulus for inflammatory cytokine production and matrix degradation (Almekinders et al 1993; Archambault et al 2002; Tsuzaki et al 2003; Wang et al 2003; Bhargava et al 2004), these studies have been performed in monolayer cultures, often including high strain magnitudes (Almekinders et al 1993; Wang et al 2003; Bhargava et al 2004), long loading histories (Archambault et al 2002; Wang et al

2003; Bhargava et al 2004), and the addition of biochemical factors (Archambault et al 2002; Tsuzaki et al 2003). Thus, the clinical relevance of such studies is unclear.

Because of these reasons, we decided to concentrate on the rat tail tendon model. This allows us to use cells in an in situ environment, numerous antibodies and techniques optimized for rat species, and maintain tissue culture viability because of the small size of the RTT (approximately 3x the thickness of a human hair).

Tendinous Injuries Overuse/Underuse

In tendinous injuries "overuse" implies a repeated strain of 4-8% on a tendon that results in the inability of the tendon to endure further tension, whereby injury occurs (Curwin & Stanish 1984; Hess et al 1989; Micheli & Fehlandt 1992; Renstrom & Johnson 1985). Central to this theory is the concept that excessive loading of tendons during vigorous physical activity is the main pathologic stimulation for degeneration of the extracellular matrix (Selvanetti et al 1997). Some investigators have suggested that it is the tendon cell's response to excessive loading that initiates the degenerative cascade that leads to tendinopathy (Skutek et al 2001; Archambault et al 2002; Tsuzaki et al 2003; Wang et al 2003). A recent study found that a general pattern of collagen deterioration and tissue degeneration was common to both ruptured and tendinopathic tendons suggesting a common, but as yet unidentified, cell-mediated, pathological mechanism acting on both of these tendon populations (Tallon et al 2001). However, the relationship between the tendon overuse and loss of tendon cell tension is unclear. The level of stimuli required to elicit these cellular responses is not clinically relevant and has only been demonstrated in cultured cells on artificial substrates (Almekinders et al 1993; Wang et al 2003; Bhargava et al 2004).

Several animal models have been utilized attemping to create overload of tendons. Such models would allow for evaluation at early stages of tissue overloading. One stimulation regimen in adult rabbits over several weeks did not result in any inflammatory or degenerative changes within or around the loaded Achilles tendon (Archambault et al 1998). Messner et al subjected rats to repetitive electrical-induced eccentric exercise and found that only half of the animals achieved histological changes in and around the tendon (Messner et al 1999). Only horses and greyhound dogs, which are undergoing forced regimens of exercise, display overuse injuries similar to those in humans, whereas other animals only display tendon overuse when extreme regimens are imposed (Patterson-Kane et al 1997; Kjaer 2004).

Experimental studies in our laboratory have documented an increase in caspase-3 gene expression and protein synthesis as well as an increase in the number of apoptotic cells following 24 hours of stress-deprivation (loss of homeostatic tension) (unpublished data). Some studies suggest that apoptosis may have a role in the pathogenesis of tendinopathy (Yuan et al 2002,2003; Hosaka et al 2005). One *ex-vivo* study was able to induce apoptosis in tendon cells following high strains (20%), but it is probable that the high strain used in the investigation damaged tendon fibers or fibrils (Scott et al 2005). This could result in the understimulation or underuse of the tendon cells associated with the damaged tissue and subsequent induction of apoptosis secondary to the loss of homeostatic tension (Grinnell et al 1999). Understimulation due to damaged rat tail tendon fasicles in our laboratory produced an up-regulation of MMP mRNA expression in the damaged area (Lavagnino et al 2006). The up-regulation of MMP by tendon cells supports the role of mechanobiological under-stimulation (loss of hemeostatic tension) of

tendon cells in gene expression which could weaken the tendon putting the ECM at risk for further damage with subsequent loading (Lavagino et al 2005).

Loss of Homeostatic Tension

Tendon fibroblasts are capable of establishing a cytoskeletal tensional homeostasis through interactions with their local extracellular environment (Brown et al 1998; Lavagnino & Arnoczky 2005). This internal cellular tension has been shown to regulate gene expression in tendon cells and establish the cell's "calibration point" (Lavagnino and Arnoczky 2005). Mechanical forces, which exert additional tension (above and beyond this homeostatic calibration point) to the cytoskeleton, will elicit an anabolic response gene, while an absence of mechanical stimuli (or a decrease below the homeostatic level) will elicit a catabolic gene response (Arnoczky et al 2004; Lavagnino et al 2003; Lavagnino & Arnoczky 2005). A decrease in extracellular strain in tendons (loss of homeostatic tension) has been associated with an increase in the up-regulation of interstitial collagenase and a subsequent decrease in the tensile properties of these tissues (Arnoczky et al 2004; Lavagnino et al 2005). Therefore, it is possible that mechanobiological understimulation or underuse of tendon cells, due to an alteration in cell–matrix interactions, could also be an inciting factor in the etiology of overuse injuries.

Previous biomechanical studies have suggested that isolated collagen fibril damage occurs near the end of the linear portion of the load deformation curves of ligaments and tendons (Viidik 1972; Kastelic et al 1980; Woo et al 1982). While this damage may not affect the ultimate tensile strength of the tissues (Panjabi et al 1996) it could alter the cell–matrix interactions within the damaged portion of the tendon. The alteration of cell–

matrix interactions secondary to isolated fibrillar damage could cause a mechanobiological understimulation (underuse) of tendon cells, which has been shown to result in an upregulation of interstitial collagenase mRNA expression and protein synthesis (Lavagnino & Arnoczky 2005). This, in turn, could weaken the tendon and put more of the extracellular matrix at risk for further damage with subsequent loading (Lavagnino et al 2005).

As noted previously Matrix metalloproteinases (MMPs) have been shown to be increased in equine and human tendinopathy cases (Clegg et al 2005; Riley et al 2005; Jones et al 2006). Increased expression of MMPs has been associated with a decrease in the material properties of tendons and other connective tissues (Lavagnino et al 2005; Zernicke et al 1995; Smith et al 1997; Hanemaaijer et al 1998). Therefore, inhibition of MMPs may play a role in limiting the degradative effects of MMPs (Woessner & Nagase 2002; Baker et al 2002).

MMP inhibitors have been shown to limit articular cartilage degradation (Billinghurst et al 1999; Smith et al 1998; Yu et al 1992) and have been used extensively in treating periodontal disease (Ryan et al 1996). Also, they have been used as promising candidates for prognostic markers in cancer as well as possible treatments. The proteolytic enzyme systems, which involve MMPS, are responsible for the turnover of the ECM in normal physiological systems but it is believed that overexpression of the enzymes in tumors render the cells capable of breaking down the ECM allowing cells to invade surrounding tissue (Wurtz et al 2005). The expression and activation of MMPs are increased in almost all human cancers (Chenard et al 1998; Nakopoulou et al 2002a; Ranuncolo et al 2003; Talvensaari-Mattila et al 2003; Leppa et al 2004) and TIMP-1 has been shown to

be upregulated in numerous malignancies including breast cancer (Ree et al 1997; McCarthy et al 1999; Nakopoulou et al 2002b; Schrohl et al 2003, 2004). The upregulation of TIMP-1 in cancer together with the association to a poor prognosis could reflect the fact that the balance between expression of MMPs and TIMP-1, although still favoring MMPs, is at a higher overall level than in non-cancerous conditions (Wurtz et al 2005). Therefore, high levels of TIMP-1 levels would be associated with a worse prognosis but would not be the cause of it (Egeblad & Werb 2002). Exogenous MMP inhibitors such as Batimastat and Merimastat have been developed for therapeutic benefits but the growth of the MMP family has made the task complex. Some tumor types will depend more heavily on MMPs for their invasive growth than others (Parks & Mecham 1998). Hydroxamate inhibitors (GI168, GI173) caused significant inhibition and necrosis of subcutaneous prostate tumors in nude mice (Conway et al 1996). These new treatments will target tumor reliance on its collaboration with nonmalignant tissue, which may be cancer's "Achilles heel" (Parks & Mecham 1998).

A study from our lab questioned whether exogenous MMP inhibitors could prevent the decrease in material properties associated with the loss of homeostatic tension in rat tail tendons. The results of the study demonstrated that short-term (7d) administration of broad-spectrum MMP inhibitors minimized the loss of material properties associated with the mechanobiological under-stimulation of tendons *in vitro* (Arnoczky et al 2007). One clinical study demonstrated the beneficial effects following the local application of a protease inhibitor in the treatment of Achilles tendinopathy (Capasso et al 1993). The results of these studies support the beneficial effects of exogenous MMP inhibitors on limiting the degradative effects of endogenous MMPs on tendons.

Therefore, the purpose of this study was to determine the effect of *in vitro* loss of homeostatic tension or cyclic loading (exercise) on the gene expression ratio of TIMP (endogenous inhibitor) to MMP in rat tail tendons since the metabolic balance between the activities of endogenous MMPs and TIMPs is responsible for regulating normal tendon remodeling (Bramono et al 2004; Dalton et al 1995; Parks et al 1998). These exercise regimes have focused on the use of eccentric loading techniques, which increases tendon strain (Bramono et al 2004; Dalton et al 1995; Parks et al 1998).

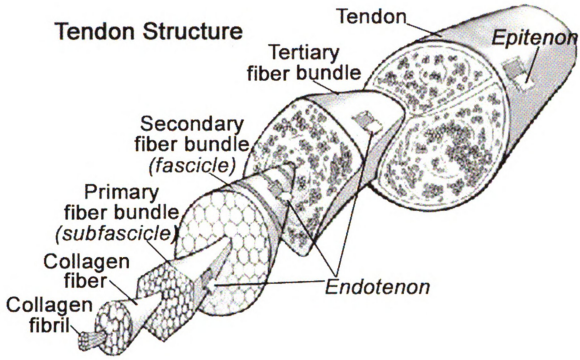

Figure 2.1 The division of tendons into fascicles and fibrils is shown in this diagram (Sharma & Maffulli 2005).

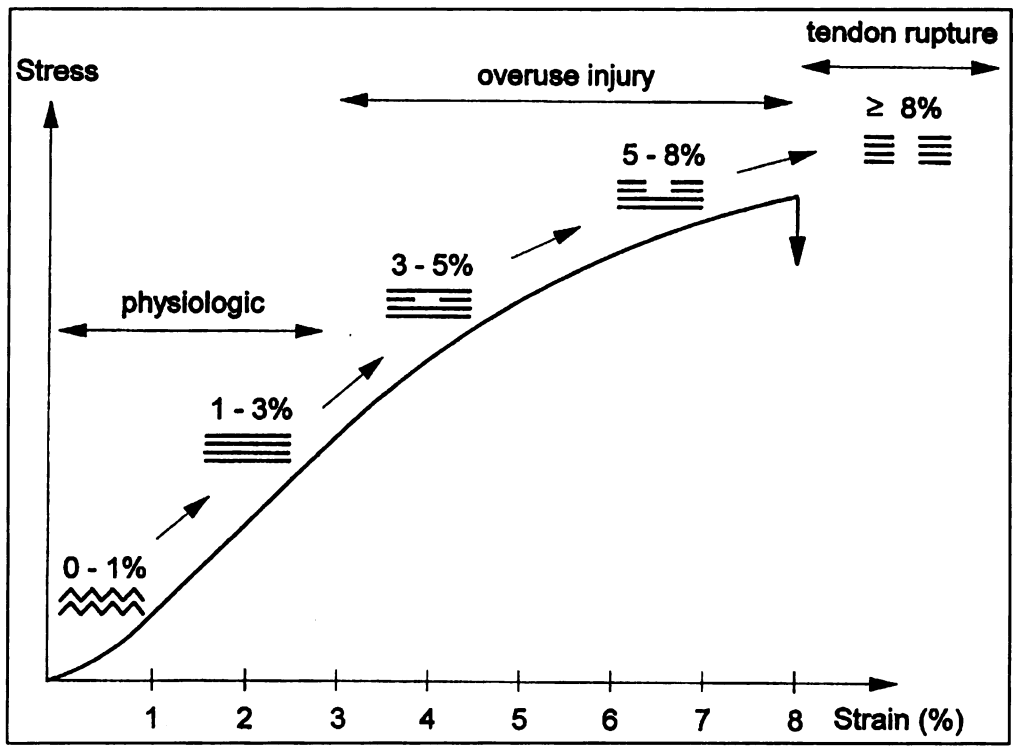

Figure 2.2 A schematic presentation of the development of chronic tendon disorders. According to current concepts, repetitive tendon strain (3-5-8% strain) may lead to cumulative fiber microtrauma. If the reparative capacity of the tendon tissue is exceeded, overuse injury can occur (Kannus 1997).

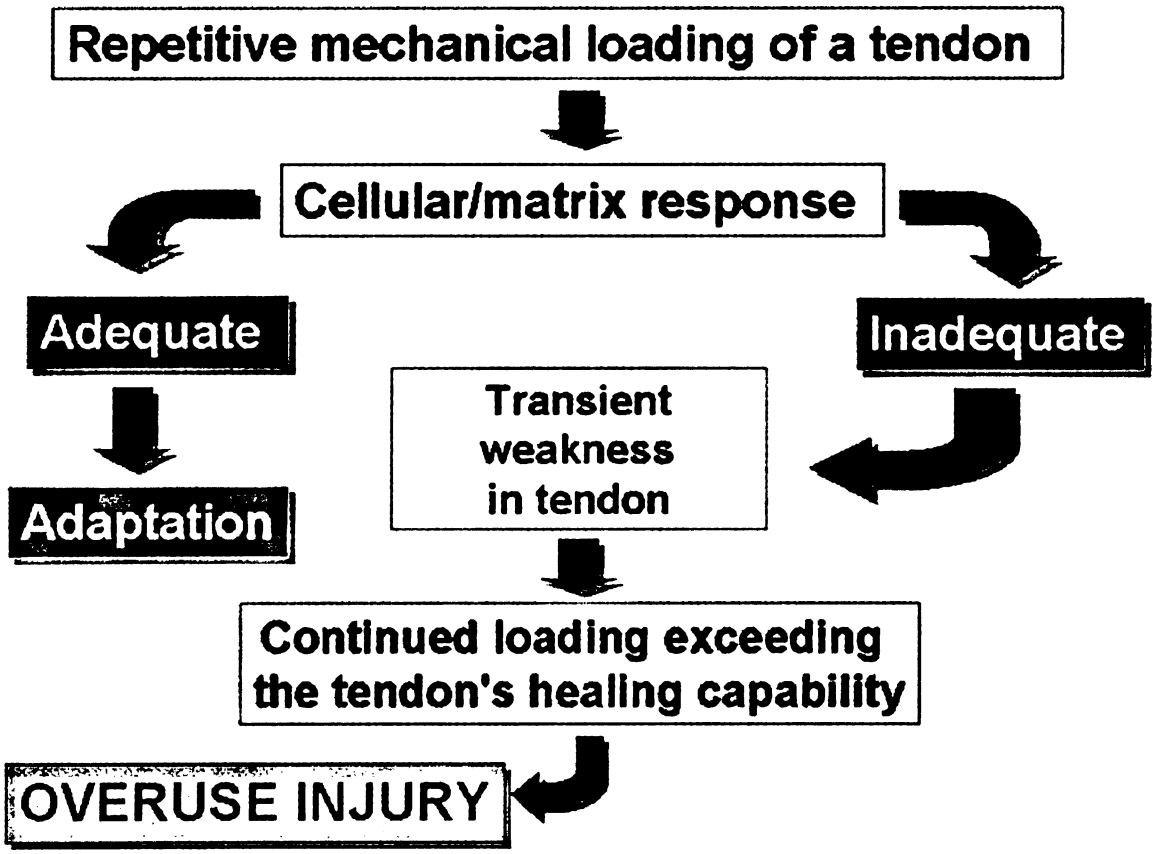

Figure 2.3 In this algorithum, repetitive loading of a tendon is thought to elicit a cellular matrix response. This cell matrix response can be adequate or inadequate. If this response is adequate the tissue will adapt to repetitive load. However, an inadequate cell matrix response leads to transient weakness in the tendon and continual loading will exceed the tendon's healing capacity resulting in an overuse injury (adapted from Archambault 1995).

References

Almekinders, L.C., Almekinders, S.V., Outcome in the treatment of chronic overuse sports injuries: a retrospective study. *J Orthop Sports Phys Ther,* 19:157-161, 1994.

Almekinders, L.C., Banes, A.J., Ballenger, C.A., Effects of repetitive motion on human fibroblasts. *Medicine and Science in Sports and Exercise,* 25:603-607, 1993.

Alfredson, H., Lorentzon, M., Backman, S., Gackman, A, Lerner, U.H., cDNA-arrays and real-time quantitative PCR techniques in the investigation of chronic Achilles tendinosis. *J Orthop Res,* 21:970-975, 2003.

Archambault, J.M., Koh, T.J., Herzog, W., Hart, D.A., Experimental model to study muscle and tendon adaptation to chronic loading. *J Musculoskeltal Res,* 2:283-288,1998.

Archambault, J., Tsuzaki, M., Herzog, W., Banes, A.J., Stretch and interleukin1 beta induce matrix metalloproteinases in rabbit tendon cells in vitro. *J Ortho Res*, 20:36-39, 2002.

Archambault, J.M., Wiley, J.P., Bray, R.C., Exercise loading of tendons and the development of overuse injuries. A review of current literature. *Sports Med,* 20(2):77-89, 1995.

Arnoczky, S.P., Lavagnino, M., Egerbacher, M., Caballero, O., Gardner, K., Matrix metalloproteinase inhibitors prevent a decrease in the mechanical properties of stress-deprived tendons: an in vitro experimental study. *Am J Sports Med,* 35(5):763-769, 2007.

Arnoczky, S.P., Lavagnino, M., Whallon, J.H., Hoonjan, A., In situ cell nucleus deformation under tensile load: a morphologic analysis using confocal laser microscopy. *J Orthop Res,* 20:29-35, 2002.

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

Aumailley, M., Gayraud, B., Structure and biological activity of the extracellular matrix. *J Mol Med,* 76(3,4):253-265, 1998.

Aumailley, M., Krieg, T., Laminins: a family of diverse multifunctional molecules of basement membranes. *J Investig Dermatol,* 106(2):209-21, 1996.

Avery, N.C., Bailey, A.J., Enzymatic and non-enzymatic cross-linking mechanisms in relation to turnover of collagen: relevance to aging and exercise. *Scand J Med Sci Sports,* 15(4):231-40, 2005.

Balint, B.J., Jozsa, L, Investigation on the contruction and spatial structure of human tendons. *Traumatologia,* 21:386-387, 1978.

Banes, A.J., Tsuzaki, M., Hu, P., Brigman, B., Brown, T., Almekinders, L., Lawrence, W.T., Fischer, T., Cyclic mechanical load and growth factors stimulate DNA synthesis in avian tendon cells. *J Biomech,* 28:1505-1513, 1995a.

Banes, A.J., Tsuzaki, M., Yamamoto, M., et al., Mechanoreception at the cellular level: the detections interpretation, and diversity of responses to mechanical signals. *Biochem Cell Biol,* 73:349-365,1995b.

Banes, A.J., Weinhold, P., Yang, X., Tsuzaki, M., Bynum, D., Bottllang, M., Brown, T., Gap junctions regulate responses of tendon cells ex vivo to mechanical loading. *Clin Orthop Relat Res,* (367 Suppl):S356-370,1999.

Barnett, C.H., Davies, D.V., MacConaill, M.A., *Synovial joints: Their structure and mechanics. Long: Longmans,* p82, 1961.

Berenson, M.C., Blevins, F.T., Plass, A.H.K., Vogel, K.G., Proteoglycans of human rotator cuff tendons. *J Orthop Res,* 14:518-525,1996.

Best, T.M., Garrett, W.E., Basic science of soft tissue; muscle and tendon. In: DeLee JC, Dres D Jr, editors. *Orthopaedic sports medicine: principles and practice. Philedelphia: Lea and Febiger*, pp59-74, 1994.

Bestwick CS and Maffulli N. Reactive oxygen species and tendon problems: review and hypothesis. *Sports Med Arthrosco,* 8:6-16, 2000.

Bhargava, M., Attia, E., Hannafin, J.A., The effect of cyclic tensile strain on MMPs, collagen, and casein degrading activities of fibroblasts isolated from anterior cruciate and medial collateral ligaments. *In: transactions of the 50th Orthopaedic Research Society*, Washington DC, p110, 2004.

Billinghurst, R.C., O'Brien, K., Poole, A.R., McIlwraith, C.W., Inhibition of articular cartilage degradation in culture by a novel nonpeptidic matrix metalloproteinse inhibitor. *Ann NY Acad Sci,* 878:594-597, 1999.

Bramono, D.S., Richmond, J.C., Weitzel, P.P., Kaplan D.L., Altman, G.H., Matrix metalloproteinases and their clinical applications in orthopaedics. *Clin Ortho,* 428:272-285, 2004.

Brown, R.A., Prajapati, R., McGrouther, D.A., Yannus, I.V., Eastwook M., Tensional homeostasis in dermal fibroblasts: mechanical responses to mechanical loading in three dimensional substrates. *J Cell Phys,* 175:323-332,1998.

Butler, D.L., Grood, E.S., Noyes, F.R., Zernicke, R.F., Biomechanics of ligaments and tendons. *Exerc Sport Sci Rev,* 6:125-181,1978.

Carr, A.J. & Norris, S.H., The blood supply of the calcaneal tendon. *J Bone Joint Surg (Br),* 71:100-101,1989.

Chenard, M.P., O'Siorain, L., Shering, S., Rouyer, N., Lutz, Y., Wolf, C., Basset, P., Belloco, J.P., Duffy, M.J., High levels of stromelysin-3 correlate with poor prognosis in patients with breast carcinoma. *International Journal of Cancer*, 69:448–451, 1998.

Chowdhury, T.T., Bader, D.L., Shelton, J.C., Lee, D.A., Temporal regulation of chondrocyte metabolism in agarose constructs subjected to dynamic compression. *Arch Biochem Biophy,* 417(1):105-111, 2003.

Clement, D., Taunton, J., Smart, G., Achilles tendonitis and peritendinitis: Etiology and treatment. *Am J Sports Med,* 12:179-184, 1984.

Conway, J.G., Trexler, S.J., Wadefield, J.A., Marron, B.E., Emerson, D.L., Bickett, D.M., Deaton, D.N., Garrison, D., Elder, M., McElroy, A., Willmott, N., Docherty, A.J.P., McGeehan, G.M., Effect of matrix metalloproteinase inhibitors on tumour growth and spontaneous metastasis. *Clin Exp Metastasis,* 14:115-124, 1996.

Cook, J.L., Khan., K.M., Etiology of Tendinopathy. *Tendinopathy in Athletes,* Blackwell Publishing, pp10-28, 2007.

Cook, J.L, Feller, J.A., Boner, S.F., Khan, K.M., Abnormal tenocyte morphology is more prevalent than collagen disruption in asymptomatic athletes' patellar tendons. *J Ortho Res*, 22(2):334-338, 2004.

Curwin, S.L., Stanish, W.D., Tendinitis: Its Etiology and Treatment. *Collamore Press*, 1984.

Dahlgren, L.A., van der Meulen, M.C., Bertram, J.E., Starrak, G.S., Nixon, A.J., Insulin-like growth factor-I improves cellular and molecular aspects of healing in a collagenase-induced model of flexor tendinitis. *J Ortho Res*, 20(5):910-9, 2002.

Dalton, S., Cawston, T.E., Riley, G.P., Bayley, I.J., Hazelman, B.L., Human shoulder tendon biopsy samples in organ culture produce procollagenase and tissue inhibitor of metalloproteinases. *Ann Rheum Dis,* 54:571-577, 1995.

Egeblad, M. & Werb, Z., New functions of the matrix metalloproteinases in cancer progression. *Nature Reviews Cancer,* 2:161–174, 2002.

Eyre, D.R., Bone biomarkers as tools in osteoporosis management. *Spine,* 22(suppl):17S-24S, 1997.

Fallon, J., Blevins, F.T., Vogel, K., Trotter, J., Functional morphology of the supraspinatus tendon. *J Orthop Res,* 20:920-926, 2002.

Fishbein, M., Middlestadt, S.E., Ottati, V., Medical problems among ICSOM musicans. *Med Probl Perform Art*, 3:1-7, 1988.

Fu, S.C., Chan, B.P., Wang, W., Pau, H.M., Chan, K.M., Rolf, C.G., Increased expression of matrix metalloproteinase 1 (MMP1) in 11 patients with patellar tendinosis. *Acta Ortho Scand,* 73(6):658-662, 2002.

Fu, S.C., Wang, W., Pau, H.M., Wang, Y.P., Chan, K.M., Rolf, C.G., Increased expression of transforming growth factor-beta1 in patellar tendinosis. *Clin Related Res,* 73:174-183, 2002.

Fyfe, J., Stanish, W.D., The use of eccentric training and stretching in the treatment and prevention of tendon injuries. *Clin Sports Med,* 11:601-624, 1992.

Gamble, J.G., Edwards, C.C., Max, S.R., Enzymatic adaptation in ligaments during immobilization. *Am J Sports Med,*12(3):221-228,1984.

Goodship, A.E., Birch, H.L., Wilson, A.M., The pathobiology and repair of tendon and ligament injury. *Vet Clin North Am Equine Pract*, 10(2):323-349, 1994.

Gordan, G.A., Stress reactions in connective tissues: a molecular hypothesis. *Med Hypotheses*, 36(3):289-94, 1991.

Grinnell, F., Zhu, M., Carlson, M., Abrams, J., Release of mechanical tension triggers apoptosis of human fibroblasts in a model of regressing granulation tissue. *Exp Cell Res,* 248:608-619, 1999.

Gruchow, H.W., Pelletier, D., An epidemiologic study of tennis elbow. Incidence recurrence, and effectiveness of prevention strategies. *Am J Sorts Med,* 7:234-238, 1979.

Hanemaaijer, R., Visser, H., Koolwijk, P., Sorsa, T., Golub, L.M., van Hinsbergh, V.W.M., Inhibition of MMP synthesis by doxycycline and chemically modified tetracyclines (CMTs) in human endothelial cells. *Adv Dent Res,* 12:114-118, 1998.

Hess, G.P., Cappiello, W.L., Poole, R.M., Hunter, S.C., Prevention and treatment of overuse tendon injuries. *Sports Med*, 8:371-384, 1989.

Higuchi, H., Shirakura, K., Kimura, M., Terauchi, M., Shinozaki, T., Watanabe, H., Takagishi, K., Changes in biochemical parameters after anterior cruciate ligament injury. *International Orthopaedics*, 30:43-47, 2006.

Hosaka, Y., Teraoka., H., Yamamoto, E., Ueda, H., Takehana, K., Mechanism of cell death in inflamed superficial digital flexor tendon in the horse. *J Comp Path,* 132:51-58, 2005.

Hynes, R.O., Integrins: versatility, modulation, and signaling in cell adhesion. *Cell,* 69:11-25, 1992.

Ingber, D.E., Integrins as mechnochemical transducers. *Opinion in Cell Biology,* 3:841-848, 1991.

Ireland, D., Harrall, R., Curry, V., Halloway, G., Hackney, R., Hazelmen, B., Riley, G., Multiple changes in gene expression in chronic human Achilles tendinopathy. *Matrix Biology,* 20:159-169, 2001.

Jones, G.C., Corps, A.N., Pennington, C.J., Clark, I.M., Edwards, D.R., Bradley, M.M., Hazleman, B.L., Riley, G.P., Expression Profiling of Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Normal and Degenerate Human Achilles Tendon. *Arthritis & Rheumatism*, 54(3):832-842, 2006.

James, L.L., Gates, B.T., Osternig, L.R., Injuries to runners. *American Journal of Sports Medicine,* 6:40-50, 1978.

Jarvinen, T.A., Jarvinen, T.L, Kaariainen, M., Kalimo, H., Jarvinen, M., Muscle injuries: biology and treatment. *Am J Sports Med. 2005,* 33(5):745-64, 2005.

Jarvinen, M., Jozsa, L., Kannus, P., Jarvinen, T.L., Kvist, M., Seadbetter, W., Histopathological findings in chronic tendon disorders. *Scand J Med & Sci Sports,* 7:86-95, 1997.

Jarvinen, M., Epidemiology of tendon injuries in sports. *Clin Sports Med,* 11:493-504, 1992.

Jarvinen, M., Kannus, P., Kvist, M., Isola, J., Lehto, M,. Jozsa, L., Macromolecular composition of the myotendinous junction. *Exp Mol Pathol,* 55:230-237, 1991.

Jones, G.C., Corps, A.N., Pennington, C.J., Clark, I.M., Edwards, D.R., Bradley, M.M., Hazleman, B.L., Riley, G.P., Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human Achilles tendon. Akura, *Arthritis & Rheumatism*, 54(3):832-842, 2006.

Jones, G.C., Corps, A.N., Pennington, C.J., Clark, I.M., Edwards, D.R., Bradley, M.M., Hazleman, B.L., Riley, G.P., Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human Achilles tendon. *Arthritis & Rheumatism*, 54(3):832-842, 2006.

Jozsa, L., Balint, B.J., Reffy, A., Demel, Z., Fine structural alterations of collagen fibers in degenerative tendinopathy. *Arch Orthop Trauma Surg*, 103(1):47-51, 1984.

Jozsa, L., Balint, J.B., Reff, A., Demel, Z., Histochemical and ultrastructural study of adult human tendon. *Acta Histochem,* 65:250-257, 1979.

Jozsa, L., Kannus, P., Human tendons: anatomy, physiology, and pathology. *Human Kinetics,* 1997.

Jozsa, L., Kannus, P., Balint, B.J., Reffy, A., Three-dimensional ultrastructure of human tendons. *Acta Anat,* 142:306-312, 1991a.

Jozsa, L, Kvist, M., Kannus, P., Vieno, T., Jarvinen, M., Lehto, M., Structure and macrovolecular composition of the myotendinous junction. *Acta Morphol Hung,* 39:287-297, 1991b.

Jozsa, L, Kvist, M., Balint, B.J., Reffy, A, Jarvinen, M., Lehto, M., Barzo, M., The role of recreational sports activity in Achilles tendon reupture. A clinical, pathoanatomical and sociological study of 292 cases. *Am J Sports Med,* 17:338-343, 1989.

Kannus, P., Etiology and pathophysiology of chronic tendon disorders in sports. *Scand J Med Sci Sports,* 7(2):78-85, 1997.

Kannus, P., Jozsa, L., Jarvinen, M., Basic science of tendons. In: Garrett WE Jr, Spreer KP, Kirkendall DT, editors. Principles and practice of orthopaedic sports medicine. *Philadelphia: Lippincott Williams and Wilkins,* pp21-37, 2000.

Kannus, P., Jarvinen, M., Posttraumatic anterior cruciate ligament insufficiency as a cause of osteoarthritis in a knee joint. *Clin Rheumatol,* 8:251-260, 1989.

Kannus, P., Niittymaki, S., Jarvinen, M., Athletic overuse injuries in children. A 30 monthprospective follow-up study at an outpatient sports clinic. *Clin Pediatr,* 27:333-337, 1988

Kannus, P., Aho, H., Jarvinen, M., Niittymaki, S., Computerized recording of visits to an outpatient sports clinic. *Am J Sports Med,* 15:79-85, 1987.

Kastelic, J., Palley, I., Baer, E., A structural mechanical model for tendon crimping. *J Biomech,* 13:887-893, 1980.

Katsumi, A., Naoe, T., Matsushita, T., Kaibuchi, K., Schwartz, M.A., Integrin activation and matrix binding mediate cellular responses to mechanical stretch. *J Biol Chem,* 280:16546-16549, 2005.

Kavorkian, L., Young, D.A., Darrah, C., Donell, S.T., Shepstone, L, Porter, S., Brockbank, S.M.V., Edwards, D.R., Parker, A.E., Clark, I.M., Expression profiling of metalloproteinases and their inhibitors in cartilage. *Arthritis Rheum,* 50(1):131-141, 2004.

Ker, R.F., The implications of the adaptable fatigue quality of tendons for their construction, repair, and function. *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology,* 133:987-1000, 2002.

Khan, K.M., Cook, J.L, Kannus, P., Maffulli, n., Bonar,S.F., Time to abandon the "tendonitis" myth. *Brit Med J,* 324:626-627, 2002.

Khan, K.M., Cook, J.L., Bonar, F., Harcourt, P., Astrom, M., Histopathology of common tendinopathies. Update and implications for clinical management. *Sports Med,* 27(6), 393-408, 1999.

Kjaer, M., Conversion of mechanical loading into functional adaptation of tendon and skeletal muscle: a role for extracellular matrix. *Physilogical Review,* 84:649-698, 2004.

Kjaer, M., Role of extracellular matrix in adaption of tendon and skeletal muscle to mechanical loading. *Physiol Rev,* 84;649-698, 2004

Komi, P.V., Physiological and biomechanical correlates of muscle function: Effects of muscle structure and stretch-shortening cycle on force and seed. In: Terjung RH (ed) *Exercise and sports sciences reviews, Vol 12. Lexington, MA:DC Heath,* pp81-123, 1984.

Kosikinen, S.A.O., Heinemeier, K.M., Olesen, J.L., Langberg, H., Kjaer, M., Physical exercise can influence local levels of matrix metalloproteinases and their inhibitors in tendon-related connective tissue. *J Appl Physio,* 96:861-864, 2004.

Kosikinen, S.A.O., Hoyhtya, M., Turpeenniemi-Hujanen, T., Martikkala, V., Makinen, T.T., Oksa, J., Rintamaki, H., Lofberg, M., Somer, H., Takala, T.E.S., Serum concentrations of collagen degrading enzymes and their inhibitors after downhill running. 11:9-15, 2001.

Kraus-Hansen, A.E., Fackelman, G.E., Becker, C., Williams, R.M., Pipers, F.S., Preliminary studies on the vascular anatomy of the equine superficial digital flexor tendon. *Equine Vet J,* 24(1):46-51, 1992.

Kujala, U., Kvist, M., Osterman, K., Knee injuries in athletes: Review of exertion injuries and retrospective study of outpatient sports clinic material. *Sports Med,* 3:447-460, 1986.

Kvist, M., Achilles tendon injuries in athletes. *Annals Chirurgie Gynecologie,* 80:88-201, 1991.

Kvist, M., Jarvinen, M., Typical injuries at an outpatient sports clinic. *Duodecim,* 94:1335-1345, 1978.

Kvist, M., Jozsa, L., Kannus, P., Isola, J., Vieno, T., Jarvinen, M., Lehton, M., Morphology and histochemistry of the myotendinous junction of the rat calf muscles. Histochemical, immunohistochemical, and electron microscopic study. *Acta Anat,* 141:199-205, 1991.

Kvist, M., Jozsa L., Jarvinen, M.J., Kvist H., Chronic Achilles paratenonitis is athletes: a histological and histochemical study. *Pathology,* 19:1-11, 1987.

Kvist, M., Jozsa, l., Jarvinen, M., Kvist, H., Fine structural alterations in chronic Achilles paratenonitis in athletes: a histological and histochemical study. *Pathology,* 19:1-11, 1987.

LaBat, R.J., Bihari, V.M., Robert, L., Extracellular matrix. *Febs Letters,* 268(2):386-393, 1990.

Lavagnino, M., Arnoczky, S.P., In vitro alterations in cytoskeletal tensional homeostasis control gene expression in tendon cells. *J Ortho Res,* 23:1211-1218, 2005.

Lavagnino, M., Arnoczky, S.P., Frank, K., Tian, T., Collagen fibril diameter distribution does not reflect changes in the mechanical properties of in vitro stress-deprived tendons. *J Biomech,* 38(1):69-75, 2005.

Lavagnino, M., Arnoczky, S.P., Tian, T., Vaupel, Z., Effect of amplitude and frequency of cyclic tensile strain on the inhibition of MMP-1 mRNA expression in tendon cells: an in vitro study. *Connect Tissue Res,* 44(3-4):181-187, 2003.

Lavagnino, M., Arnoczky, S.P., Egerbacher, M., Gardner, K., Burns, M.E., Isolated fibrillar damage in tendons stimulates local collagenase mRNA expression and protein synthesis. *J Biomech,* 39:2355-2362, 2006.

Lawler, J.W., Review: The structural and functional properties of thrombospondin. *Blood,* 67:1192-1204, 1986.

Leadbetter, W.B., Cell-matrix response in tendon injury. *Clin Sports Med,* 11:533-578, 1992.

Leppä, S., Saarto, T., Vehmanen, L., Blomqvist, C., Elomaa, I., A high serum matrix metalloproteinase-2 level is associated with an adverse prognosis in node-positive breast carcinoma. *Clinical Cancer Research,* 10:1057–1063, 2004.

Lian, O., Scott, A., Engebretsen, L., Bahr, R., Duronio, V., Khan, K., Excessive apoptosis in patellar tendinopathy in athletes. *Am J Sports Med,* (4):605-11, 2007.

Lorentzon, R., Causes of injuries: Intrinsic factors. In: Dirix A, Knuttgen HG, Tittel K (ed) *The Olympic Book of Sports Medicine. Oxford:Blackwell Scientific Publications,* pp376-390, 1988.

Mackey, A.L., Donnelly, A.E., Turpeenniemi-Hujanen, T., Roper, H.P., Skeletal muscle collagen content in humans after high-force eccentric contractions. *J Appl Physiol,* 97:197-203, 2004.

Maffulli, N., Ewen, S.W., Waterston, S.W., Reaper, J., Barrass, V., Tenocytes from ruptured and tendinopathic Achilles tendons produce greater quantities of Type III collagen than tenocytes from normal Achilles tendons. An *in vitro* model of human tendon healing. *Am J Sports Med,* 28:499-505, 2000.

Maffulli, N., Khan, K.M., Puddu, G., Overuse tendon conditions: time to change a confusing terminology. *Arthoscopy,* 14:840-843, 1998.

Maffulli, N., Wong, J., Almekinders, L.C., Types of epidemiology of tendinopathy. *Clin Sports Med,* 22:675-692, 2003.

Magra, M., Maffulli, N., Molecular events in tendinopathy: A role for metalloproteinases. *Foot Ankle Clin N Am,* 10:267-277, 2005.

Malmivaara, A., Viikari-Juntura, E., Huuskonen, M., Aho, K., Kivi, P., Koskimies, S., Kuosma, E., Kurppa, K., Rheumatoid factor and HLA antigens in wrist tenosynovitis and humeral epicondylitis. *Scand J Rheumatol,* 24:154-156, 1995.

Mann, R.A., Biomechanics of running. In: *Mack RP (eds) Symposium of Foot and Leg in Running Sports. St. Louis: CV Mosby,* pp1-29, 1982.

Markison, R.E., Tendinitis and related inflammatory conditions seen in musicians. *J Hand Therapy,* 9:80-83, 1992.

Markison, R.E., Tendinitis and related inflammatory conditions seen in musicians. *Hand Clin,* 6:525-544, 1990.

McCarthy, K., Maguire, T., McGreal, G., McDermott, E., O'Higgins, N., Duffy, M.J., High levels of tissue inhibitor of metalloproteinase-1 predict poor outcome in patients with breast cancer. *International Journal of Cancer (Pred. Oncol.),* 84:44–48, 1999.

McCormack, R.R., Inman, R.D., Wells, A., Bernstein C., Imbus, H.R., Prevalence of tendonitis and related disorders of the upper extremity in a manufacturing workforce. *J Rheumatol,* 17:958-964, 1990.

Merrilees, M.J., Flint, M.H., Ultrastructural study of tension and pressure zones in a rabbit flexor tendon. *Am J Anat,* 157(1):87-106, 1980.

Messner, K., Wei, Y., Anderson, B., Gillquist, J., Rat model of Achilles tendon disorder. *Cell Tissue Org,* 165:30-39, 1999.

Micheli, L.J., Fehlandt, A.F., Overuse injuries to tendons and apophyses in children and adolescents. *Clin Sports Med,* 11:713-726, 1992.

Miller, R.R., McDevitt, C.A., Thrombospondin in ligament, meniscus and intervertebral disc. *Biochim Biophys Acta,* 1115(1):85-88, 1991.

Mostafavi-Pour, Z, Askari, J.A., Parkinson, S.J., Parker, P,J., Ng, T.T., Humphries, M.J., Integrin-specific signaling pathways controlling focal adhesion formation and cell migration. *J Cell Biol,* 161(1):155-167, 2003.

Nakama, L.H., King, K.B., Abrahmsson, S., Rempel, D.M., Evidence of tendon microtears due to cyclical loading in an in vivo tendinopathy model. *J Ortho Res*, 23(5):1199-1205, 2005.

Nakopoulou, L., Panayotopoulou, E.G., Giannopoulou, I., Alexandrou, P., Katsarou, S., Athanassiadou, P., Keramopoulos, A., Stromelysin-3 protein expression in invasive breast cancer: relation to proliferation, cell survival and patients' outcome. *Modern Pathology* 15:1154–1161, 2002a.

Nakopoulou, L., Giannopoulou, I., Stefanaki, K., Panayotopoulou, E., Tsirmpa, I., Alexandrou, P., Mavrommatis, J., Katsarou, S., Davaris, P., Enhanced mRNA expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) in breast carcinomas is correlated with adverse prognosis. *Journal of Pathology,* 197:307–313, 2002b.

Nigg, B., Causes of injuries: Extrinsic factors. In: Dirix A, Knuttgen HG, Tittel K (eds) *The Olympic Book of Sports Medicine.* Oxford: Blackwell Scientific Publications, pp363-375, 1998.

Nirschl, R.P., Elbow tendinosis/tennis elbow. The surgical treatment of lateral epicondylitis. *J Bone Joint Surg (Am),* 61:832-839, 1992.

Noonan, T.J., Garrett, W.E., Injuries at the myotendinous junction. *Clin Sports Med,* 11:783-806, 1992.

Noyes, F.R., Functional properties of knee ligaments and alterations induced by immobilization: a correlative biomechanical and histological study in primates. *Clin Orthop Relat Res,* 123:210-42, 1977.

O'Brien, M., Structure and metabolism of tendons. *Scand J Med Sci Sports*, 7:55-61, 1997.

Panjabi, M.M., Yoldas, E., Oxland, R.R., Crisco III, J.J., Subfailure injury of the rabbit anterior cruciate ligament. *J Ortho Res,* 14:24-31, 1996.

Parks, W.C., Mecham, R.P., *In: Matrix Metalloproteinases.* Academic Press, 1998.

Parks, W.C., Sudbeck, B.D., Doyle, G.R., Saariahlo-Kere, U.K., Matrix metalloproteinases in tissue repair. *In: Matrix Metalloproteinases.* Academic Press:263-297, 1998.

Patterson-Kane, J.C., Wilson, A.M., Firth, E.C., Parry, D.A., Goodship, A.E., Camparison of collagen fibril population in the superficial digital flexor tendons of exercised and nonexercised thoroughbreds. *Equine Vet J,* 29:121-125, 1997.

Puxkandl, R., Zizak, I., Paris, O., Keckes, J., Tesch, W., Bernstorff, S., Purslow, P., Fratzl, P., Viscoelastic properties of collagen: synchrotron radiation investigations and structural model. *Philos Trans R Soc Lond B Biol Sci,* 357(1418):191-7, 2002.

Ranuncolo, S.M., Armanasco, E., Cresta, C., Bal De Kier Joffe, E., Puricelli, L., Plasma MMP-9 (92 kDa-MMP) activity is useful in the follow-up and in the assessment of prognosis in breast cancer patients. *Int J Cancer,* 106:745–751, 2003.

Ree, A.H., Florenes, V.A., Berg, J.P., Mælandsmo, G.M., Nesland, J.M., Fodstad ø., High levels of messenger RNAs for tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-1) in primary breast carcinomas are associated with development of distant metastases. *Clinical Cancer Research,* 3:1623–1628, 1997.

Renstrom, P., Diagnosis and management of overuse injuries. In: Dirix A, Knuttgen HG, Tittel K (eds) *The Olympic Book of Sports Medicine.* Oxford: Blackwell Scientific Publications, pp446-468, 1988.

Renstrom, P., Johnson, R.J., Overuse injuries in sports. A review. *Sports Med,* 2(5):316-333, 1985.

Renstrom, Per A.H.F., Woo, S.L., Tendinopathy: A Major Medical Problem in Sport. *Tendinopathy in Athletes,* Blackwell Publishing, 1-9, 2007.

Reynolds, N.L., Worrell, T.W., Chronic Achilles peritendinitis: Etiology, pathophysiology, and treatment. *J Orthop Sorts Phys Ther,* 171-176, 1991.

Riley, G.P., Gene expression and matrix turnover in overused and damaged tendons. *Scand J Med Sci Sports,* 15:241-251, 2005.

Riley, G.P., Curry, V., DeGroot, J., van El, B., Verzijl, N., Hazleman, B.L., Bank, R.A., Matrix metalloproteinase activities and their relationship with collagen remodeling in tendon pathology. *Matrix Biol,* 21(2):185-195, 2002.

Ritty, T.M., Roth, R., Heuser, J.E., Tendon cell array isolation reveals a previously unknown fibrillin-2 containing macromolecular assembly. *Structure,* 11:1179-1188, 2003.

Ross, R.S., Molecular and mechanical synergy: cross-talk between integrins and growth factor receptors. *Cardiovasc Res,* 63:381-390, 2004.

Ryan, M.E., Ramarmurthy, N.S., Golub, L.M., Matrix metalloproteinases and their inhibition in periodontal treatment. *Curr Opin Periodontol,* 3:85-96, 1996.

Sachs, F., Mechanical transduction in biological systems. *Critical Rev Biomed Eng,* 15:141-169, 1988.

Sano, H., Uhthoff, H.K., Backman, D.S., Brunet, J.A., Trudel, G., Phamb, B., Ishii, H., Structural disorders at the insertin of the supraspinatius tendon. Relation to tensile strength. *J Bone Joint Surg (Br),* 80:720-725, 1998.

Schrohl, A.S., Christensen, I.J., Pedersen, A.N., Jensen, V., Mouridsen, H., Murphy, G., Foekens, J.A., Brünner, N., Holten-Andersen, M.N., Tumor tissue concentrations of the proteinase inhibitors tissue inhibitor of metalloproteinases-1 (TIMP-1) and plasminogen activator inhibitor type 1 (PAI-1) are complementary in determining prognosis in primary breast cancer. *Molecular & Cellular Proteomics,* 2:164–172, 2003.

Schrohl, A.S., Holten-Andersen, M.N., Peters, H.A., Look, M.P., Meijervan Gelder, M.E., Klijn, J.G.M., Brünner, N., Foekens, J.A., Tumor tissue levels of tissue inhibitor of metalloproteinase-1 as a prognostic marker in primary breast cancer. *Clinical Cancer Research,* 10:2289–2298, 2004.

Schwartz, M.A., Schaller, M.D., Ginsberg, M.H., Integrins: emerging paradigms of signal transduction. *Annu Rev Cell Dev Biol,* 11:549-599, 1995.

Scott, A., Khan, K.M., Cook, J.L., Duronio, V., Human tendon overuse pathology: histopathologic and biochemical findings. *Tendinopathy in Athletes,* Blackwell Publishing, pp69-84, 2007.

Scott, A., Khan, K.M, Heer, J., Cook, J.L, Lian, O., Duronio, V., High strain mechanical loading rapidly induces tendon apoptosis: an *ex vivo* rat tibialis anterior model. *Br J Sports Med,* 39:e25, 2005.

Sell, D.R., Aging of long-lived proteins: extracellular matrix (collagens, elastins, proteoglycans) and lens crystallins. In: *Handbook of Physiology. 11 Aging* (Masaro, E., ed.). Oxford University Press, New York, 1995.

Selvanetti, A., Cipolla, M., Puddu, G., Overuse tendon injuries: basic science and classification. *Oper Tech Sports Med,* 5:110-117,1997.

Sharma, P., Maffulli, M., Basic biology of tendon injury and healing. *Surgeon,* 3(5):309-16, Oct. 2005.

Shirakura, K., Ciarelli, M., Arnoczky, S.P., Whallon, J.H., Deformation induced calcium signaling. *Trans. Combined Orthop Res Soc,* 2:94, 1995.

Skutek, M., van Greinsven, M., Zeichen, J., Grauer, N., Bosch, U., Cyclic mechanical stretching enhances secretion of interleukin 6 in human tendon fibroblasts. *Knee Surgery Sports Traumatology and Arthroscopy,* 9:322-326, 2001.

Smart, G., Taunton, J., Clement, D., Achilles disorders in runners-a review. *Med Sci Sports Exerc,* 12:231-243, 1980.

Smith, G.N.Jr., Yu, L.P.Jr., Brandt, K.D., Capello, W.N., Oral administration of doxycycline reduces collagenase and gelatinase activities in extracts of human osteoarthritic cartilage. *J Rheumatol,* 35:1150-1159, 1998.

Stanish, W.D., Curwin, S., Rubinovich, M., Tendinitis: The analysis and treatment for running. *Clin Sports Med,* 4:593-613, 1985.

Steadman, J.R., Forster, R.S., Silferskiold, J.P., Rehabilitation of the knee. *Clin Sports Med,* (3):605-627, 1989.

Stone, M.H., Connective tissue and bone response to strength training. In: PV (ed) *Strength and power in sport,* Oxford: Blackwell ,pp279-290, 1992.

Squier, C.A., Bausch, W.H., Three-dimensional organization of fibroblasts and collagen fibrils in rat tail tendon. *Cell and Tissue Research,* 238(2):319-327, 1984.

Tallon, C., Maffulli, N., Ewen S.W., Ruptured Achilles tendons are significantly more degenerated than tendinopathic tendons. *Med Sci Sports Exer,* 33:1983-1990, 2001.

Talvensaari-Mattila, A., Pääkko, P., Turpeenniemi-Hujanen, T., Matrix metalloproteinase-2 (MMP-2) is associated with survival in breast carcinoma. *British Journal of Cancer* 89:1270–1275, 2003.

Tayebjee, M.H., Lip, G.Y.H., Blann, A.D., MacFadyen, R.J., Effects of age, gender, ethnicity, diurnal variation and exercise on circulating levels of matrix metalloproteinases (MMP)-2 and –9, and their inhibitors, tissue inhibitors of matrix metalloproteinases (TIMP)-1 and –2. *Thrombosis Research,* 115:205-210, 2005.

Tillander, B., Franzen, L, Norlin, R., Fibronectin, MMP-1 and histologic changes in rotator cuff disease. *J Ortho Res,* 20:1358-1364, 2002.

Tsuzaki, M., Bynum, D., Almekinders, L., Yang X., Faber, J., Banes, A.J., ATP modulates load-inducible IL-1beta, COX 2, and MMP-3 gene expression in human tendon cells. *Journal of Cellular Biochemistry,* 89:556-562, 2003.

Viidik, A., Biomechanics and functional adaptation of tendons and joint ligaments. In: Evans GF (ed) *Studies on the anatomy and function of bone and joints.* New York: Springer, pp17-39, 1966.

Viidik, A., Interdependence between structure and function in collagenous tissues. *In: Biology of Collagen*, Academic Press, New York:257-280, 1972.

Vogel, K.G., Tendon structure and response to changing mechanical load. *J Musculoskel Neuron Interact,* 3(4):323-325, 2003.

Wang, J.H., Thampatty, B.P., Lin, J.S., Im, H.J., Mechanoregulation of gene expression in fibroblasts. *Gene,* 391(1-2):1-15, 2007.

Wang, N., Butler, J.P., Ingber, D.E., Mechanotransduction across the cell surface and through the cytoskeleton. *Science,* 260:1124-1127, 1993.

Wang, J.H., Jia, F., Yang, G., Yang, S., Campbell, B.H., Stone, D., Woo, S.L., Cyclic mechanical stretching of human tendon fibroblasts increases the production of prostaglandin E2 and levels of cyclooxygenase expression: a novel in vitro model study. *Conn Tissue Res,* 44:128-133, 2003.

Watson, P.A., Function follows form: generation of intracellular signals by cell deformation. *FASEB J,* 5:2013-2019, 1991.

Woessner, J.F., Nagase, H., *Matrix Metalloproteinases and TIMPs.* Oxford University Press. 2002.

Wolfe, S. L. *Molecular and Cellular Biology.* Belmont, CA: Wadsworth Publishing Co., pp. 274-287, 1993.

Woo, S.L, Gomez, M.A., Woo, Y.K., Keson, W.H., Mechanical properties of tendons and ligaments. II. The relationships of immobilization and exercise on tissue remodeling. *Biorheology,* 19(3):397-408, 1982.

Wurtz, S.O., Schrolhl, A-S., Noller Sorensen, N., Lademann, U., Christensen, J., Mouridsen, H., Brunner, N., Tissue inhibitor of metalloproteinase-1 in breast cancer. *Endocr Relat Cancer,* 12(2):215-227, 2005.

Yu, L.P.Jr., Smith, G.N.Jr., Brandt, K.D., Myers, S.L., O'Connor, B.L., Brandt, D.A., Reduction of the severity of canine osteroarthritis by prophylactic treatment with oral doxycycline. *Arthritis Rheum,* 35:1150-1159, 1992.

Yuan, J., Murrell, G.A., Wei., A.Q., Wang, M.X., Apoptosis in rotator cuff tendinopathy. *J Ortho Res,* 20:1372-1379, 2002.

Yuan, J., Wang, M.X., Murrell, G.A., Cell death and tendinopathy. *Clinics in Sports Med,* 22:693-701, 2003.

Yurchenco, P.D., Birk, D.E., Mecham, R.P., Extracellular matrix assembly and structure. *Acad Press, San Diego,* 1994.

Zareins, B., Ciullo, J.V., Acute muscle and tendon injuries in athletes. *Clin Sports Med,* 2:167-182, 1983.

Zeichen, J., van Griensven, M., Bosch, U., The proliferative response of isolated human tendon fibroblasts to cyclic biaxial mechanical strain. *Am J Sports Med*, 28(6):888-892, 2000.

Zernicke, R.F., Salem, G.J., Barnard, R.J., Woodward, J.S., Meduski, J.W., Meduski, J.D., Adaptations of immature trabecular bone to exercise and augmented dietary protein. *Med Sci Sports Exerc,* 27(11):186-193, 1995.

Chapter III

The effect of stress-deprivation and cyclic loading on the TIMP/MMP ratio in tendon cells: an *in-vitro* experimental study

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Introduction

Tendinopathy, a syndrome of tendon pain, localized tenderness, and swelling that impairs performance, is a major medical problem that accounts for 30-50% of all sports-related injuries (Renstrom 1991) and 48% of reported occupational maladies (NIOSH 1996). While many factors have been linked to the development of tendinopathy, very little is understood about its pathogenesis. Recent clinical and experimental studies have suggested that matrix metalloproteinases (MMPs) may play a significant role in the catabolic cascade that has been associated with tendinopathy (Alfredson et al 2000; Fu et al 2002; Ireland et al 2001; Jones et al 2006; Lo et al 2004; Magra & Maffulli 2005; Riley 2004; Riley et al 2002; Sharma & Maffulli 2005). While a variety of MMPs have been shown to be increased in clinical cases of tendinopathy (Alfredson et al 2000; Fu et al 2002; Ireland et al 2001; Jones et al 2006; Lo et al 2004; Magra & Maffulli 2005; Riley 2004; Riley et al 2002; Sharma & Maffulli 2005; Riley 2005), the mechanism(s) that trigger this increase are unclear. It has been suggested that the destructive mechanism(s) that precedes overt pathological development of tendinopathy may, in fact, be a MMP-mediated catabolic response by the tendon cells to the local loss of homeostatic strain as a result of isolated, microscopic, collagen fiber damage (Jones et al 2006; Arnoczky et al; Arnoczky et al 2007). Several *in vitro* experimental studies have demonstrated an increase in MMP-13 (interstitial collagenase) mRNA and protein expression in rat tail tendon cells following loss of homeostatic tension secondary to stress-deprivation (Arnoczky et al 2007; Arnoczky et al 2004; Lavagnino et al 2005; Lavagnino et al 2003). This increase in interstitial collagenase activity in stress-deprived tendons has produced a series of catabolic events (collagen degeneration and disarray as well as significant loss

of material properties), which have been reported to occur in tendinopathy (Jarvinen et al 1997; Jozsa et al 1990; Kannus & Jozsa 1991).

The regulation of extracellular matrix catabolism in connective tissues is mediated by a family of homologous proteins referred to as tissue inhibitors of metalloproteinases (TIMPs) (Brew et al 2000). TIMPs are multifunctional proteins that inhibit the activation of MMPs by binding in 1:1 stoichiometric reversible complexes with the MMPs (Willenbrock & Murphey 1994). MMPs and TIMPs are involved in a wide variety of physiological and pathological conditions that involve the turnover of the extracellular matrix (Koskinen et al 2004) and an imbalance in the TIMP/MMP ratio has been implicated in the etiopathogenesis of tendinopathy (Fu et al 2002; Jones et al 2006; Lo et al 2004; Dalton et al 1995; Magra & Maffulli 2005). A clinical study found a higher percentage of TIMP-1 positive cells compared to MMP-1 (human interstitial collagenase) positive cells in normal human patellar tendons (Fu et al 2002). However, this ratio was reversed when patellar tendons from tendinopathy patients were examined (Fu et al 2002).

A recent experimental study has demonstrated that exogenous broad-spectrum MMP inhibitors could be used to prevent the catabolic effects caused by excessive levels of interstitial collagenase in stress-deprived tendons (Arnoczky et al 2007). A clinical study has also demonstrated beneficial effects following the local application of a protease inhibitor in the treatment of Achilles tendinopathy (Capasso et al 1993). These results would suggest that increasing endogenous TIMP expression in tendons might be beneficial in the prevention and/or treatment of tendinopathy. Clinical studies have demonstrated an increase in levels of TIMP-1 in the serum and peritendinous space

following exercise (Koskinen et al 2004; Mackey et al 2004; Tayabjee et al 2005), suggesting that cyclic loading may stimulate TIMP-1 mRNA and protein expression in tendon cells. Such a response could reverse the imbalance of TIMP/MMP seen in tendinopathy (Fu et al 2002; Jones et al 2006) and provide a mechanistic explanation for the beneficial effects seen in some exercise regimes used to treat tendinopathy (Canell et al 2001; Jensen & Di Fabio; Jonsson & Alfredson 2005; Kongsgaard et al 2006; Purdam et al 2004; Young et al 2005).

The purpose of this study was to determine the effect of *in vitro* stress deprivation (loss of tendon cell homeostatic tension) or cyclic loading on the TIMP-1/MMP-13 (rat interstitial collagenase) mRNA expression ratio in rat tail tendon cells. We hypothesized that normal tendons have a positive TIMP-1/MMP-13 ratio and that loss of homeostatic tension (stress-deprivation) will inverse this ratio through a significant increase in MMP-13 expression. We also hypothesize that cyclic strain will increase the ratio of TIMP-1/ MMP-13 mRNA expression above basal levels in a dose dependent manner.

Materials and Methods

Drugs and Chemicals

Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), gentamicin, and penicillin streptomycin-fungizone solution were obtained from Gibco (Grand Island, NY). Ascorbate was from Sigma (St. Louis, MO) and ilomastat was obtained from BIOMOL International L.P. (Plymouth Meeting, Pa).

Rat Tail Tendons

Following institutional animal care and use approval, tendons were obtained from the tails of euthanized adult Sprague-Dawley rats that were approximately six months old.

The tendons were gently teased from the distal portion of each tail with forceps and placed into a petri dish containing complete media, which consists of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% antibiotic/antimycotic solution, 0.2% gentamicin and 7.5g/L ascorbate. The tendons were then allocated to the following experiments:

TIMP-1/MMP-13 mRNA expression ratio with stress deprivation

To determine the effect of stress deprivation on the TIMP-1/MMP-13 ratio, tendons were divided into 4 groups with 10 tendons per group; (1) 0 time (fresh) controls, (2) stress-deprived 24hrs, (3) stress-deprived 48hrs, and (4) stress deprived 72hrs. Stress-deprived tendons were maintained in the above noted media under tissue culture conditions at 37°C and 10% CO_2. The experiment was repeated four times. Significant differences between groups were determined by running multiple (6) paired t-tests with significance set at $p<0.008$.

TIMP-1/MMP-13 mRNA expression ratio with stress deprivation and 50μM ilomastat

To determine if the increase in MMP-13 mRNA expression seen with stress-deprivation seen in previous studies (Lavagnino et al 2003; Arnoczky et al 2004) had any effect on TIMP-1 mRNA expression, tendons were stress-deprived in the presence or absence of 50μM ilomastat (BIOMOL, International L.P., Plymouth Meeting, PA). A previous study has shown that ilomastat, a broad-spectrum MMP inhibitor, will inhibit MMP-13 mRNA expression in stress-deprived RTTs (Arnoczky et al 2007). Ten tendons were examined in each group (1) 0 time (fresh) controls, (2) stress-deprived 24hrs, and (3) stress-deprived 24hrs in the presence of 50μM ilomastat. Tendons were maintained in the above noted media under tissue culture conditions at 37°C and 10% CO_2. The

experiment was repeated three times. Significant differences between groups were determined by running an ANOVA with significance set at $p<0.05$.

TIMP-1/MMP-13 mRNA expression ratio with exercise

To determine the effect of cyclic loading on the TIMP-1/MMP-13 ratio, RTTs (10 per group) were cyclically loaded at 0.17Hz at one of three strains: 1%, 3%, or 6% for 24hrs and compared to fresh (0 time) controls and 24hrs of stress deprivation. A sawtooth-shaped waveform of cyclic strain was applied to the tendons using a custom-designed, computer-controlled, stepper motor-driven device (Lavagnino et al 2003). The grip-to-grip length was set to 40mm using digital calipers. Tendons were placed in the device until all visible slack was removed to approximate 0% strain. Tendons were then clamped in the grips to prevent slipping before undergoing 1, 3 or 6% cyclic strain with a step size of 25 um and a frequency of 0.17Hz. Tendons were maintained in complete media and under tissue culture conditions during the loading period and the experiment repeated 3 times. At the end of the loading protocol, the entire lengths of the unclamped tendon segments (~40mm) were collected for RNA extraction. To determine the significance of loading on the TIMP-1/MMP-13 ratio, a linear regression analysis was performed with significance set at $p<0.05$.

TIMP-1 and MMP-13 mRNA Expression

Rat interstitial collagenase (MMP-13) and TIMP-1 mRNA were evaluated for each treatment group. Ten tendons from each group were placed in 1.0ml of RNAlater® (Quiagen, Valencia, CA) at 4°C for at least 24hrs prior to processing. Total RNA was extracted using the Quiagen RNEasy kit with protocol provided for fibrous tissues. RNA (200-400ng) was converted into cDNA using the Invitrogen SuperScript III Reverse

Transcription system (Carlsbad, CA). Real Time Quantitative PCR was performed using TaqMan Gene Expression Assay from Applied Biosystems (ABI, Foster City, CA). Samples were run in a 96-well plate (20ul final volume per reaction) on an ABI 7500-Fast Q-PCR apparatus. The endogenous control used for all Q-PCR experiments was 18s rRNA. Results were analyzed using the Sequence Detection System software from ABI. TaqMan probe and primer sets were obtained for MMP-13 (Rn01448197_m1), TIMP-1 (Rn00587558_m1) and 18s rRNA (Hs99999901_s1) from ABI's Gene Expression Assay database (http://allgenes.com).

TIMP-1/MMP-13 Ratio Calculations

Assuming optimal amplification reaction efficiency, it is estimated that ten copies of a gene are generated every 3.32 PCR cycles. Based on the difference between a hypothetical Ct value equivalent to one gene copy, 39, and the Ct value for the 0hrs (control) samples, an estimated copy number can be calculated for the 0 hours samples (Livak & Schmittgen 2001). This then allows for the estimation of the number of gene copies for the treated samples. The TIMP/MMP ratios were calculated by dividing the number of TIMP-1 copies by the number of MMP-13 copies for each group.

Results

TIMP-1/MMP-13 mRNA expression ratio with stress deprivation

The ratio of TIMP-1/MMP-13 mRNA expression in fresh rat tail tendons was 3.73:1 $\pm$ 0.73. Twenty-four hours of stress deprivation resulted in a significant decrease in (0.25:1 $\pm$ 0.04), and an inversion of, the TIMP-1/MMP-13 ratio (Figure 1). The TIMP-1/MMP-13 ratio continued to decrease significantly with increasing time of stress deprivation (Figure 1).

TIMP-1/MMP-13 mRNA expression ratio with stress deprivation and 50μM ilomastat

The ratio of TIMP-1/MMP-13 mRNA expression in stress-deprived tendons treated with 50μM ilomastat was 2.50:1 ± 1.17. This was not statistically different from fresh controls. Tendons that were stress-deprived for 24hrs in the absence of ilomastat had an inversed TIMP-1/MMP-13 ratio (0.37:1 ± 0.35) (Figure 2).

TIMP-1/MMP-13 mRNA expression ratio with exercise

Cyclic loading at 1, 3, or 6% strain at 0.17Hz for 24hrs resulted in a weak (r^2=0.35), but significant (p=0.016) dose-dependent increase in the TIMP-1/MMP-13 mRNA expression ratio when compared to zero hour controls (Figure 3).

Discussion

Matrix metalloproteinases (MMPs) belong to a diverse group of enzymes that are not only involved in restructuring the extracellular matrix, but also play a major role in various pathophysiological conditions by virtue of their complicated expression, activation, and regulation processes (Acharya et al 2004). The activity of MMPs is inhibited reversibly by tissue inhibitors of metalloproteinases (TIMPs), which are endogenous inhibitors of MMPs (Bramono et al 2004). The balance between TIMPs and MMPs regulates extracellular matrix turnover and remodeling during normal development and pathology (Chirco et al 2006). Disruption of the TIMP-MMP balance has been associated with a number of pathological processes including tumor invasion and metastasis, rheumatoid arthritis, osteoarthritis, and ligament injuries (Fehrenbacher et al 2003; Hijilla et al 2003; Kleiner & Stetler-Stevenson 1999; Sprindler et al 1996). An imbalance in the TIMP/MMP ratio has also been implicated in the pathogenesis of tendinopathy (Fu et al 2002; Jones et al 2006; Lo et al 2004; Dalton et al 1995; Magra &

Maffulli 2005) and the resultant increase in MMP activity reported in tendinopathy (Alfredson et al 2000; Fu et al 2002; Ireland et al 2001; Jones et al 2006; Lo et al 2004; Magra & Maffulli 2005; Riley 2004; Riley et al 2002; Sharma & Maffulli 2005) is thought to be responsible for the progression of extracellular matrix catabolism seen in tendinopathy.

A clinical study examining tissues from normal and tendinopathy patients found a higher percentage of TIMP-1 immunopositive cells compared to MMP-1 (human interstitial collagenase) positive cells (24% to 18% respectively) in healthy control tendons (Fu et al 2002). However, this positive TIMP-1/MMP-1 ratio became inversed (3.4% to 47%) when tendinopathy tissues were examined (Fu et al 2002). A recent study profiling gene expression in tendinopathy patients also noted a 1000 fold increase in MMP-1 expression in ruptured tendons compared to only an 8 fold increase in TIMP (Jones et al 2006). The imbalance of TIMPs and MMPs in favor of the MMPs seen in tendinopathy tissues may explain the collagenolysis reported in the histological evaluations of these pathologic tissues (Jarvinen et al 1997; Jozsa et al 1990; Kannus & Jozsa 1991). Because absolute values of TIMP and/or MMP mRNA expression can differ between individuals and with the extent of pathology (Jones et al 2006; Riley et al 2002), some investigators have suggested that the relative expression of TIMPs and MMPs may be more important in determining the presence of collagenolysis in patients with tendinosis (Fu et al 2002). Therefore, in the current study, we chose to express the results as the ratio between TIMP-1 and MMP-13 (rat interstitial collagenase) mRNA expression.

While the TIMP family consists of four distinct members, TIMP-1, TIMP-2, TIMP-3, and TIMP-4, we chose TIMP-1 as the representative TIMP in our study. TIMP-1 expression is known to be inducible (Chirco et al 2006), has broad MMP inhibitory activity, and has a specific effect in inhibiting interstitial collagenase activity (Bramono et al 2004; Cawston et al 1994). Previous studies from our laboratory utilizing a rat tail tendon model have documented an increase in both MMP-13 mRNA and protein expression following loss of cellular homeostatic tension (Arnoczky et al 2004; Lavagnino et al 2005; Lavagnino et al 2003; Lavagnino et al 2006). We have postulated that the etiopathologic stimulus for the degenerative cascade that precedes the overt pathological development of tendinopathy is the catabolic response of tendon cells to mechanobiologic *understimulation* as a result of microscopic damage to the collagen fibers of the tendon and loss of homeostatic cellular tension (Arnoczky et al 2007). Therefore, we chose interstitial collagenase (MMP-13) as our representative MMP in this study.

The results of the current study reveal that, similar to results reported in healthy human tendons (Fu et al 2002), there is a positive ratio of TIMP-1 to MMP-13 mRNA expression in normal rat tail tendons. However, following only 24 hours of stress deprivation, this ratio becomes inversed. The change in TIMP-1/MMP-13 mRNA expression seen with stress-deprivation appears to be as a result of a significant increase in MMP-13 mRNA expression and not a decrease in TIMP-1 expression. Indeed, inhibiting MMP-13 mRNA expression with a broad-spectrum MMP inhibitor, ilomastat, did not result in any significant change in TIMP-1 expression in the stress-deprived tendons. The inversion in the ratio of TIMP-1/MMP-13 mRNA expression has been

shown to have deleterious effects on the mechanical properties of these stress-deprived tendons over time (Lavagnino et al 2005) and has been implicated in the pathogenesis of tendinopathy (Fu et al 2002; Jones et al 2006; Lo et al 2004; Dalton et al 1995; Magra & Maffulli 2005). Therefore, inhibition of MMP activity in tendinopathy patients via exogenous and/or endogenous routes may represent a novel therapeutic approach to this problem.

A recent *in vitro* experimental study has shown that two broad-spectrum MMP inhibitors, doxycycline and ilomastat, were able to prevent the degradative histological and biomechanical changes in stress-deprived rat tail tendons (Arnoczky et al 2007). Presumably, this was accomplished through the inhibition of MMP activation by these compounds. However, the ability of exogenous MMP inhibitors to minimize collagenolysis in tendinopathy patients has yet to be determined. A preliminary clinical study has reported significant improvement in patients with Achilles tendinopathy with the use of a locally administered aprotinin, a protease inhibitor (Capasso et al 1997). However, the exact mechanism(s) (inhibition of proteolytic enzymes, anti-inflammatory effect, etc.) by which aprotinin exerted this beneficial effect has yet to be determined.

Increasing the expression of endogenous TIMPs in patients with tendinopathy may also be beneficial. The results of the current study demonstrated a significant effect of cyclic loading in increasing TIMP-1/MMP-13 mRNA expression ratio. Previous clinical studies have demonstrated an increase in TIMP-1 protein synthesis in the blood and peritendinous space in individuals following treadmill exercise (Koskinen et al 2004; Tayebjee et al 2005). Another clinical study has shown that a single bout of high force, eccentric exercise increased serum levels of TIMP-1 (Mackey et al 2004). These clinical

observations, coupled with the results of the current experimental study, could provide a mechanistic explanation for the reported beneficial effects of some rehabilitation regimes that have been recently advocated in the non-operative treatment of tendinopathy patients (Cannell et al 2001; Jensen & Di Fabio 1989; Jonsson & Alfredson 2005; Kongsgaard et al 2006; Purdam et al 2004; Young et al 2005). Recent studies have documented the clinical efficacy of eccentric exercise programs in the non-operative treatment of tendinopathy (Cannell et al 2001; Jensen & Di Fabio 1989; Jonsson & Alfredson 2005; Kongsgaard et al 2006; Purdam et al 2004; Young et al 2005). Several of these studies (Jonsson & Alfredson 2005; Purdam et al 2004; Young et al 2005) have demonstrated significant clinical improvement in patients with patellar tendinopathy following 12 weeks of eccentric training performed on a 25° decline board when compared to standard eccentric exercise performed on a flat surface. However, the precise physiological mechanisms responsible for this improvement have not been identified. A recent study has demonstrated that the use of a 25° decline during eccentric loading actually increases the load and strain on the patellar tendon when compared to eccentric loading on a flat surface (Kongsgaard et al 2006). The results of the current study suggest that one potential benefit of increased tendon strain in the treatment of tendinopathy may be related to an increase in the TIMP/MMP ratio seen with increasing tendon strain. In tendinopathy patients, the higher tendon strains produced during eccentric loading may inhibit further catabolism and encourage homeostasis of the extracellular matrix by re-establishing a normal, positive TIMP/MMP ratio. Additional clinical studies are needed to monitor the TIMP/MMP ratios in tendinopathy patients to determine if there is a

correlation between this ratio and the extent of pathology and/or the level of clinical improvement following therapy.

As noted previously, the current experimental study focused only on the relationship between TIMP-1 and MMP-13 (rat interstitial collagenase) mRNA expression. While these two genes represent the major inhibitor and effector, respectively, of type I collagenolysis, the significance of potential imbalances between other MMP and TIMP levels in the pathogenesis of tendinopathy must still be explored.

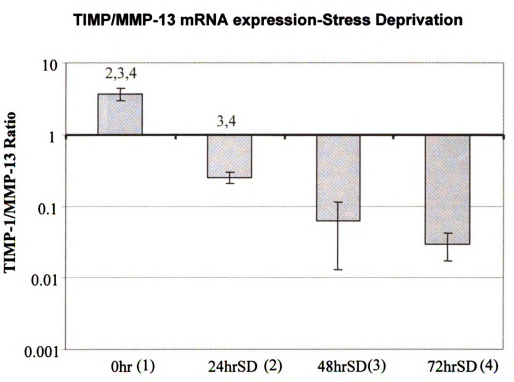

Figure 3.1 Graph of TIMP-1/MMP-13 mRNA expression ratios based on transcript numbers calculated from quantitative PCR, (1) 0 hour stress-deprived tendons, (2) 24 hour stress-deprived tendons, (3) 48 hour stress-deprived tendons, and (4) 72 hour stress-deprived tendon. Ten tendons per treatment group and the experiment was replicated three times. A ratio of 1 represents equal amounts of TIMP-1 and MMP-13 expression. Samples with bars above 1 indicate a greater expression of TIMP-1 than MMP-13. Bars below 1 indicate less expression of TIMP-1 than MMP-13. The numbers above each bar represent statistically significant differences.

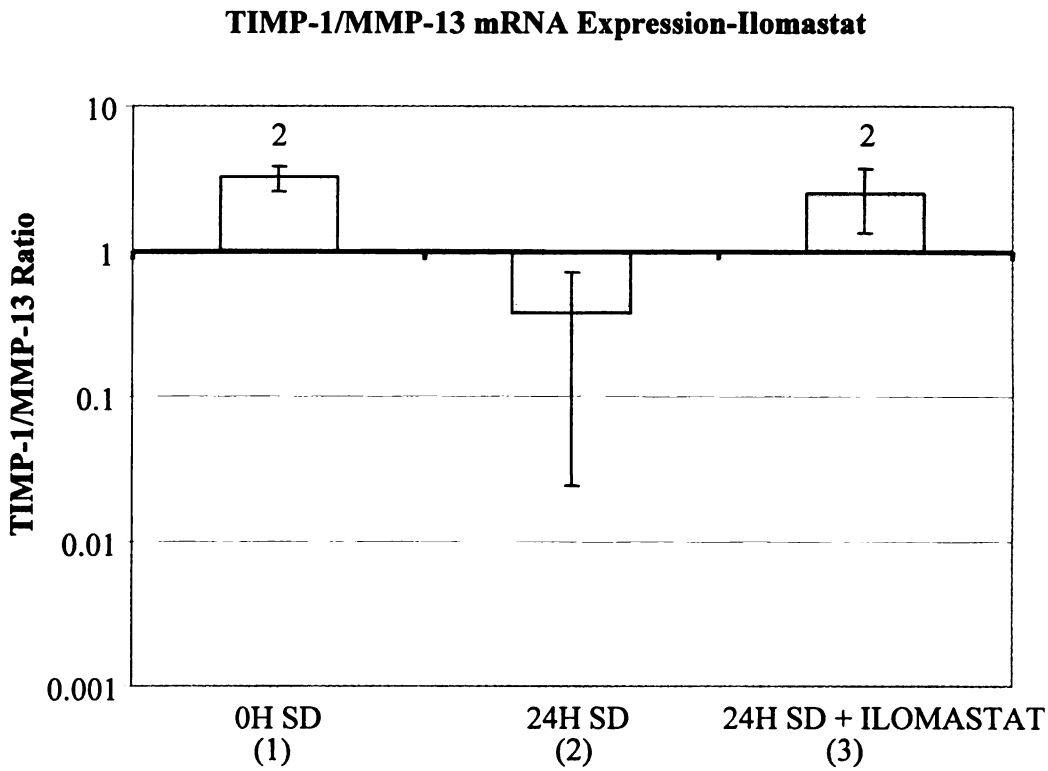

Figure 3.3 Graph of TIMP-1/MMP-13 mRNA expression ratios based on transcript numbers calculated from quantitative PCR, (1) 0 hour stress-deprived tendons, (2) 24 hour stress-deprived tendons, and (3) 24 hour stress-deprived tendons treated with 50uM ilomastat. The numbers above each bar represent statistically significant differences.

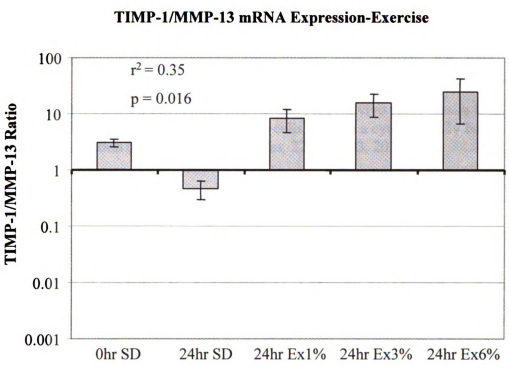

Figure 3.2 Graph of TIMP-1/MMP-13 mRNA expression ratios of control (0hr), 24hr stress-deprived (SD), and cyclically loaded for 24 hr at 1%, 3%, and 6% strain based on transcript numbers calculated from quantitative PCR. Ten tendons per treatment and the experiment was replicated three times.

REFERENCES

Acharya MR, Venitz J, Figg WD, Sparreboom A. Chemically modified tetracyclines as inhibitors of matrix metalloproteinases. *Drug Resist Updat*; 7(3):195-208, 2004.

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

Arnoczky, S.P., Lavagnino, M., Egerbacher, M., The mechanobiological aetiopathogenesis of tendinopathy: is it over-stimulation or the understimulation of tendon cells? *International J Exp Path,* (in press), 2007.

Arnoczky, S.P., Lavagnino, M., Egerbacher, M., Caballero, O., Gardner, K., Matrix metalloproteinase inhibitors prevent a decrease in the mechanical properties of stress-deprived tendons: an in vitro experimental study. *Am J Sports Med,* 35(5):763-769, 2007.

Arnoczky, S.P., Lavagnino, M., Gardner, K.L., Tian, T., Vaupel, Z.M., Stick, J.A., In vitro effects of oxytetracycline on matrix metalloproteinase-1 mRNA expression and on collagen gel contraction by cultured myofibroblasts obtained from the accessory ligament of foals. *Am J Vet Res,* 65(4):491-496, 2004.

Alfredson, H., Lorentson, M., Backman, S., Lerner, U.H., cDNA-arrays and real-time quantitative PCR techniques in the investigation of chronic Achilles tendinosis. *J Orthop Res,* 21(6):970-975, 2003.

Brew, K., Dinakarpandian, D., Nagase, H., Tissue inhibitors of metalloproteinases: evolution, structure and function. *Biochim Biophys Acta,* 1477(1-2):267-283, 2000.

Bramono DS, Richmond JC, Weitzel PP, Kaplan DL, Altman GH. Matrix metalloproteinases and their clinical applications in orthopaedics. *Clin Orthop Relat Res* (428):272-85, 2004.

Cannell, L.J, Taunton, J.E., Clement, D.B., Smith, C., Khan, K.M., A randomized clinical trial of the efficacy of drop squats or leg extension/leg curl exercises to treat clinically diagnosed jumper's knee in athletes: pilot study. *Br J Sports Med,* 35(1):60-64, 2001.

Capasso, G., Maffulli, N., Testa, V., Sgambato, A., Preliminary results with peritendinous protease inhibitor injections in the management of Achilles tendonitis. *J Sports Traumatol Rel Res,* 15:37-43, 1993.

Capasso, G., Testa, V., Maffulli, N., Gifulco, G., Aprotinin, corticosteroids and normosaline in the management of patellar tendinopathy in athletes: a prospective randomized study. *Sports Exercise and Injury,* 3:111-115, 1997.

Cawston T, Plumpton T, Curry V, Ellis A, Powell L. Role of TIMP and MMP inhibition in preventing connective tissue breakdown. *Ann N Y Acad Sci*; 732:75-83, 1994.

Chirco, R., Xu-Wen, L., Ki-Kyung, J., Hyeong-Reh, C.K., Novel functions of TIMPs in cell signaling. *Cancer Metastasis Rev,* 25:99-113, 2006

Dalton, S., Cawston, T.E., Riley, G.P., Bayley, I.J., Hazelman, B.L., Human shoulder tendon biopsy samples in organ culture produce procllagenase and tissue inhibitor of matalloproteinases. *Ann Rheum. Dis,* 54:571-577, 1995.

Fehrenbacher, A.; Steck, E.; Rickert, M.; Roth, W.; and Richter, W. Rapid regulation of collagen but not metalloproteinase 1, 3, 13, 14 and tissue inhibitor of metalloproteinase 1, 2, 3 expression in response to mechanical loading of cartilage explants in vitro. *Arch Biochem Biophys*, 410(1):39-47, 2003.

Fu, S.C., Chan, B.P., Wang, W., Pau, H.M., Chan, K.M., Rolf, C.G., Increased expression of matrix metalloproteinase 1 (MMP1) in 11 patients with patellar tendinosis. *Acta Orthop Scand,* 73(6):658-662, 2002.

Hojilla, C.V.; Mohammed, F.F.; and Khokha, R. Matrix metalloproteinases and their tissue inhibitors direct cell fate during cancer development. *Br J Cancer*, 89(10):1817-21, 2003.

Ireland, D., Harrall, R., Curry, V., Halloway, G., Hackney, R., Hazleman, G., Riley, G., Multiple changes in gene expression in chronic human Achilles tendinopathy. *Matrix Biol,* 20(3):159-169 2001.

Kleiner, D.E., and Stetler-Stevenson, W.G., Matrix metalloproteinases and metastasis. *Cancer Chemother Pharmacol*, 43 Suppl:S42-51, 1999.

Jarvinen, M., Jozsa, L., Kannus, P., Jarvinen, T.L., Kvist, M., Seadbetter, W., Histopathological findings in chronic tendon disorders. *Scand J Med & Sci Sports,* 7:86-95, 1997.

Jensen, K., Di Fabio, R.P., Evaluation of eccentric exercise in treatment of patellar tendonitis. *Phys Ther,* 69(3):211-216, 1989.

Jones, G.C., Corps, A.N., Pennington, C.J., Clark, I.M., Edwards, D.R., Bradley, M.M., Hazleman, B.L., Riley, G.P., Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human Achilles tendon. *Arthritis & Rheumatism*, 54(3):832-842, 2006.

Jonsson, P., Alfredson, H., Superior results with eccentric compared to concentric quadriceps training in patients with jumper's knee: a prospective randomized study. *Br J Sports Med,* 39(11):847-850, 2005.

Jozsa, L., Reffy, A., Kannus, P., Demel, S., Elek, E., Pathological alterations in human tendons. *Arch Orthop Trauma Surg,* 110(1):15-21, 1990.

Kannus, P., Jozsa, L., Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. *J Bone Joint Surg Am,* 73(10):1507-1525, 1991.

Kongsgaard M, Aagaard P, Roikjaer S, Olsen D, Jensen M, Langberg H, Magnusson SP. Decline eccentric squats increases patellar tendon loading compared to standard eccentric squats. *Clin Biomech* (Bristol, Avon); 21(7):748-54, 2006.

Kosikinen, S.A.O., Heinemeier, K.M., Olesen, J.L., Langberg, H., Kjaer, M., Physical exercise can influence local levels of matrix metalloproteinases and their inhibitors in tendon-related connective tissue. *J Appl Physio,* 96:861-864, 2004.

Lavagnino, M., Arnoczky, S.P., Tian, T., Vaupel, Z., Effect of amplitude and frequency of cyclic tensile strain on the inhibition of MMP-1 mRNA expression in tendon cells: an in vitro study. *Connect Tissue Res,* 44(3-4):181-187, 2003.

Lavagnino, M., Arnoczky, S.P., In vitro alterations in cytoskeletal tensional homeostasis control gene expression in tendon cells. *J Ortho Res,* 23:1211-1218, 2005.

Lavagnino, M., Arnoczky, S.P., Egerbacher, M., Gardner, K.L., Burns, M.E., Isolated fibrillar damage in tendons stimulates local collagenase mRNA expression and protein synthesis. *J. of Biomechanics,* 39:2355-2362, 2006.

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*; 25(4):402-8, 2001.

Lo, I.K., Marchuk, L.L., Hollinshead, R., Hart, D.A., Frank, C.B., Matrix metalloproteinase and inhibitor of matrix metalloproteinase mRNA levels are specifically altered in torn rotator cuff tendons. *Am J Sports Med,* 32(5):1223-1229, 2004.

Mackey, A.L., Donnelly, A.E., Turpeenniemi-Hujanen, T., Roper, H.P., Skeletal muscle collagen content in humans after high-force eccentric contractions. *J Appl Physiol,* 97:197-203, 2004.

Magra, M., Maffulli, N., Matrix metalloproteinase: a role in overuse tendinpathies. *Br J Sports Med,* 39(11):789-791, 2005.

Magra, M., Maffulli, N., Molecular Events in Tendinopathy: A Role for Metalloproteinases. *Foot Ankle Clin N Am,* 10:267-277, 2005.

Purdam CR, Jonsson P, Alfredson H, Lorentzon R, Cook JL, Khan KM. A pilot study of the eccentric decline squat in the management of painful chronic patellar tendinopathy. *Br J Sports Med*; 38(4):395-7, 2004.

Renstrom, P., Sports traumatology today. A review of common current sports injury problems. *Ann Chir Gynaecol,* 80(2):81-93, 1991.

Riley, G.P., Gene expression and matrix turnover in overused and damaged tendons. *Scand J Med Sci Sports,* 15:241-251, 2005.

Riley, G., The pathogenesis of tendinopathy. A molecular perspective. *Rheumatology (Oxford),* 43(2):131-142, 2004.

Riley, G.P., Curry, V., DeGroot, J., van El, B., Verzijl, N., Hazleman, B.L., Bank, R.A., Matrix metalloproteinase activities and their relationship with collagen remodelling in tendon pathology. *Matrix Biol,* 21(2):185-95, 2002.

Sharma, P., Maffulli, N., Tendon injury and tendinopathy: healing and reapair. *J Bone Joint Surg Am,* 87(1):187-202, 2005.

Spindler, K.P., Clark, S.W., Nanney, L.B., Davidson, J.M., Expression of collagen and matrix metalloproteinases in ruptured human anterior cruciate ligament: an in situ hybridization study. *J Orthop Res,* 14(6):857-861, 1996.

Tayebjee, M.H., Lip, G.Y.H., Blann, A.D., MacFadyen, R.J., Effects of age, gender, ethnicity, diurnal variation and exercise on circulating levels of matrix metalloproteinases (MMP)-2 and –9, and their inhibitors, tissue inhibitors of matrix metalloproteinases (TIMP)-1 and –2. *Thrombosis Research,* 115:205-210, 2005.

Young MA, Cook JL, Purdam CR, Kiss ZS, Alfredson H. Eccentric decline squat protocol offers superior results at 12 months compared with traditional eccentric protocol for patellar tendinopathy in volleyball players. *Br J Sports Med*; 39(2):102-5, 2005.

Willenbrock, F., Murphy, G., Structure-function relationships in the tissue inhibitors of metalloproteinases. *Am J Respir Crit Care Med,* 150(6 Pt 2):S165-170, 1994.

IV. DISCUSSION

Understanding the etiology of tendinopathy is a critical aspect of improving knowledge to help with the debilitating symptoms in this disease. Many causes have been theorized (Jozsa & Kannus 1997; Sharma & Maffulli 2005), but central to these theories is the concept that excessive loading of tendons during rigorous physical activity is the main pathologic stimulation for degeneration of the extracellular matrix (Selvanetti et al 1997). Data from this study and others from our laboratory suggest that it is actually an absence of mechanical stimuli, secondary to microtrauma, which is the mechanobiologic stimulus for the degradative cascade that leads to tendinopathy.

The overall conclusions from this study coincide with the observations that tissues from tendinopathy patients have demonstrated an increase in MMP expression and degeneration of the extracellular matrix. Our research has sought to provide a mechanistic explanation for these clinical findings. Based on previous research from our laboratory we concluded that the loss of tendon cell homeostatic strain has been shown to result in an increase in MMP mRNA expression and protein synthesis (Arnoczky et al 2004, Lavagnino & Arnoczky 2006). Also, isolated tendon fibril failure secondary to tendon over-strain results in a localized alteration of cell matrix interactions and subsequent loss of tendon cell homeostatic strain. This loss of tendon cell homeostatic strain within the isolated fibril leads to a localized increase in MMP mRNA expression and protein synthesis (Lavagnino et al 2006). In this current research, the loss of tendon cell homeostatic strain has led to an inverse TIMP-1/MMP-13 mRNA expression ratio. The imbalance in the TIMP/MMP mRNA expression ratio may be responsible for the degenerative changes in the extracellular matrix associated with loss of tendon cell

homeostatic strain. Therefore, we theorize that during repetitive activities one or more abnormal cycles may occur, during which individual collagen fibrils can be damaged leading to a loss of tendon cell homeostatic strain. This in turn initiates the catabolic cascade, which has been associated with tendinopathy.

Results from previous studies suggest there are several potential treatment modalities for tendinopathy that may be a consequence of our research. We have shown that exogenous MMP inhibitors minimize the degradative loss of tendon material properties associated with the loss of tendon cell homeostatic strain (Arnoczky et al 2006). We have shown that cyclic loading increases the ratio of TIMP-1/MMP-13 mRNA expression due to increased levels of endogenous tissue inhibitor of metalloproteinase-1 (TIMP-1) in these current studies. This suggests that the increased TIMP levels seen with exercise may provide a scientific basis for the beneficial effects seen with the eccentric loading rehabilitation regimes for tendinopathy, which favor increased strain amplitudes (Cannell et al 2001; Jensen & Di Fabio 1989; Jonsson & Alfredson 2005; Kongsgaard et al 2006; Purdam et al 2004; Young et al 2005).

This research can assist in our understanding some of the clinical applications for the treatment of tendinopathy. Therapeutic regulation of MMPs for the treatment of various orthopedic conditions, including tendinopathies, is currently being explored. Initial clinical studies using paratendinous injections of aprotinin, a polyvalent inhibitor of MMPs, have shown promising results in limiting extracellular matrix degeneration and thus decreasing pain (Capasso et al 1993, 1997; Sayana & Maffulli 2007). Therefore, increasing the local production of TIMPs or partial inhibition the MMPs may be alternative therapeutic options. In conclusion, my research has shown that the stimulus

and inhibition of MMP-13 production in tendon cells may have key implications in the etiopathogenesis and treatment of tendinopathy.

Future Research Directions

Investigations into the effect of age on TIMP/MMP mRNA expression ratios would make an interesting study, now that is has been established that this ratio decreases with loss of homeostatic tension and reverses with cyclic loading. A hypothesis could be formed stating that, the TIMP/MMP ratio decreases with increasing age, predisposing older animals to tendinopathy. This study could be set up with three age groups of rats, young (30 days), middle age (6 mo), and old (12 mo). Ratios of TIMP-1/MMP-13 mRNA expression could be determined using quantitative PCR. An age related TIMP/MMP study could lead to further investigation into the benefit of exercise in older rats. Older rats are likely more sedentary than younger rats, therefore, controlled exercise protocols may increase the TIMP/MMP ratios, decreasing the predisposition of older rats for tendinopathy. These are just a few studies that could continue the examination of TIMP/MMP ratios in tendons. Various other ratio studies extending from this research could examine the effects of exercise protocols (*in vivo and in vitro)*, other age ranges, alternate tendons, and blood versus tissue levels of TIMP/MMP.

References

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

Arnoczky, S.P., Lavagnino, M., Egerbacher, M., Caballero, O., Gardner, K., Matrix Metalloproteinase Inhibitors Prevent a Decrease in the Mechanical Properties of Stress-Deprived Tendons: An In Vitro Experimental Study. *Am J Sports Med,* 35(5):763-769, 2007.

Cannell, L.J, Taunton, J.E., Clement, D.B., Smith, C., Khan, K.M., A randomized clinical trial of the efficacy of drop squats or leg extension/leg curl exercises to treat clinically diagnosed jumper's knee in athletes: pilot study. *Br J Sports Med,* 35(1):60-64, 2001.

Capasso, G., Maffulli, N., Testa, V., Sgambato, A., Preliminary results with peritendinous protease inhibitor injections in the management of Achilles tendonitis. *J Ortho Res,* 15:37-43, 1993.

Capasso, G., Testa, V., Maffulli, N., et al. Aprotinin, corticosteroids and normosaline in the management of patellar tendinopathy in athletes: a prospective randomized study. *Sports Exerc Injury*, 3:111-115, 1997

Jensen, K., Di Fabio, R.P., Evaluation of eccentric exercise in treatment of patellar tendonitis. *Phys Ther,* 69(3):211-216, 1989.

Jonsson, P., Alfredson, H., Superior results with eccentric compared to concentric quadriceps training in patients with jumper's knee: a prospective randomized study. *Br J Sports Med,* 39(11):847-850, 2005.

Jozsa, L.G., Kannus, P., *Human Tendons: Anatomy, Physilogy, and Pathology. Human Kinetics,* Champaign, IL:164-253.

Kongsgaard M, Aagaard P, Roikjaer S, Olsen D, Jensen M, Langberg H, Magnusson SP. Decline eccentric squats increases patellar tendon loading compared to standard eccentric squats. *Clin Biomech* (Bristol, Avon); 21(7):748-54, 2006.

Lavagnino, M., Arnoczky, S.P., Egerbacher, M., Gardner, K.L., Burns, M.E., Isolated fibrillar damage in tendons stimulates local collagenase mRNA expression and protein synthesis. *J Biomech,* 39:2355-2362, 2006.

Purdam CR, Jonsson P, Alfredson H, Lorentzon R, Cook JL, Khan KM. A pilot study of the eccentric decline squat in the management of painful chronic patellar tendinopathy. *Br J Sports Med*; 38(4):395-7, 2004.

Sayana, M.K., Maffulli, N., Eccentric muscle training in non-athletic patients with Achilles tendinopathy. *J Sci Med Sport,* 10(1):52-58, 2007.

Sharma, P., Maffulli, M., Basic biology of tendon injury and healing. *Surgeon*, 3(5):309-16, Oct. 2005.

Selvanetti, A., Cipolla, M., Puddu, G., Overuse tendon injuries: basic science and classification. *Oper Tech Sports Med,* 5:110-117, 1997.

Young MA, Cook JL, Purdam CR, Kiss ZS, Alfredson H. Eccentric decline squat protocol offers superior results at 12 months compared with traditional eccentric protocol for patellar tendinopathy in volleyball players. *Br J Sports Med*; 39(2):102-5, 2005.


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