VARYING WHOLE BODY VIBRATION AMPLITUDE DIFFERENTIALLY AFFECTS TENDON AND LIGAMENT STRUCTURAL AND MATERIAL PROPERTIES

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ABSTRACT

BENJAMIN V. KELLER: Varying Whole Body Vibration Amplitude Differentially Affects Tendon and Ligament Structural Properties and Material Properties
(Under the direction of Dr. Paul Weinhold)

Whole Body Vibration (WBV) is becoming increasingly popular for bone maintenance and muscle strengthening applications. Low-magnitude WBV (<1 G) has shown evidence for increasing bone density, bone formation rate, as well as bone strength. High-magnitude WBV (>1 G) has also been found to increase muscle size, strength, and reaction time. Our study is the first to examine the biomechanical effects of both low-magnitude and high-magnitude WBV on tendons and ligaments. It was hypothesized that both low vibration (0.3 G) and high vibration (2 G) would strengthen ligament and tendon but that low vibration would be more effective because high vibration may be near the threshold of tissue overloading. A total of 36 rats were divided into three groups: control, low-vibration (0.3 G), high-vibration (2 G). Experimental groups were vibrated 20 minutes a day, 5 days a week, over 5 weeks with a 30 Hz sinusoidal stimulus. Tensile testing and histological examination were carried out on the rats' Achilles tendons, patellar tendons, anterior cruciate ligaments, and medial collateral ligaments. Differences in biomechanical data and histology of the high-vibration group suggest that high-magnitude vibration may be harmful to ligaments and tendons. No significant differences in the biomechanical properties were observed with the low-vibration group relative to the control suggesting that little effect exists or that a longer duration or higher-magnitude stimulus may be required to observe significant effects.

I would like to dedicate this thesis to my parents, Richard and Abigail,

whose love and strength guide me.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table

LIST OF FIGURES

Figure

LIST OF ABBREVIATIONS AND SYMBOLS

ACL: Anterior Cruciate Ligament

AT: Achilles Tendon

CSA: Cross-Sectional Area

Col1A1: Collagen Type 1, Alpha 1

ECM: Extracellular Matrix

FATC: Femur-ACL-Tibia Complex

FMTC: Femur-MCL-Tibia Complex

GAG: Glycosaminoglycan

MCL: Medial Collateral Ligament

MTS: Material Testing System

LIPUS: Low Intensity Pulsed UltraSound

LMHFV: Low-Magnitude High-Frequency Vibration

OTJ, Osteotendinous Junction

PCR: Polymerase Chain Reaction

PT: Patellar Tendon

WBV: Whole Body Vibration

CHAPTER 1

Introduction

1.1 Introduction

Hippocrates once said "that which is used develops, and that which is not used wastes away" when describing musculoskeletal tissues of the human body. Hippocrates' observation has now been termed "Wolff's Law." Wolff's Law, named after the 19th century German anatomist/surgeon Julius Wolff, states that normal tissues will undergo structural or material remodeling as a result of the loading environment. In general, unloaded or immobilized tissues become thinner and weaker whereas loaded or exercised tissues become larger and stronger. In an attempt to harness the body's marvelous ability to adapt, clinicians and researchers have explored the use of various types of exercise and therapies on musculoskeletal tissues. Over the past two decades, a new form of loading therapy called Whole Body Vibration (WBV) has emerged and been used for an array of medical conditions. Some of WBV's most promising results have come in the musculoskeletal field, specifically bone and muscle. Now the time has come to explore WBV's effects on the other musculoskeletal tissues: tendons and ligaments.

1.2 Significance of Research

Whole Body Vibration (WBV) is becoming increasingly popular for bone maintenance and muscle strengthening applications. Low-magnitude WBV (<1G) has shown evidence for increasing bone density, bone formation rate, as well as bone strength. High-magnitude WBV (>1G) has also been found to increase muscle size, strength, and reaction time. To the best of our knowledge, our study is the first to examine both the effects of low-magnitude (0.3 G) and high-magnitude (2 G) WBV on tendons and ligaments. If vibration therapies were to show similar efficacy as in bone and muscle then they could provide clinicians and trainers with a means to prevent future injuries in their patients' tendons and ligaments. WBV has been shown to accelerate fracture healing and under the right conditions the same could hold true for tendons and ligaments. However, if vibration therapies cause weakening of tendons and ligaments then the clinical and recreational use of whole body vibration platforms should be challenged. Also, if negative effects are similar to overuse injuries then it could serve as a tendinopathy animal model.

1.3 Statement of Objectives

1) Evaluate histology to determine any morphological changes that may occur in tendon and ligament as a result of WBV at the two magnitude levels.
2) Using tensile testing and cross-sectional area protocols, determine any differences in structural or material properties of the tendons and ligaments exposed to WBV at the two magnitude levels.

1.4 Hypothesis

Both low-magnitude (0.3 G) and high-magnitude (2 G) vibration should strengthen ligament and tendon with low vibration being more effective because high vibration may be near the threshold of tissue overloading.

CHAPTER 2

Background

2.1 Tendon & Ligament Anatomy

Tendons and ligaments are musculoskeletal tissues which share very similar structure. Tendons and ligaments should be thought of as composite materials because their microstructure consists of collagen fibers, proteoglycans, fibroblast cells, and water. Collagen is the main component of these two tissues, making up 70-90% of the dry weight [Screen et al. 2008]. Collagen is arguably one of the most important structural proteins that the body produces and is a key component in all musculoskeletal tissues. There are many different types of collagen which serve various functions. In tendon and ligament tissues, collagen type I creates a hierarchy of collagen fibers (Figure 5) which are neatly organized to create a highly functional macrostructure. The collagen fibril is the smallest functional collagen fiber unit with diameters ranging from 20-150 nm [Jozsa & Kannus 1997]. Collagen fibrils assembly themselves in quarter-staggered array formation, bound together by lysine cross-links. This structure allows for greater stiffness and resiliency during loading. Cross-linked fibrils form fascicles which are on the order for 50-300 μm [Magnusson et al. 2010]. Furthermore, fascicles form collagen fibres which are typically 100-500 μm and when grouped together within endotenon and epitenon sheaths they become tendons or ligaments. Endotenon and epitenon sheaths are important because they thought to contain fibroblasts, nerve supply, as well as blood and lymph vessels.

The matrix that surrounds the collagen fibers is predominately proteoglycans (PGs), glycoproteins, fibroblast cells, and water. Proteoglycans' negatively charged glycosaminoglycan (GAG) side chains are hydrophilic. Water bound to proteoglycans gives tendons and ligaments a natural lubricating mechanism between and within collagen fibers. Proteoglycans are also known for their ability to resist high compressive and tensile forces [Jozsa & Kannus 1998] and have been referred to as "pulse dampeners" [Banes et al. 1999].

Tendons are ligaments were once thought to be avascular and metabolically inactive. However, over time researchers have shown that tendons and ligaments do have a small but vital blood supply [Jozsa & Kannus 1998]. It is thought that tendons receive their blood supply from three locations: (1) the myotendinous junction, (2) the osteotendinous junction, and (3) the paratenon and epitenon layers which supply the blood vessels that infiltrate the middle third of the tendon [Kjaer 2004]. Blood supply for ligaments is virtually the same but instead of a myotendinous junction, ligaments have two osteotendinous junctions. Ligaments have been shown to have a better blood supply and as a result, they are often more cellular [Amiel et al. 1984]. The resident fibroblasts, called tenocytes, require oxygen and nutrients delivered by blood supply. Tenocytes are responsible for maintenance of the collagen fiber microstructure and the surrounding matrix. There are also resident tendon cells found on the tendon surface, in the epitenon. These tendon surface cells are critical during injury, especially tendon or ligament ruptures since they are responsible for cell recruitment and repopulation [Banes et al. 1995].

2.2 Tendon & Ligament Biomechanics

Despite having remarkably similar structure, the function of tendons and ligaments differ. Tendons connect muscle to bone and passively transmit forces between the two during locomotion. Ligaments are also passive connective tissues that connect bone to bone and function as joint stabilizers. Both tendons and ligaments are characterized for having great uniaxial tensile strength and viscoelastic properties. The collagen fiber structure allows for the tensile strength and resiliency. When relaxed the collagen fibres have been described to have a crimped structure [Screen et al. 2008]. Upon stretching the fibres uncrimp which is exhibited by the non-linear toe region in a stress-strain curve (Figure 6). The toe region is often less stiff than the linear region which usually occurs between 2-6% strain (Figure 6). In the linear region, the collagen fibers are thought to slide between and within each other [Screen et al. 2008, Magnusson et al. 2010]. Daily physiological loading is thought to occur mainly within the toe region with occasional loads researching the linear region [Curwin 2011]. When strains start eclipsing the 6% strain threshold, the fibers themselves start to stretch and it is no longer a collective response (Figure 6). Partial failures may occur at strains between 5-8% whereas catastrophic tendon or ligament failures usually will not occur until 8-10% strain (Figure 6).

Proteoglycans provide the lubricating environment that surrounds the collagen fibers. Therefore, proteoglycans (PGs) and the water that binds to their glycosaminoglycan (GAG) side chains create the viscoelastic properties. Creep, stress relaxation, and hysteresis are viscoelastic characteristics which are often tested when assessing tendon and ligament biomechanics. The viscoelastic properties of tendon and ligament are unique and allow the tissues to store and release energy during locomotion. Interestingly, in regions of

compression tendon fibroblasts produce more PGs and GAGs instead of collagen fibers which are designed for tensile loading [Jozsa & Kannus 1998]. One study has shown that if an enzyme is allowed to digest the proteoglycans than it greatly reduces the viscoelastic properties; reaffirming the role of PGs [Millesi et al., 1995]. However, Screen et al. [2008] found that collagen fibre sliding and possibly collagen fibril sliding partly contribute to the stress-relaxation response. The friction created between the collagen fibers and the surrounding matrix can cause energy dissipation or a hysteresis effect [Screen et al. 2008]. When a tendon or ligament is repeatedly loaded it is not uncommon to witness the stress-strain curve shift to the right as a result of hysteresis (Figure 7).

2.3 Response to Loading

Tendon and Ligaments respond similarly to loading as bone and muscle, although at a slower rate. The resident fibroblasts, known as tenocytes, sense the stretching of their cell membranes which triggers a cascade of catabolic and anabolic signals [Kjaer 2004]. Many cytokines, growth factors, and hormones are affected by loading and act extracellularly and/or intracellularly. Collagen turnover, degradation versus production, is often the first major response in tendons and ligaments exposed to external loading [Magnusson et al. 2010, Curwin 2011]. Matrix metalloproteinases (MMPs) are enzymatic regulators of collagen fibers and matrix proteins. Shortly after loading or exercise they have been shown to increase in quantity and clear the way for new collagen deposits [Kjaer 2004, Magnusson et al. 2010, Frizziero et al. 2011]. Collagen synthesis and degradation occurs simultaneously after loading as displayed in Figure 4 [Magnusson et al. 2010]. Figure 4 shows that acute loading can be net-negative for collagen production in the first 36 hours afterwards. Overuse

tendinopathies can occur if recovery between exposures to loading is limited. Overuse tendinopathies are characterized by hypertrophic fibroblasts and unorganized collagen. These observations suggest that when tendons are reloading prematurely they may keep fibroblasts focused on collagen turnover and prohibit the cells from conducting their subsequent organizational duties. However, after 36 hours, collagen formation overtakes collagen degradation creating a net-positive affect (Figure 4). Furthermore, the same researchers have concluded that chronic exercise results in a reduction in the degradation response while the formation response remains elevated [Langberg et al. 2001]. Loading has also been shown to increase new fibroblast counts [Jozsa and Kannus 1998]. These new fibroblasts, known as tenoblasts, not only produce new collagen but also help organize the existing collagen. Collagen production and organization during post-loading recovery is vital for maintaining and strengthening ligaments and tendons.

Tenocytes, like osteocytes, are mechano-sensitive cells which cause biochemical changes as a result of direct exposure to microstrains felt by the cell's membrane. Collagen fiber production initiates within the cell from signals received from integrin receptors [Kjaer 2004]. The endoplasmic reticulum produces peptides that become united in triplicates to form triple helical molecules which are known as procollagen (Figure 8). The procollagen molecule is secreted from the cell in a process known as exocytosis. In the extracellular space, the procollagen molecule is processed with the removal of its "loose-ends." The new molecule is known as tropocollagen and is cross-linked with other tropocollagens in a quarter-staggered array to form a fibril (Figure 8). Loading has been shown to increase collagen fibril numbers and size [Michna & Hartmann 1989]. Therefore exercise or an appropriate loading regimen can be a way to strengthen tendon and ligament microstructure.

Over longer periods of time with continually upregulation of collagen type I production the macrostructure will gradually change. Tendons that become stronger because of an expansion in tissue size go through a process known as tissue hypertrophy. Disuse or unloading has been shown to cause the opposite effect, tissue atrophy [Kjaer 2004]. Ruptured tendons also have exhibited smaller quantities and smaller diameters of fibrils than normal, healthy tendon [Magnusson et al. 2002, Kongsgaard et al. 2005]. Aging has also been associated with a decline in collagen fibril diameter [Kjaer 2004] but no decline in overall tendon cross-sectional area or other structural properties [Couppe et al. 2009]. The results by Couppe et al. [2009] revealed that elderly people maintain tissue strength and stiffness through collagen cross-linking instead of new collagen fiber production. Many researchers have proposed the idea that tendons and ligaments in younger individuals adapt to loading via structural strengthening (tissue hypertrophy) whereas older individuals adapt via material strengthening [Reeves et al. 2003, Kjaer et al. 2004, Narici et al. 2008]. Several studies have demonstrated that tendons and ligaments exposed to an increased loading environment can improve tissue material strength through collagen cross-linking [Kubo et al. 2001, Buchanan & Marsh 2001] and greater collagen packing density [Woo et al. 1980]. Current literature is inconclusive and contradictory when it comes to identifying how loading affects tendons and ligaments. Some studies point to structural strengthening [Rosager et al. 2002, Magnusson & Kjaer 2003, Kongsgaard et al. 2005, Kongsgaard et al. 2007, Arampatzis et al. 2007, Couppe et al. 2008], others suggest material strengthening [Buchanan and Marsh 2001, Kubo et al. 2002, Reeves et al. 2003], and sometimes both [Woo et al. 1980]. With many differences in species, age, tissue choice, and loading type amongst the studies general conclusions are challenging. Our research explores the possibility of whole

body vibration (WBV) as a potential loading regimen for strengthening intact tendons and ligaments.

2.4 Bone & WBV

In the early 1990's, Clinton Rubin and his colleagues popularized Whole Body Vibration (WBV) as an alternative therapy to traditional musculoskeletal loading. Their goal was to bombard the skeletal system with thousands of micro-loads which would be felt by the long bones of the human body. They found that WBV had osteogenic potential and could be used to reverse bone loss in cases of osteopenia and osteoporosis [Fritton et al. 1997]. More recent studies have used WBV to accelerate fracture healing and improve callus quality [Leung et al. 2008]

WBV excites many mechanisms throughout the body and within bone. WBV is thought to induce microstrains in the extracellular matrix (<10 με) felt by the osteoclasts, osteoblasts, and bone lining cells which results in the up-regulation of bone matrix production and osteoprogenitor differentiation. Interestingly, the increased blood flow generated by the vibration can also create shear stresses on the trabeculae of cancelleous bone [Dickerson et al. 2008]. Vibration's ability to enhance blood flow [Stewart et al. 2005] could contribute to an accelerated healing response. Vibration may also indirectly affect bone by eliciting hormonal responses. Two studies [Bosco et al. 2000 and Kvorning et al. 2006] have found elevated levels of growth-stimulating hormones (endogenous growth hormone and testosterone) and depressed levels of growth-inhibiting hormones (cortisol) after 9 weeks of WBV. The hormonal responses are much less transient and will persist long after the completion of the vibration protocol.

Hypogravity (<1 G) or low-magnitude accelerations are commonly used in bone applications because only micrometer sized vibrations are needed to promote bone formation and attenuate bone resorption [Qin et al. 1998, Prisby et al. 2008]. The optimum acceleration for bone maintenance has tentatively been determined to be around 0.3 G since it results in a better bone formation rate over other *hypogravity* levels [Garman et al. 2007]. One study compared vibration with *hypogravity* and *hypergravity* accelerations levels and revealed that all vibration groups were able to increase bone mineralization, bone formation rate, and ultimate strength in ovariectomized rats [Oxlund et al. 2003]. Interestingly the same study showed an increase in cortical bone formation in the *hypergravity* (3 G, 45 Hz) group, which has been since been confirmed by Rubinacci et al. [2008] operating at 3 G and 30 Hz. *Hypogravity* accelerations have not been shown to alter cortical bone formation but only cancellous bone which responds to vibration with increases in trabeculae size and numbers [Rubin et al. 2004].

2.5 Muscle & WBV

For sedentary, injured, and elderly people, lower-magnitude WBV may provide a promising surrogate to exercise and promote healthy musculoskeletal tissue. A few researchers [Rubin et al. 2002, Oxlund et al. 2003] have proposed that the elderly tend to lose fast oxidative-type muscle fibers which cause microstrains at above 20 Hz. This condition is known as sarcopenia and can be thought of as the muscle analog of osteoporosis. The two atrophic tissue conditions have a strong linear correlation with one another and may stem from the loss of active muscle tissue. Interestingly, WBV delivers the high frequency

microstrains (~5 microstrain) that the fast oxidative-type muscles once provided [Rubin et al. 2002, Oxlund et al. 2003].

For healthy individuals WBV platforms are also used for muscle strengthening and training exercises [Gilsanz et al. 2006, Roelants et al. 2004, Xie et al. 2008]. *Hypogravity* (<1 G) vibrations are not effective in increasing muscle strength so there is a need for higher magnitudes of vibration. Most commercially available platforms developed for muscle training deliver *hypergravity* accelerations (>1 G), between 2-6 Gs but can be as high as 15 Gs. Bogaerts et al. [2007] found that a year of daily vibration could cause muscular strength and mass gains which were equivalent to patients who engaged in a fitness program. When used appropriately there have been many positive findings including increases in proprioception, posture, balance, gait, as well as muscle strength and power [Adams et al. 2009, Moezy et al. 2008, Brunetti et al. 2006, Cardinale & Bosco 2003]. However, misuse can result in muscle fatigue, attenuated perception, and reduced response timing [Adamo et al. 2002, Cardinale & Wakeling 2005]. It is still unclear whether or not *hypergravity* accelerations are safe for all tissue types.

Whole body vibration generally involves transmitting vibrations through the body via a vibratory plate that is stood upon. WBV has several parameters which may be adjusted: frequency, acceleration amplitude, and duration. The optimization of these parameters is still underway through the process of trial and error. The common ranges for WBV parameters are available in Table 4.

2.6 Tendon & WBV

Unlike bone and muscle, studies examining the effects of vibratory loading environments on tendon and ligament adaptation are rare and conflicting. Sandhu et al. [2011] found that low-magnitude vibration (0.3 G, 30 Hz) increased rat flexor tendon cross-sectional area as well as stiffness. It was suspected that the 41% increase in stiffness was a result of structural changes, suggested by the 32% increase in cross-sectional area. Legerlotz et al. [2007] examined how high-magnitude (2 G, 25 Hz) vibration affects rat Achilles tendon but did not find any significant changes with respect to cross-sectional area or mechanical properties. However, the daily duration of the vibration was not consistent and was only activated when the rats attempted to eat. Another study subjected rats to four hours of high-vibration (13.2 G, 81 Hz) which was found to be traumatic to tissue [Hansson et al. 1988]. They reported a hypercellularity of large, plump fibroblasts that appeared to be in a prolonged synthesizing state which endured ten days after vibration trauma. These are the only three known studies exploring the effects of WBV on tendon. We believe that the magnitude of the vibration plays an important factor in determining how musculoskeletal tissue responds to WBV.

CHAPTER 3

Experiment: The Effects from Different Amplitude Levels of Whole Body Vibration on Tendon and Ligament Structural and Material Properties

3.1 Summary

Background: *Whole Body Vibration* (WBV) is becoming increasingly popular for helping maintain bone mass and strengthening muscle. Vibration regimens optimized for bone maintenance often employ low-magnitude (<1 G) high-frequency vibration (LMHFV) while regimens optimized for muscle strengthening often employ high-magnitude (> 1G) high-frequency vibration with frequencies between 15-60 Hz. Excessive vibratory loads, above some critical threshold, may be injurious. We hypothesized that both low vibration (0.3 G) and high vibration (2 G) would strengthen ligament and tendon with low vibration being more effective because high vibration may be near the threshold of tissue overloading.

Methods: 36 retired-breeder female Sprague-Dawley rats were equally divided into three test groups: control, low vibration, and high vibration. All rats were placed in a four chamber platform for 20 minutes a day, five days a week, for five weeks. For the low vibration and high vibration groups, the chambers were coupled to electromagnetic shakers. The low vibration operated at 30 Hz and 0.3 G peak-to-peak acceleration. The high vibration

operated at 30 Hz and 2 G peak-to-peak acceleration. Upon sacrifice, the medial collateral ligament (MCL), anterior cruciate ligament (ACL), patellar tendon (PT), and the Achilles Tendon (AT) were isolated with insertion sites intact. Three left limbs from each group were designated for histological examination. The remaining 27 left limbs were used for MCL tensile testing. The 36 right limbs were used for ACL, PT, and AT tensile testing.

Results: The AT and ACL appeared to be relatively unaffected by the two vibration levels after five weeks. Significant differences in structural and material properties were seen in the MCL. MCLs exposed to high vibration were on average 21% and 25% weaker than the no vibration and low vibration groups, respectively. There was also a significant decrease in energy density to failure in the MCLs exposed to high vibration. No differences in biomechanical data between the control group and the low vibration group were found. Histological examination of the MCL and PT showed a hypercellular tissue response to WBV. The tissues exposed to high vibration also showed a disorganization of fibers when compared with control and low vibration samples.

Conclusions: To our knowledge, this is the first vibration study comparing hypogravity and hypergravity levels of vibration on tendons and ligaments. By five weeks, there was evidence of weakness in the tendons and ligaments exposed to high vibration (2 G). The high vibration group displayed diminished biomechanical properties, most significantly in the MCL. The tissue response to high vibration exhibited a proliferation of active fibroblasts and disorganized collagen microstructure. Dense fibrous tissues exposed to WBV having a magnitude of 2 G or higher may have a response similar to overuse tendinopathies.

3.2 Background

Recently there have been a large number of studies examining the potential therapeutic effects of whole body vibration (WBV). Numerous studies have shown that WBV can be anabolic for bone and muscle. Both high (>2 G) and low (0.3 G) vibratory stimuli have been reported to initiate an increase in bone density, bone formation rate, and subsequently bone strength [Oxlund et al. 2003, Rubin et al. 2001]. Vibration has been applied to both animals and humans with notable increases in muscle cross-sectional area as well as strength [Gilsanz et al. 2006, Roelants et al. 2004, Xie et al. 2008]. However, the effects of WBV regimens remain relatively unexplored in tendon and ligament tissues. Legerlotz et al. [2007] investigated the response of rat Achilles tendons to 2 G WBV and found no effect on biomechanical properties [Legelotz 2007]. However, they applied between 2-7 minutes of vibration a day which is significantly less than that used in other studies or in clinical applications. Sandhu et al. [2011] found that 20 minutes of 0.3 G vibration applied daily increased rat flexor tendon cross-sectional area as well as stiffness [Sandhu 2011]. It was suspected that the 41% increase in stiffness was a result of structural changes, suggested by the 32% increase in cross-sectional area.

The goal of our study was to determine the effects of a low and a high vibration level on the structural and material properties of intact ligaments and tendons. The low magnitude vibration stimulus (0.3 G) had identical frequency and amplitude level as that used in Sandhu et al. [2011]. The 0.3 G level of whole body vibration has been proven to be anabolic for bone maintenance and bone healing applications [Rubin et al. 2001, Oxlund et al. 2003, Leung et al. 2006]. The high magnitude vibration stimulus (2 G) had similar frequency and amplitude level as used in Legerlotz et al. [2007]. The high vibration was chosen to

represent vibration commonly experienced by whole body vibration exercise platforms intended to improve muscle performance. Commercially available platforms generally operate between 2-6 Gs but some platforms can generate vibrations as large as 16 Gs.

Loading of musculoskeletal tissues has been shown to be anabolic but sometimes the magnitude levels can be understimulating or excessive; causing overuse injury. Ideally, the loading environment should activate the mechanosensitive tendon fibroblasts to upregulate collagen production and encourage linear fiber organization. We hypothesized that both low vibration (0.3 G) and high vibration (2 G) would strengthen ligament and tendon with low vibration being more effective because high vibration may be near the threshold of tissue overloading.

3.3 Methods

3.3.1 Experimental Setup:

After approval by UNC's Institute of Animal Care and Use Committee, 36 retired-breeder female Sprague Dawley rats were divided into three groups of 12. Rats were assigned to groups so that the average rat weight in each group was 330 g ± 2 g. The study consisted of one control group and two experimental groups: Low Vibration Stimulus (0.3G acceleration peak-to-peak at 30Hz) and High Vibration Stimulus (2 G acceleration peak-to-peak at 30Hz). The vibration regimen was applied 20 minutes a day, five days a week, for five weeks. Rats were placed in a four chamber vibration platform which was coupled with an electromagnetic shaker (Model N-300, Agac-Derritron Inc., Alexandria, VA). The shaker received a 30 Hz amplified sine wave signal which was created by a function generator. An accelerometer (352C65, PCB Piezotronics, Depew, NY) was used to ensure the vibration

chambers operated at the proper frequency and amplitude levels. The control rats were placed in chambers in an identical housing unit but did not receive any vibration.

After sacrifice, the rats were reweighed and their hind limbs removed. Three left limbs from each group were designated for histology and immediately were fixed with a 10% neutral buffered formalin solution. The remaining 27 left limbs were designated for medial collateral ligament (MCL) biomechanical testing. All 36 right limbs were designated for biomechanical testing of the anterior cruciate ligament (ACL), patellar tendon (PT), and the Achilles tendon (AT). Each limb was wrapped in saline soaked gauze, placed in an individual zip-lock bag, labeled, and frozen at -20 ºC. Prior to preparation and biomechanical testing, tissues were allowed to defrost for an hour. General preparation involved the removal of surrounding soft tissue, dissection of the hindfoot with the Achilles tendon, and potting of the femur and tibia in PVC tubes with poly-methyl methacrylate (PMMA) bone cement.

3.3.2 Biomechanical Test Setup:

Prior to biomechanical testing, the cross-sectional areas of the tissues were assessed by one of two methods. For tendons, an area micrometer was used to determine the cross-sectional area while a standard compression pressure of 0.12 MPa was applied to the midsubstance [Butler et al 1984]. For the MCL, an optical method was employed using a Dino-Lite Digital Microscope Pro (BigC, Torrance, CA). The tibia was secured in a vertical drill chuck which could be rotated 360 degrees and the femur was allowed to suspend freely. Minimum and maximum thickness images were taken 90 degrees apart at the midsubstance of the MCL. For calibration, a small metric ruler was held in the same plane as the tissue

during the collection of each image. The cross-sectional area was calculated as a rectangle after assuming a ribbon-like geometry. The cross-sectional area and gauge length of the ACL was not evaluated due to difficulty in visualizing it within the intraarticular space.

All tissue types were tensile tested with a materials testing system (Instron 8500 Plus, Instron Corporation, Norwood, MA). Tensile loading was applied along the long axis of the tissue in order to generate uniform tissue fiber tensioning. Custom jigs were designed to grip the PVC tubes in the following orientations during testing: MCL at 45º knee flexion with femur in-line with actuator and tibia angled at 45º [Lechner & Dahners 1991], ACL at 90º of knee flexion with femur and tibia both 45º off the loading axis [Kanaya et al. 2007, Warden et al. 2006]. The patellar tendon and Achilles tendon were tensioned in-line with the potted tibia and calcaneus, respectively. The calcaneus was grasped by a drill chuck during testing. A cryoclamp was used to grip the quadriceps muscle during patellar tendon testing and gastrocnemius/soleus muscle complex during Achilles tendon testing. For the tendons, the gauge length was measured from the tendon insertion site to the cryoclamp edge. For the MCL, the gauge length was calculated from the bone insertion site on the femur to the bone insertion site on the tibia.

All samples were pretensioned, preconditioned, and pretensioned again before testing to failure. Ligaments (MCL, ACL) were pretensioned to 0.5 N whereas tendons (PT, AT) were pretensioned to 2 N. All tissue types were preconditioned at 2% strain for 10 cycles. Two parallel strain marker lines were applied perpendicular to the long-axis of the tissue using India ink. A high speed camera (ES310, Redlake Inc., Tallahassee, FL) was used to capture live strain values during failure testing. All ligaments were tensioned to failure at a rate of 0.2 mm/sec. All tendons were tensioned to failure at a rate of 0.4 mm/sec. The region

of failure was recorded for all specimens. As the test was being executed, load and actuator displacement data were recorded. From the load-displacement curve several structural parameters were derived: ultimate load, stiffness, displacement at failure, and energy at failure. Individual stiffness values were calculated by fitting a regression equation to the slope of the load-displacement curves between 20% and 60% of the ultimate load.

3.3.3 Histological Examination:

Each medial collateral ligament was clipped from both the femur and the tibia, with a sliver of bone which allowed the insertion sites to remain intact. The patellar tendon was removed from each limb by excising the tendon precisely at its interface with the tibia and keeping the quadriceps muscle intact. Both tissue types were immediately placed in 10% neutral buffered formalin for 48 hours. Once removed from the fixation solution, the tissues were stored in 70% ethanol solution. Prior to paraffin embedding, the tissue was decalcified since the patella remained on the PT samples and the tibial bone-tendon interface on the MCL samples. These bony insertion sites were left to provide a visual landmark and to examine the activity at transition zones. The longitudinal cross-sections were taken in the sagittal plane for the patellar tendon and in the coronal plane for the MCL. Both tissues were stained with H&E and viewed with an Olympus BX40 Microscrope (Olympus America, Center Valley, PA).

The histology slides were assessed for cellularity and collagen organization. Cellularity assessment was conducted by three blinded graders using a 40x objective. Each specimen had two sections separated by 100 μm and five regions per section were evaluated by fibroblast counting. The regions were chosen based on two criteria: (1) The region filled

the whole viewing field and (2) the region had little to no section artifact. The fibroblast counts were averaged in the view and the counts were averaged across the three graders.

3.3.4 Statistical Analysis

A one-way ANOVA test was used to assess group differences of all biomechanical properties. An alpha level of 0.05 was used to determine significance. If significant, a Student-Newman-Keuls post-hoc test was deployed to determine significance between specific groups.

3.4 Results

None of the three groups experienced significant weight change during the study. Neither gauge lengths nor cross-sectional area measurements (Table 1) differed significantly between groups for any given tissue.

No significant differences in biomechanical data occurred between the control group and the low vibration group in any of the examined tissues. There was a pattern for higher means in the structural properties of the low vibration group (Table 1). There was also a trend for greater stiffness in the PTs exposed to low vibration in comparison to the other groups (Table 1). The high vibration group had a pattern for lower means in the structural properties of all tissues (Table 1). The high vibration ultimate load values found in the MCL data were 21% and 25% weaker than no vibration and low vibration groups, respectively (Figure 1b).

Material parameters were not affected by vibration, with the exception of the MCL which had a weakening of material properties in the vibration groups, with greater weakness

occurring in the high vibration group (Table 2). A near significant trend for tensile strength (Figure 1c) and a significant difference between groups for energy density were exhibited (Figure 1d).

The region of failure was recorded for all tissue types. No significant differences were found between the groups with respect to their failure location. In general, 92% of ACLs failed in the ligament midsubstance, 70% of MCLs failed at the tibial insertion site, and 90% of all tendons tested failed at the osteotendinous junction. There were a combined total of eight tibial growth-plate avulsions during ACL and PT tests. Tibial growth-plate avulsions occurring during ACL testing compromised the tibia insertion of the patellar tendon, preventing PT testing.

Histological assessment of the MCL and patellar tendon tissues revealed hypercellularity in both of the vibration groups (Table 3). When compared to the control cell counts, there were noticeable differences in the vibration groups but they failed to achieve statistical significance due to the small number of specimens (n=3/group) dedicated to histology. The cells that were counted were fibroblasts and displayed differing cell morphologies. In the control and low vibration group, the cells were narrow and had spindle-shape geometry (Figure 2a, 2b). In the high vibration group, all graders reported round plump fibroblasts (Figure 2c). The low vibration group tended to have the longest aligned chains of fibroblasts whereas the control and high vibration groups had intermittent groups of aligned fibroblasts. Another important observation made by graders regarded collagen fiber organization. Control and low-magnitude vibration tissues were reported to have well-aligned collagen fibers whereas high-magnitude vibration tissue was generally characterized as having unaligned, disorganized collagen fibers (Figure 2).

3.5 Discussion

Based on our findings, a low-magnitude WBV stimulus may be approaching an anabolic stimulus whereas a high-magnitude WBV stimulus may be catabolic to tendon and ligament tissues. Depending on the intensity of the stimulus, loading can under-stimulate or overload the tissue. However, a carefully optimized mechanical loading regimen may still exist which could aid in the strengthening or healing of ligaments and tendons. Our data suggests that low magnitude vibration (0.3 G peak-to-peak) either is ineffective or may simply be below the loading threshold needed to cause an anabolic tissue response. On the contrary, there is evidence of weakness in the biomechanical data corresponding to high magnitude vibration (2 G). In the MCL, high-magnitude vibration caused significantly reduced ultimate load and energy density values. Despite having a smaller sample size, all of the significant differences were observed in the MCL [Miller et al. 2005, Magnusson et al. 2010]. One explanation could be that the loads experienced by the MCL during high vibration are much higher than normal physiological conditions whereas they may only be slightly higher for the tendon tissues. Ligaments normally do not experience high loads because they serve as joint stabilizers whereas tendons function to transmit muscle loads to bone to create bodily movement. Another explanation for the MCL being more responsive than tendons is that ligaments have been shown to be more cellular and have a better blood supply [Amiel et al. 1984]. The MCL has better access to a blood supply than the ACL, as is evident from recovering knee injuries [Bray et al. 2002]. The MCL has multiple blood vessels integrated throughout the epitenon and into the midsubstance whereas the ACL is

thought to receive a majority of its nourishment through the synovial fluid. Furthermore, synovial fluid may dampen the transmission of the vibrations.

It still remains unclear whether the trends and significant differences between treatment groups are a result of material or structural changes. A number of other studies have concluded that repetitive mechanical stimuli can increase tendon strength and stiffness through tissue hypertrophy [Inglemark 1945, Michna & Hartmann 1989, Ronsager et al. 2002, Magnusson et al. 2003, Kongsgaard et al. 2007, Couppe et al. 2008]. In a preliminary study conducted by Sandhu et al. [2011], experimental rats that were exposed to the same vibration regimen as our low vibration group exhibited a 41% increase in stiffness and a 32% increase in cross-sectional area of wrist flexor tendons with respect to control rats. However, the vibrated and the control tissues in the study revealed no difference in material properties. This evidence suggests that the low-magnitude WBV may cause tissue hypertrophy. In our study, careful examination of the PT and MCL data revealed elevated ultimate load, stiffness, and cross-sectional area mean values in the low vibration group compared to the controls, but failed to reach significance. The hypercellularity in the low vibration MCL and PT samples further supports the likelihood of tissue hypertrophy. However, the lack of significant differences requires that further work be done. The current data suggests that the short-term application of low-magnitude WBV is ineffective at strengthening tendon and ligament.

Several high intensity endurance exercise studies have documented a decline in the biomechanical strength of tendons and ligaments [Sommer 1987, Soslowsky et al. 2002]. Sommer [1987] observed a decrease in ultimate tensile strength of the Achilles tendon despite increases in cross-sectional area and total collagen content after intensive running training in rats. Another overuse study in rat supraspinatus tendons also demonstrated

increased cross-sectional area measurements accompanied by decreases in maximum stress and tissue modulus values [Soslowsky et al. 2002]. The only known study investigating the response of rat tendons to high-magnitude vibration (2 G, 25 Hz) found no effect on biomechanical properties of the rat Achilles tendon [Legerlotz et al 2007]. Rats were only exposed to 2-7 minutes of vibration a day which is significantly less than other studies as well as clinical applications. Our study revealed findings suggesting that high vibration could be detrimental to tendons and ligaments. Significant decreases were seen in the ultimate load data for MCLs exposed to high vibration. This decrease in tissue strength was not accompanied by a reduction in tissue size, therefore a change in the tissues' material properties occurred. A reduction in energy density at failure in the high-vibration MCL ligaments also supports the idea that high vibration may lead to deteriorating material properties. The results from the high vibration groups, specifically the MCL's, suggest that current high vibration protocol is ineffective at promoting ligament or tendon strengthening and may be detrimental.

Histology of both vibration groups revealed a hypercellular response. Hypercellularity is associated with the proliferation stage of tendon healing, just prior to the remodeling stage [Chamberlain et al. 2009, Killian et al. 2012]. Interestingly, the fibroblast morphology and overall tissue organization between the low vibration and the high vibration appeared different. In the low vibration group the fibroblasts were spindle shaped and resembled fibroblasts seen in the control group. These spindle-shaped fibroblasts generally aligned themselves in long linear arrays, in greater quantity than the control. The collagen fibers in the low-vibration tissue were also neatly organized like the control tissues. Recorded observations from graders suggested a disorganization of collagen fiber structure

and fibroblasts in the high vibration group. The high vibration group had large plump fibroblasts which appeared to be in a more active state. Hansson et al. [1988] reported similar changes in tendon fibroblasts after exposure to high vibration (81 Hz, 13.2 G), describing them as "enlarged" and "hypertrophic." Hansson et al. [1988] believed that the fibroblasts were in a prolonged "synthesizing state" which "may induce chronic damage in the tendons." The hypercellularity of round plump fibroblasts and unorganized collagen structure seen in our work and Hansson et al. [1988] resembles the tendon histology in overuse models [Soslowsky et al. 2002, Soslowsky et al. 1996, Glazebrook et al. 2008, Backman et al. 1990, Cho et al. 2011]. We speculate that tissues receiving high vibration are being held captive at the proliferative stage, preventing the tissue from advancing to the last stage of healing: remodeling. This idea is supported by work done by Langberg et al. [1999, 2001] and Miller et al. [2005] which showed collagen turnover is the greatest in the first 24 hours post-loading. Interestingly, the net collagen turnover at 24 hours after loading is negative and it is not until about 36 hours after loading that collagen turnover becomes net positive [Magnusson et al. 2010]. Reloading the tissue within 24 hours of the last loading episode could be especially damaging because it may result in an additive net negative response. Without proper recovery time, the tissue may remain in a constant state of overactivity without ever getting the chance to reorganize newly deposited collagen. If vibration interrupts reorganization, collagen turnover could be positive and tissue properties could still decline. This may explain why the high vibration resulted in an overactive tissue morphology and weaker MCL biomechanics. The animals in our study were exposed to WBV in five-day blocks for five weeks which allowed little time for the tissue to recover and

restructure. It is possible that the 2 G magnitude of high vibration may not be catabolic if the tissues were given more time to respond.

Our study has several limitations that should be overcome in future studies. We believe that our sacrifice time point at five weeks may not have allowed for significant changes to take place. Short-term exercise has been shown to have little to no effect on tendon properties while longer term regimens have shown significant improvements [Inglemark 1945, Jozsa & Kannus 1997, Sommer 1987]. The response curves shown in Figure 3, adapted from Woo et al., shows that a stimulus applied to intact ligaments and tendons needs a long application period in order to achieve large differences in biomechanical properties. However, the same stimulus may cause a greater or more rapid response when delivered to injured or unloaded tissues. Future work needs to be done to investigate the potential for whole-body vibration on ligament and tendon healing. Our cross-sectional area measurements were taken at the midpoint of the ligament or tendon and evaluation at other locations may have helped detect treatment differences. Couppe et al. [2008] examined patellar tendons in world-class fencers and showed no differences in cross-sectional area at the midpoint of their patellar tendons. However, in the same study there was a significant 28% and 20% increase in the cross-sectional area of the patellar tendon near its proximal and distal insertion sites on the leading leg, respectively [Couppe et al. 2008]. Other studies have also found similar region-specific hypertrophy near the osteotendinous junction of PTs [Kongsgaard 2007] and ATs [Magnusson and Kjaer 2003] that had experienced long-term exercise. Another limitation of our study was recording consistent strain values when using the strain markers. For consistency, the actuator's

displacement was used for tissue displacement calculations, but real-time strains would have been preferred.

In conclusion, this study investigated how different magnitude levels of WBV affect the tissue responses in tendons and ligaments. The low vibration stimulus (0.3 G) was found to be insignificantly stimulating for all tissues analyzed. However, greater mean values in cross-sectional area, ultimate load, and stiffness of the low vibration groups in some tissues suggest that WBV may be causing a limited tissue hypertrophy response. Hypertrophic responses have been shown to take a long time and could explain why no differences were seen at five weeks. The high vibration stimulus (2 G) showed more negative effects on tissue strength and health. There were significant declines in the ultimate load and energy density of the MCL group exposed to high vibration. Histology of the high vibration group revealed hypercellularity of large fibroblasts as well as unorganized collagen structures. It is possible that increased duration or amplitude may further deteriorate tissue strength and health. Daily sessions of high-magnitude WBV, at 2 G or greater, may be used to create an accelerated animal model of overuse tendinopathy. Currently we are unable to determine whether the negative tissue response comes from the acceleration level or an insufficient amount of time between sessions for tissue healing or both. The 2 G WBV may have created loads which overloaded the tendons and ligaments. However, if subsequent WBV sessions were spaced farther apart then the tissue could have more time to respond, repair, and restructure.

3.6 Acknowledgements

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3.7 Tables and Figures

TABLE 1: STRUCTURAL PROPERTIES

		CSA (mm2)	Gauge Length (mm)	Ultimate Load (N)	Stiffness (N/mm)	Displacement at failure (mm)	Energy at failure (mJ)
AT	No Vibration (n = 11)	2.29 ± .59	14.35 ± 1.76	87.65 ± 14.20	82.37 ± 14.56	1.43 ± .21	64.57 ± 16.34
	Low Vibration (n = 12)	2.32 ± .39	14.12 ± 1.14	87.23 ± 13.27	84.55 ± 29.65	1.53 ± .61	66.89 ± 28.28
	High Vibration (n = 12)	2.25 ± .63	14.6 ± 1.60	80.99 ± 16.85	84.00 ± 16.88	1.89 ± .24	50.04 ± 19.83
		P-value: 0.960	P-value: 0.749	P-value: 0.495	P-value: 0.968	P-value: 0.140	P-value: 0.161
PT	No Vibration (n = 10)	2.26 ± .32	9.32 ± 1.00	82.37 ± 12.34	87.93 ± 17.67	1.05 ± .37	49.88 ± 21.30
	Low Vibration (n = 12)	2.34 ± .27	9.24 ± .59	86.17 ± 13.20	101.91 ± 20.45	1.07 ± .50	51.17 ± 20.73
	High Vibration (n = 12)	2.12 ± .25	9.04 ± .90	81.21 ± 15.75	83.96 ± 22.29	1.05 ± .30	47.77 ± 16.12
		P-value: 0.160	P-value: 0.727	P-value: 0.666	P-value: 0.095	P-value: 0.994	P-value: 0.911
ACL	No Vibration (n = 12)	Unable to accurately record ACL's cross-sectional area and gauge length. Hence no ACL material data.		49.87 ± 16.24	53.19 ± 20.38	1.07 ± .31	30.79 ± 15.05
	Low Vibration (n = 12)			50.92 ± 14.81	57.74 ± 24.15	1.07 ± .21	28.13 ± 9.74
	High Vibration (n = 12)			47.65 ± 14.68	50.66 ± 22.05	1.13 ± .29	26.75 ± 11.18
				P-value: 0.736	P-value: 0.866	P-value: 0.847	P-value: 0.722

	No Vibration (n = 9)	0.63 ± .16	9.12 ± 1.63	32.83 ± 6.25	27.53 ± 4.00	1.42 ± .32	6.66 ± 2.45
MCL	Low Vibration (n = 7)	0.77 ± 0.25	9.60 ± 1.02	34.60 ± 5.39	31.22 ± 8.02	1.44 ± .63	6.64 ± 2.72
	High Vibration (n = 7)	0.77 ± .29	9.16 ± 1.53	26.03 ± 7.20 ***, #**	25.26 ± 7.00	1.45 ± 1.05	4.94 ± 2.59
		P-value: 0.386	P-value: 0.780	**P-value: 0.044**	P-value: 0.232	P-value: 0.996	P-value: 0.360

Table 1: Mean values +/- standard deviation for structural properties in Achilles Tendon (AT), Patellar Tendon (PT), Anterior Cruciate Ligament (ACL), and Medial Collateral Ligament (MCL). Statistical differences between control (*) and low-vibration (#) [Student-Newman-Keuls].

TABLE 2: MATERIAL PROPERTIES

		Tensile Strength (MPa)	Energy Density (mJ/mm^3)	Elastic Modulus (MPa)
	No Vibration	40.00 ± 9.60	2.08 ± .64	537.18 ± 141.34
AT	Low Vibration	39.16 ± 11.84	2.13 ± 1.42	568.65 ± 113.03
	High Vibration	37.97 ± 11.85	1.67 ± .88	547.685 ± 123.92
		P-value: 0.909	P-value: 0.542	P-value: 0.827
	No Vibration	36.77 ± 5.59	2.37 ± .86	360.24 ± 126.45
PT	Low Vibration	36.95 ± 5.32	2.37 ± .90	358.33 ± 106.27
	High Vibration	38.5 ± 6.90	2.60 ± 1.16	349.11 ± 99.79
		P-value: 0.749	P-value: 0.812	P-value: 0.967
	No Vibration	56.39 ± 20.61	1.29 ± .59	368.96 ± 144.43
MCL	Low Vibration	47.88 ± 13.04	0.97 ± .49	379.03 ± 208.73
	High Vibration	36.14 ± 11.03	0.568 ± .30 *****	297.53 ± 92.55
		P-value: 0.065	**P-value: 0.043**	P-value: 0.563

Table 2: Mean values +/- standard deviation for material properties in Achilles Tendon (AT), Patellar Tendon (PT), Anterior Cruciate Ligament (ACL), and Medial Collateral Ligament (MCL). Statistical differences between control (*) and low-vibration (#) [Student-Newman-Keuls]

PT: Average Cell Count +/- S.D.	
Control	68.4 ± 3.78
Low Vibration	92.5 ± 30.1
High Vibration	86.3 ± 9.98

MCL: Average Cell Count +/- SD	
Control	127.8 ± 21.8
Low Vibration	153.8 ± 29.9
High Vibration	163.7 ± 59.3

Table 3: Fibroblast cell count means and standard deviations from three independent graders observing the same field of view. A) Left: shows the mean fibroblast counts in the patellar tendon (PT) for each experimental group, P= 0.321. B) Right: shows the mean fibroblast counts in the medial collateral ligament (MCL) for each experimental group, P= 0.562.

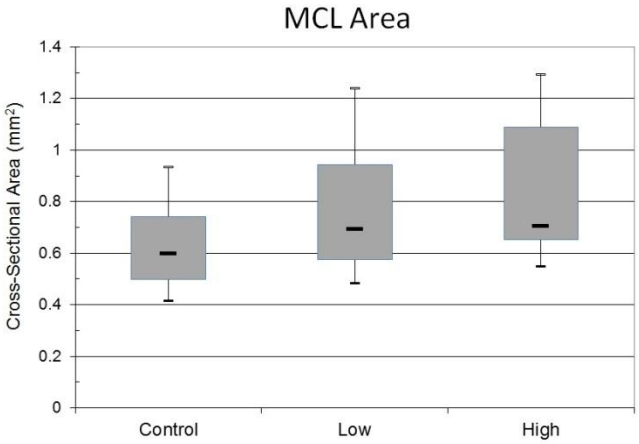


Figure 1a) Box Plot displaying cross-sectional area data for the MCL groups. P = 0.386

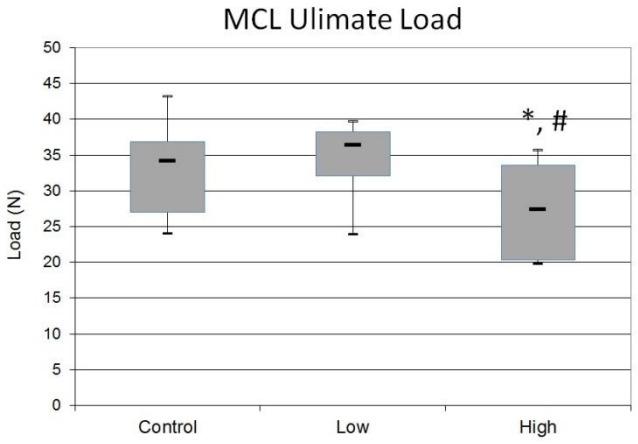


Figure 1b) Box Plot displaying ultimate load data for the MCL groups. P = 0.044, statistical differences between control (*) and low-vibration (#) [Studen-Newman-Keuls].

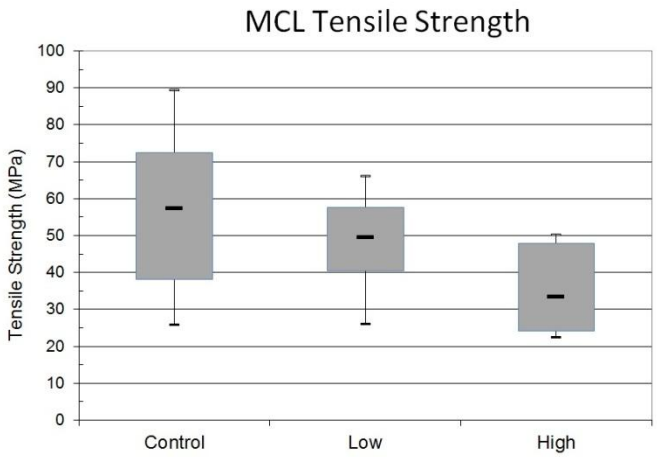


Figure 1c) Box Plot displaying ultimate load data for the MCL groups. P = 0.065.

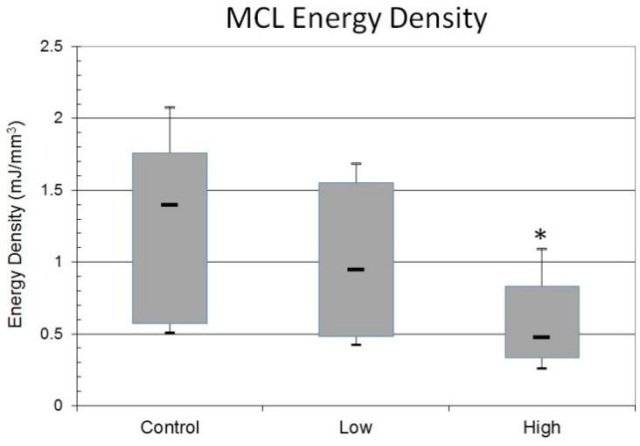
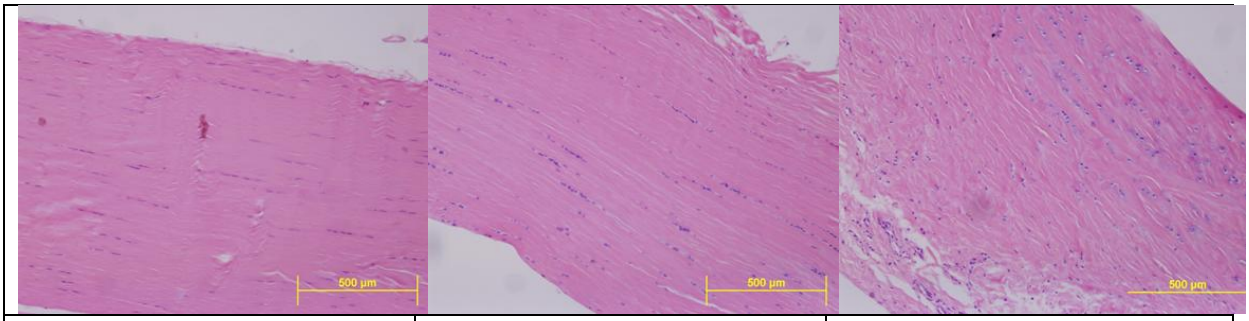


Figure 1d) Box Plot displaying energy density data for the MCL groups. P = 0.043, statistical differences between control (*) and low-vibration (#) [Studen-Newman-Keuls].



2a) Control MCL	2b) Low Vibration MCL	2c) High Vibration MCL

Figure 2: H&E stained MCL used for fibroblast counting and collagen fiber organization. 2a) Control MCL view. 2b) Low Vibration MCL View. 2c) High Vibration MCL View

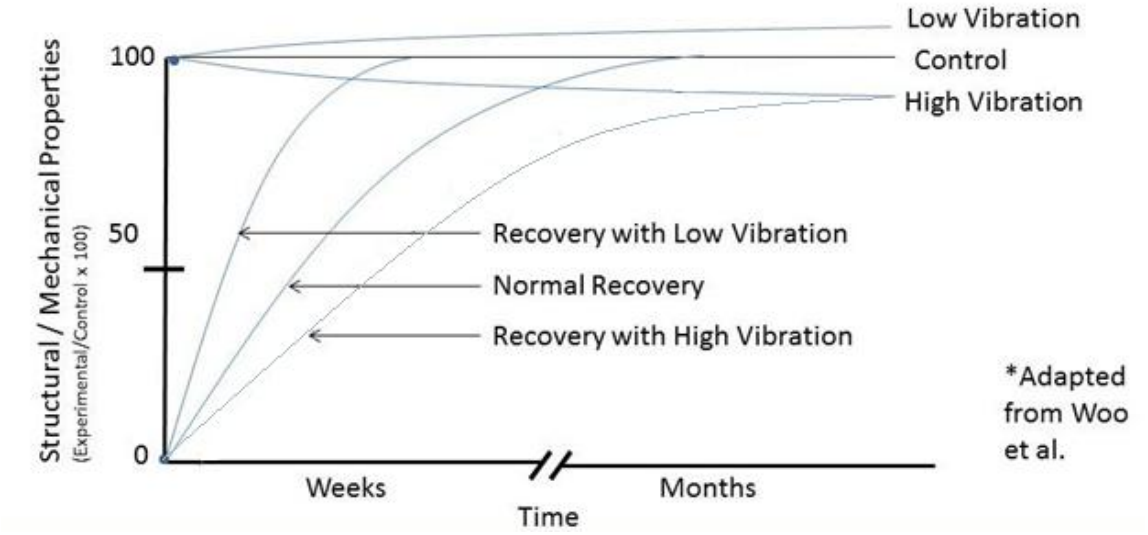


Figure 3: Structural and Mechanical Properties adapting to physical stimulus over time. The figure shows predicted responses for both intact tissues and tissues recovering from an injury. This figure was adapted from a similar figure created by Woo et al.

CHAPTER 4

Discussion

4.1 Discussion

It is hard to draw universal conclusions about whole body vibration (WBV) from available research because of the variation in WBV's parameters: frequency, magnitude, duration, and timing. Our study was focused on how the magnitude of WBV may affect tendons and ligaments. The results from this study suggest that exposure to WBV, especially high-magnitude, can result in structural, material, and cellular changes in tissue. Histology of MCL and PT samples revealed a hypercellular response in both low and high vibration groups. The high vibration group also displayed disorganized collagen microstructure which may explain the decline in ultimate load values seen in the MCL tissue. Over five weeks, the high vibration group displayed diminished structural and material properties. However, the structural properties of the low vibration were not significantly different from the controls. To our knowledge, we have conducted the first study examining the effects of amplitude of WBV on tendons and ligaments. Since there are very few related WBV studies, the rest of the discussion will draw most of its comparisons from studies that examined endurance training on ligaments and tendons.

Even though only a small proportion of our results achieved statistical significance, much may be learned from our study. Our experimental groups received identical vibration protocols with the exception of the magnitude of acceleration. By choosing previously used

acceleration magnitudes, we allowed for our work to be compared to past work. Since our chosen acceleration levels were below and above normal gravity conditions (1G, 9.81 m/s2), we were able to deliver both hypogravity and hypergravity loads. The daily dose of low vibration created 36,000 hypogravity loads of magnitude which should be lower than the loads experienced by rats while walking. Since the high vibration group remained at the same frequency, a similar bout of 36,000 loads was given at a hypergravity level. Because magnitude of loading is critical for generating musculoskeletal tissue responses, multiple thresholds have been commonly discussed [Cardinale & Wakeling 2005, Curwin 2011, Arampatzis et al. 2007]. The lowest threshold is that at which the magnitude of the loads is just large enough to maintain the normal physiological environments and this is known as the *homeostatic threshold*. The next threshold occurs when loads become just large enough to anabolically affect tissue and is termed the *anabolic threshold*. The third threshold occurs when loads are no long anabolic, start to become excessive, causing damaging and this is termed the *overload threshold*.

The high vibration stimulus resulted in a few biomechanical changes as well as some interesting histologic changes in the morphological examination. The stiffness of the high vibration groups in the PT, MCL, and ACL all declined when compared to the low vibration and control groups (Figures 9, 10). The high vibration groups also exhibited a decrease in the ultimate load across the board, for all examined tissues (Figures 9, 10). However, only the MCL revealed a significant decrease in ultimate load values of the high vibration group from the control and low vibration groups (Figure 10). We believe that the tendons may be more able to endure the vibration loads because they are force transmitters from muscle to bone. Muscles may absorb a majority of the vibration reducing the transmissibility of vibration to

the tendons. Ligaments are designed to be joint stabilizers and are attached to rigid bodies making them less able to withstand large repetitive loads. Therefore, the loads experienced by the MCL during high vibration are likely to be greater from normal physiological loading conditions whereas the loads experienced by tendon tissues may be similar to the physiological loading conditions. If greater loads are transmitted to the MCL they will induce larger strains which could cause fiber damage. Vibration may create ever-increasing strains due to the hysteretic nature of ligament and tendon viscoelasticity. The MCL might also be more responsive because ligaments display better vascularity and cellular density than tendons [Amiel et al. 1984, Bray et al. 2002]. MCLs in both the low and high vibration groups were hypercellular when compared to the control (Figure 11). Both of these groups appear to be in a proliferative stage characterized by fibroblast proliferation [Killian et al. 2012]. Normally this proliferative stage lasts for weeks in humans and up to three weeks in rats [Chamberlain et al. 2009]. Chamberlain et al. [2009] reported that total cell count was largest at seven days post- injury and that apoptotic cells appeared most frequently at fourteen days, coinciding with the start of the remodeling phase (Figure 12). Since our histological samples from the vibrated groups are still hypercellular at five weeks, this suggests that they may still remain in the proliferative stage. However, two observations differentiate the high vibration group from the low vibration group. 1) Large, round, plump fibroblasts were seen in the high vibration group which differed from the small, spindle-like fibroblasts observed in the control and low vibration groups (Figure 11). 2) Collagen organization was much more disorderly in the high vibration group than in the control and low vibration groups (Figure 11). New collagen that is not stressed, or over-stressed during the proliferation stage, may be deposited “haphazardly” [Kannus et al. 1997.] Histology of

tendinopathies have also revealed rounder fibroblasts, hypervascularization, and disorganized collagen fibers [Magnusson et al. 2010]. It appears that tissues from our high vibration group have several of the histological characteristics associated with overuse tendinopathies (Figure 11). Hansson et al. [1988] also exposed tendons to high vibration (81 Hz, 13.2 G, 4 hours) and saw "enlarged", "hypertrophic" fibroblasts which appeared to be in an overactive "synthesizing state". They went on to state that the synthesizing and secretory state that the fibroblasts remained in for 10 days was abnormal and it could "induce chronic damage in tendons and other structures in the connective tissue." Although their vibration was more traumatic than our stimulus (due to the duration and amplitude), both high vibration protocols appear to create tendinopathy-like tissue environments. The biomechanical and histological results from the high vibration group led us to believe that a 2 G amplitude level creates loads that approach or surpass the *overload threshold*.

The low vibration stimulus used in our study resulted in no statistically significant differences in the biomechanical data as compared to the control tissues. The histological examination revealed a tendency toward hypercellarity (figure 11) of the MCL and PT tissues exposed to low vibration (table 3). However, no difference could be detected in the cell counts, since only three specimens for each tissue type were dedicated to histology. It is also possible that the forces generated by the 0.3 G low vibration were below the *anabolic threshold*, thus not strong enough to elicit a tissue response. Arampatzis et al. [2007] declared that load regimens that deliver small strains which do not go beyond those triggered by daily activity loading are insufficient to elicit a tissue response. While we cannot say this with certainty, the four tissues that we investigated may experience larger loads during their daily activity than during exposure to our low vibration protocol.

Correct frequency selection is essential for the transmission of whole body vibration. A wide range of frequencies have been used in WBV studies ranging from 10 Hz to 90 Hz. The higher frequencies are usually avoided because much of the signal is absorbed by the soft tissues [Issurin & Tenenbaum 1999], never reaching the targeted tissues. The applied vibration should also remain above 25 Hz because patients experiencing frequencies below that threshold have complained of motion sickness-like discomfort [Kiiski et al. 2008, Cardinale et al]. Both vibration groups in our study used a 30 Hz vibration signal because a past study in our lab [Sandhu et al. 2011] exhibited a significant tendon hypertrophy response from a 30 Hz, 0.3 G WBV stimulus. Inssurin & Tenenbaum [1999] suggest that a frequency between 40-50 Hz would be optimal for vibration transmission and muscle excitation. Recently, some have suggested the vibration frequency should be matched with the natural frequency of the skeletal muscles which typically fall between 10-50 Hz [Cardinale & Wakeling 2005, Issurin & Tenenbaum 1999]. Both animal [Elliott 1965] and human studies [Kongsgaard et al. 2005] have shown that tendon size can be directly influenced by muscle size and loading. Our 30 Hz appears to be an acceptable frequency for stimulating tendon and ligament tissues but frequencies between 40-50 Hz may be effective too.

Stimulus duration and timing are two confounding variables that may be of critical importance. The overall duration of the study may have been too short to allow for substantial tissue remodeling. Healthy, intact musculoskeletal tissues respond slower to mechanical stimuli than injured tissues (Figure 3). For many studies it takes weeks, months, if not years of exercise before structural or material changes are noticeable [Kongsgaard et al. 2007, Woo et al. 1980,1981, Hansen et al. 2003, Couppe et al. 2008]. In one animal study, it took 12 weeks before the exercise group showed significant changes in cross-sectional area

(CSA) and strength from the control group, in rat Achilles tendons [Sommer 1987]. In untrained humans, six to nine months of daily running was not sufficient to increase Achilles tendon cross-sectional area [Magnusson et al. 2001, Hansen et al. 2003]. However, Achilles tendons of long-time runners (5+ years) were determined to be much larger in cross-sectional area than untrained subjects [Rosager et al., Magnusson & Kjaer 2003]. Other studies have observed region-specific hypertrophies of the PT [Couppe et al. 2008, Kongsgaard et al. 2007] and Achilles tendon [Magnusson & Kjaer 2003, Kongsgaard et al. 2005] after long-term exercising. Interestingly, the CSA of the tendon midsubstance remains relatively unchanged, whereas the regions of the tendon near the osteotendinous junctions (OTJ) have been enlarged by 32% (AT) [Magnusson and Kjaer 2003] and 28% (PT) [Couppe 2008] compared to their untrained counterparts. Since the region-specific hypertrophy often occurs near the OTJs (Figure 13), it could mean compressive loads are causing a synthesis of proteoglycans and other ECM components in those regions [Magnusson & Kjaer 2003]. On the other hand, the more traditional view of tendon hypertrophy could also be occurring. Like the osteocyte, the tenocyte is a mechano-sensitive cell which when mechanically stimulated will activate both anabolic and catabolic pathways. One of the earliest responses is the recruitment and multiplication of tenoblasts. The hypercellularity of tenoblasts is accompanied by increases in collagen I formation, alignment, and density [Jozsa & Kannus 1997, Kjaer 2004, Arampatzis et al. 2007, Couppe et al. 2008]. Our histology displayed highly aligned collagen fibers and increased fibroblast cell counts in the low-vibration group. Recurrent loading can create both an increase in the number of fibrils as well as fibril size [Jozsa & Kannus 1997, Woo et al. 1980, 1982]. Over time, the expansion of the collagen microstructure results in a larger cross-sectional area of tissues. It is possible that increases

in cross-sectional area and structural properties may have occurred if the study was carried out longer. Similar to endurance exercise training, we believe low-magnitude vibration applied over a long-term period may cause structural adaptation of tissue in the form of tissue hypertrophy.

As for the vibration timing, it is possible that every daily bout of WBV reinjures the tissue and prevents the tissue from transitioning to the remodeling stage. Recent studies have shown that collagen synthesis in humans peaks at approximately 24 hours after strenuous exercise and returns to homeostatic levels at 72 hours [Miller et al. 2005, Magnusson et al. 2010]. Surprisingly, a larger collagen degradation response occurs alongside collagen synthesis but peaks earlier (figure 4). Consequently, a net degradation of collagen occurs in the first 24-36 hours, followed by a net synthesis from 36-72 hours. Magnusson et al. [2010] makes a vital inference from this work: "net increase in collagen requires a certain restitution period, and that without sufficient rest a continuous loss of collagen is likely to occur, which might render the tendon vulnerable to injury." This finding could have implications for our study. Our WBV sessions were delivered to the rats at the same time each day, resulting in 24 hours between sessions (except on weekends). According to figure 4 provided by Magnusson et al. [2010], 24 hours is not long enough to result in a net synthesis of collagen. This could explain the hypercellularity of overactive fibroblasts present in the high vibration histology. Since collagen turnover precedes collagen organization, a daily application of WBV may keep the fibroblasts in a continual synthesizing state without ever sending cues to restructure. A negative overall tissue effect could eventually accrue from constantly having a net negative collagen turnover rate and a lack of restructuring.

4.2 Path Forward

The information found in this study will help design future studies exploring the effects of whole body vibration. The results also open the door to many new questions.

1) *If we vibrated for five weeks straight (no weekends off) would the negative results in the high vibration group become even more pronounced?* This question could be answered by including a 2 G group that is vibrated for 35 straight days. The current high vibration group received no vibration on weekends which may have allowed their tissue time to recover. This could be useful if we believe that WBV can create an accelerated animal tendinopathy model.
2) *How would the results change if WBV was applied every other day or once every three days?* Allowing for tendon and ligament tissue to recovery between WBV sessions may make the stimulus more anabolic. As we suggested previously, collagen microstructure from tissues exposed to high vibration seemed very unorganized. If WBV acts on the same mechanism as strenuous activity, then collagen synthesis persists days after the stimulus. It may be advantageous for the tissue to go through the full collagen turnover process and proceed to the restructuring process before delivering another session of WBV. To investigate this phenomenon, there should be two or three groups which have varying recovery time between WBV sessions. The WBV stimulus should be applied at the same frequency and magnitude, with 2 G being preferable.

3) *If we vibrated a longer total duration would the vibrated tissue eventually advance to a remodeling stage or would it remain in an overly-active proliferative stage?* This would be an important next step if we believe that long-term application of the low vibration stimulus would result in tissue hypertrophy. Tissue hypertrophy usually occurs over months not weeks. However, it would probably be wise to optimize stimulus timing before doing a longer study.
4) *What effect would WBV have on tendon or ligament healing?* Fracture healing has been accelerated using WBV and it has even resulted in stronger calluses. We can only speculate on which mechanisms of tendon or ligament healing would be affected by WBV. However, we have completed one WBV study examining the effects of low-magnitude vibration on MCL healing. The manuscript for this study can be found in appendix B.

4.3 Conclusion

The mechanical loading of musculoskeletal tissues requires a certain dose just as a pharmaceutical remedy would. Frequency, amplitude, duration, and stimulus timing are all critical for achieving a desired tissue response. As its name suggests, whole body vibration may affect multiple organ systems at once and the fine-tuning of parameters for one tissue could have detrimental results on another. A good example may be the use of high-magnitude (>2 G) WBV for muscle strengthening purposes without considering the chance of a catabolic response in the metabolically slower tendon tissues [Miller et al. 2005]. Evidence from our research suggests that ligaments and tendons experiencing repeated bouts of hypergravity vibrations could weaken over time. Currently, we believe that the

mechanism for the weakness is a proliferation of overactive fibroblasts which appear to focus more on collagen turnover than collagen organization. Both the histology from our study and Hansson et al. [1988] support this idea that vibrations with hypergravity accelerations can create tissue overactivity in tendon and ligament tissues. Interestingly, these tissue responses appear to resemble those from tendinopathy studies. Repeated daily exposure to high vibration may be a novel way to create a tendinopathy animal model.

The low vibration does not trigger as great of a response as the high vibration. It is likely that low-magnitude (0.3 G) vibration is not strong enough to generate loads above the *anabolic threshold*. Although no statistical significance was found in the biomechanical results, the low vibration groups of our ATs, PTs, and MCLs displayed greater mean values for stiffness and cross-sectional area. An increase in stiffness coinciding with larger cross-sectional areas would suggest a hypertrophic response. It should be noted that cross-sectional area measurements from our patellar tendons and Achilles tendons were gathered at the tendon mid-substances. If cross-sectional area measurements were also collected near the OTJ, there may have been significant differences at five weeks. However, if loading from whole body vibration is anything like endurance training then a significant hypertrophy of tissue may take months to develop. A larger cross-sectional area is the best prevention for injury because loads will be dissipated through the larger volume, generally resulting in smaller stresses on the tissue.

Our conclusions may be incorrect if stimulus timing is not explored. Studies have shown that a net negative collagen response may occur in the first 24 hours after strenuous exercise. Between 36-72 hours after the loading there is a net positive collagen response, return to more normal collagen turnover levels, and subsequent tissue healing. By delivering

daily bouts of vibration we may be causing continuous collagen turnover without any time or energy allotted for tissue restructuring. It is possible that the high vibration may elicit anabolic improvements in biomechanical strength and tissue structure if the tissue was allowed more recovery time. Further investigations will need to take place in order to evaluate whole body vibration's effect on tendon and ligament tissues.

APPENDIX A

Table 4: Whole Body Vibration (WBV)	
Parameter:	**Value:**
Frequency	15-90 Hz
Acceleration	0.3-15 G
Waveform	Sine
Loading Regimen	<30 mins/day for 1-12 months

Table 4: Common ranges of whole body vibration parameters.

PT Failure Frequency

# of total Samples	# failed at MS	# failed at patella	# failed at tibial insertion	# failed at tibia growplate
34	3	12	13	6
No Vib (10)	20%	30%	30%	20%
Low Vib (12)	8%	25%	50%	17%
High Vib (12)	0%	50%	33%	17%

Table 5: Patellar Tendon (PT) failure locations. *Note: Only 34 samples because #3 and #24 both failed at Tibia Growth plate during ACL testing

AT Failure Frequency

# of total samples	# failed at MS	# failed at BT insertion
36	4	32
No Vib (12)	17%	83%
Low Vib (12)	17%	83%
High Vib (12)	0%	100%

Table 6: Achilles Tendon (AT) failure locations.

MCL Failure Frequency

# of total samples	# failed at MS	# failed at femoral insertion	# failed at tibial insertion
23	4	3	16
No Vib (9)	22%	11%	67%
Low Vib (7)	0%	14%	86%
High Vib (7)	29%	14%	57%

Table 7: Medial Collateral Ligament (MCL) failure locations. *Note: 23 samples because 12 used for histology and one was compromised during bone cementing

ACL Failure Frequency

# of total samples	# failed at MS	# failed at growth plate
36	33	3
No Vib (12)	83%	17%
Low Vib (12)	100%	0%
High Vib (12)	92%	8%

Table 8: Anterior Cruciate Ligament (ACL) failure locations. *Note: 2 growth plate failures occurred at tibial growth plate and other occurred at femoral growth plate

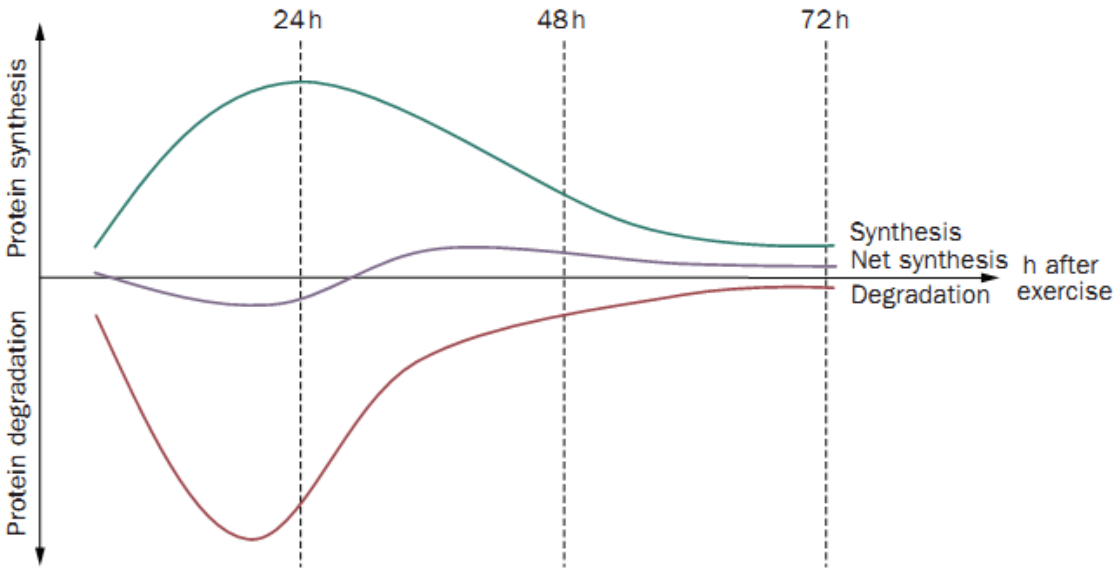


Figure 4: Collagen turnover after strenuous exercise.

Borrowed from Magnusson et al. 2010.

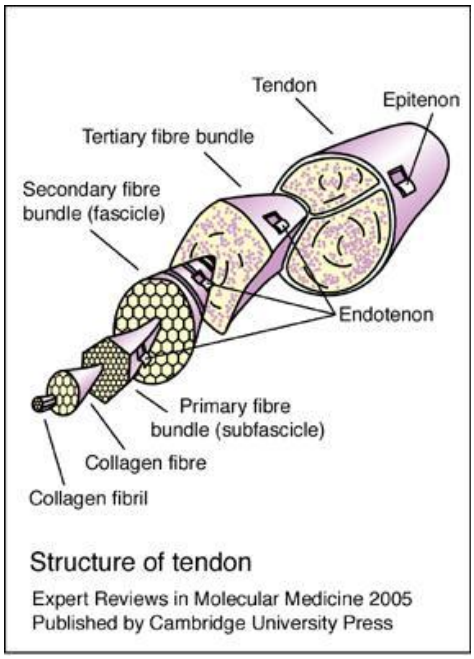


Figure 5: Collagen Fiber Hierarchy.

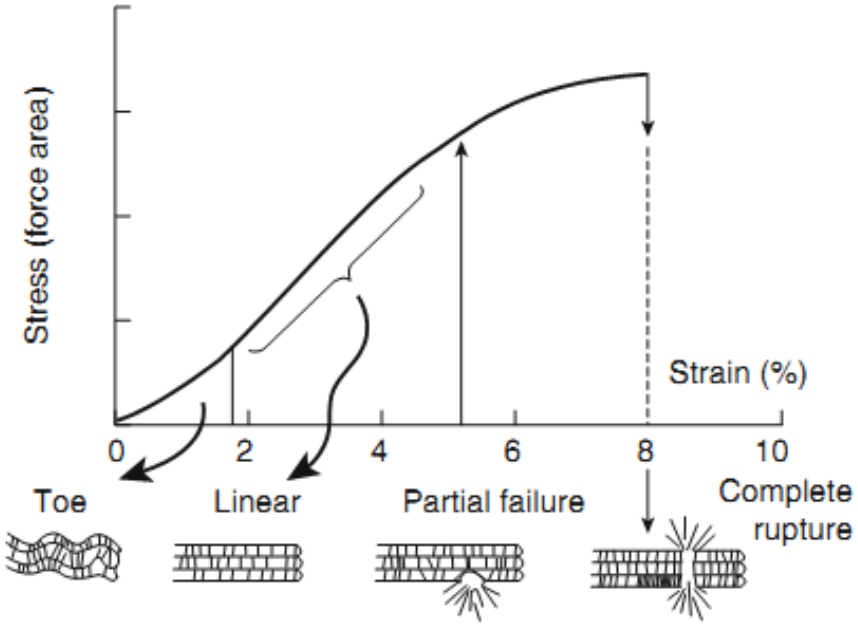


Figure 6: Stress-Strain curve for a ligament or tendon in tension. Borrowed from Curwin 2011.

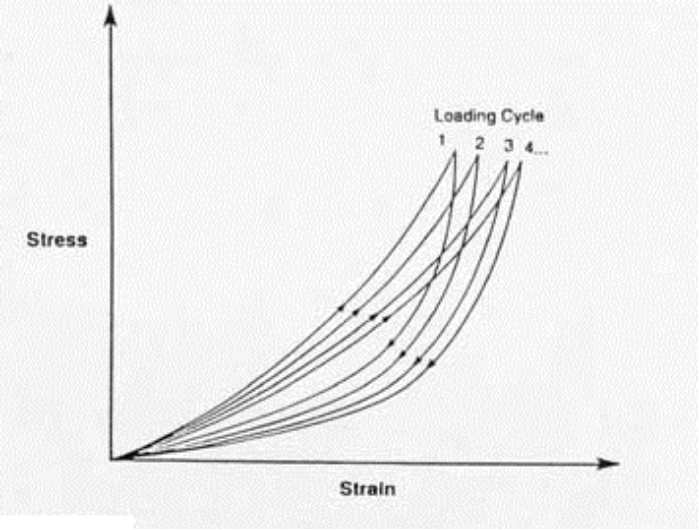


Figure 7: Hysteresis effect due to friction within the composite structure of tendons and ligaments.

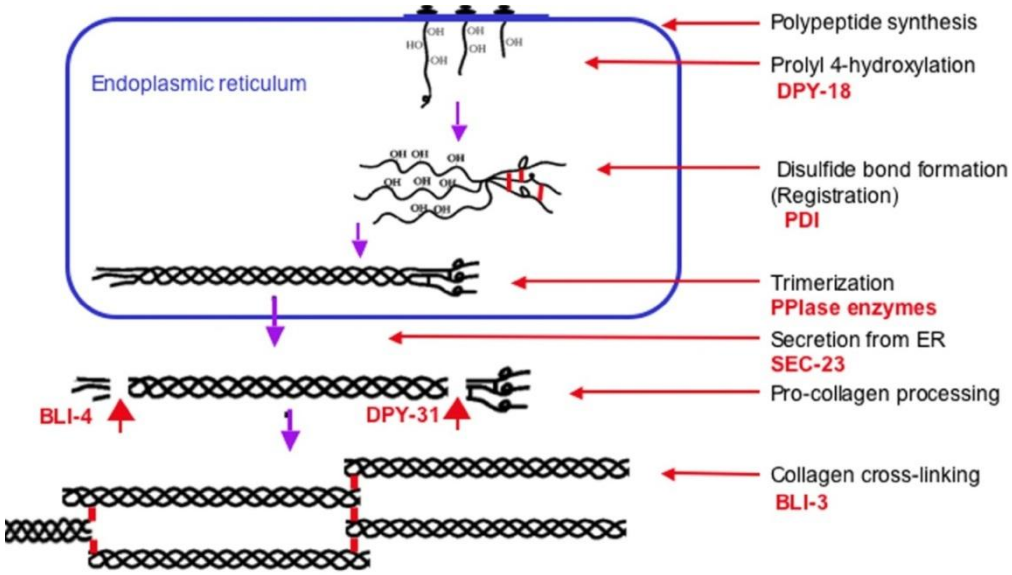


Figure 8: Intracellular and extracellular collagen formation response.

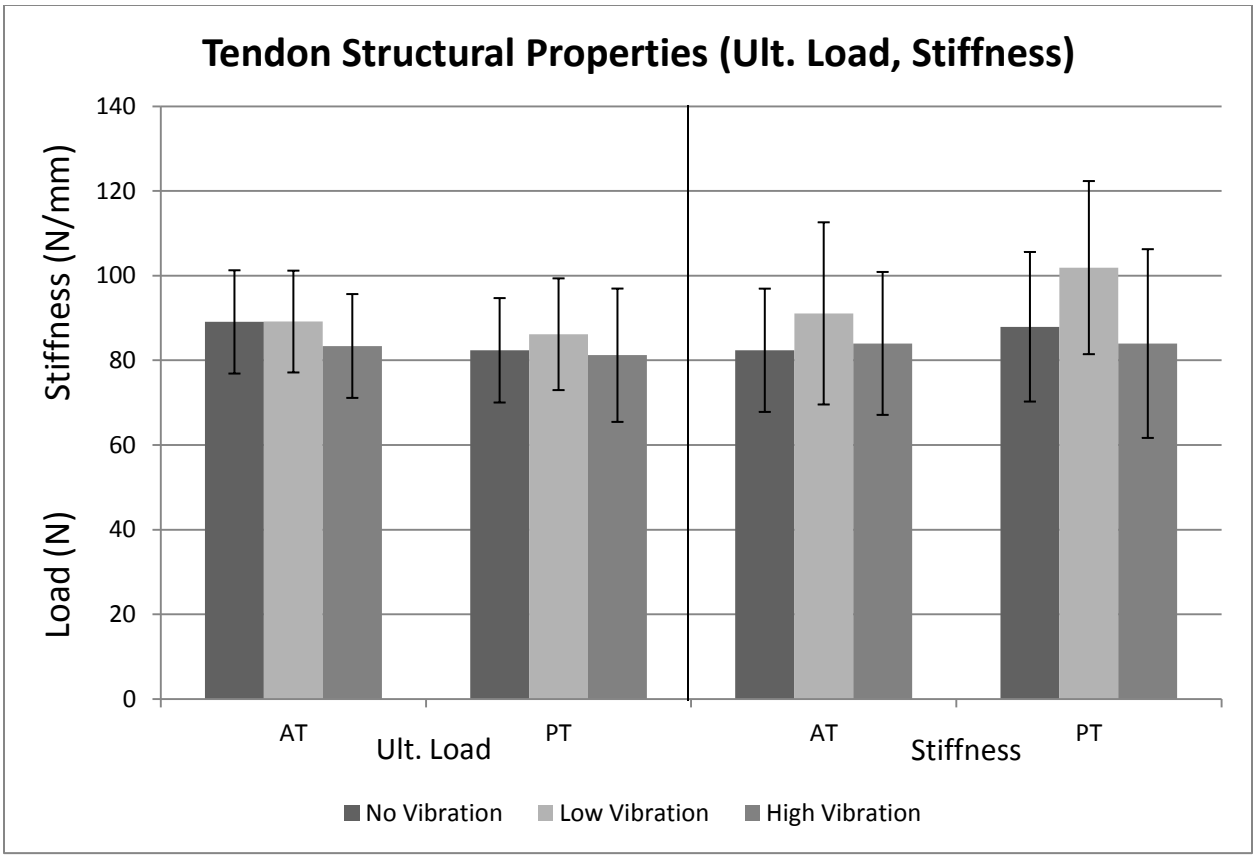


Figure 9: Mean ultimate load and stiffness values for both the Achilles tendon (AT) and the patellar tendon (PT) with standard deviation bars.

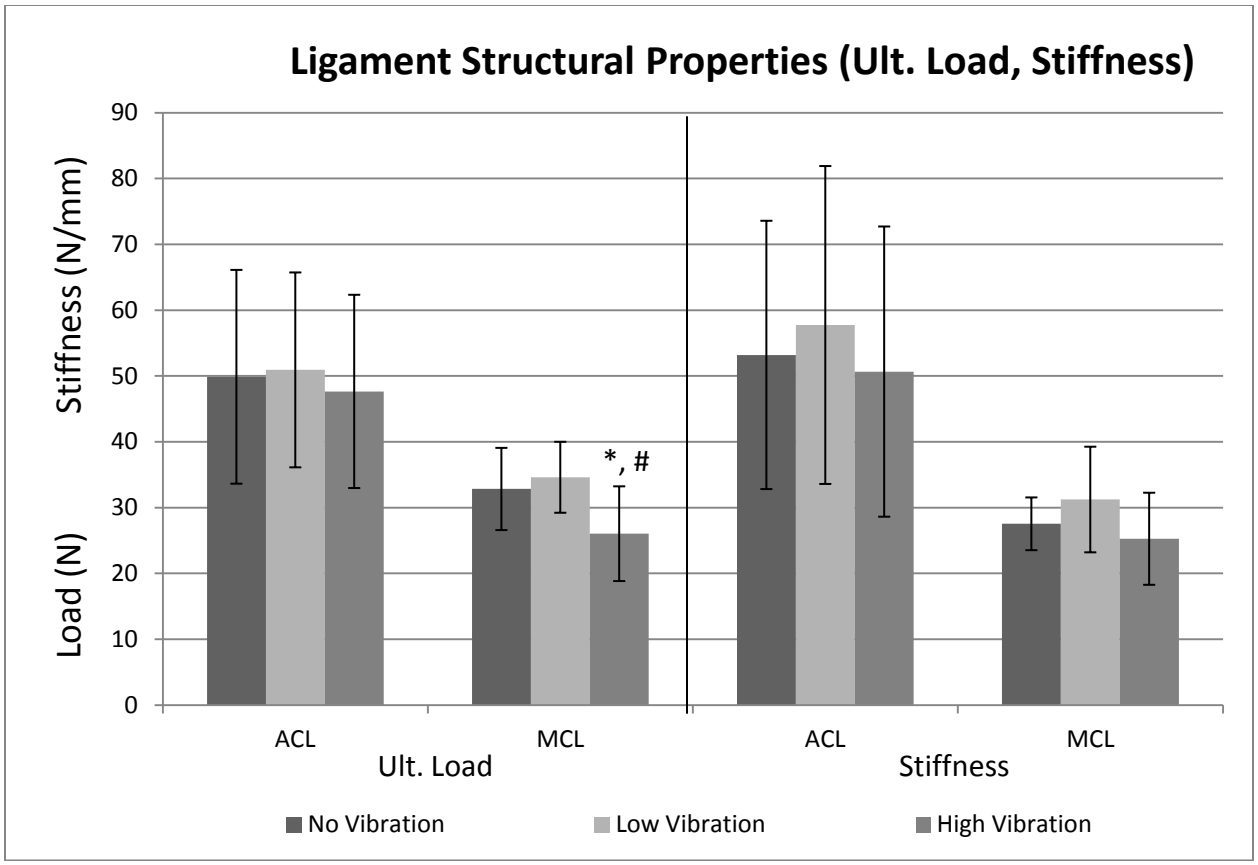


Figure 10: Mean ultimate load and stiffness values for both the anterior cruciate ligament (ACL) and the medial collateral ligament (MCL) with standard deviation bars. Student-Newman-Keuls post-hoc test was deployed to show differences between control (*) and low-vibration (#).

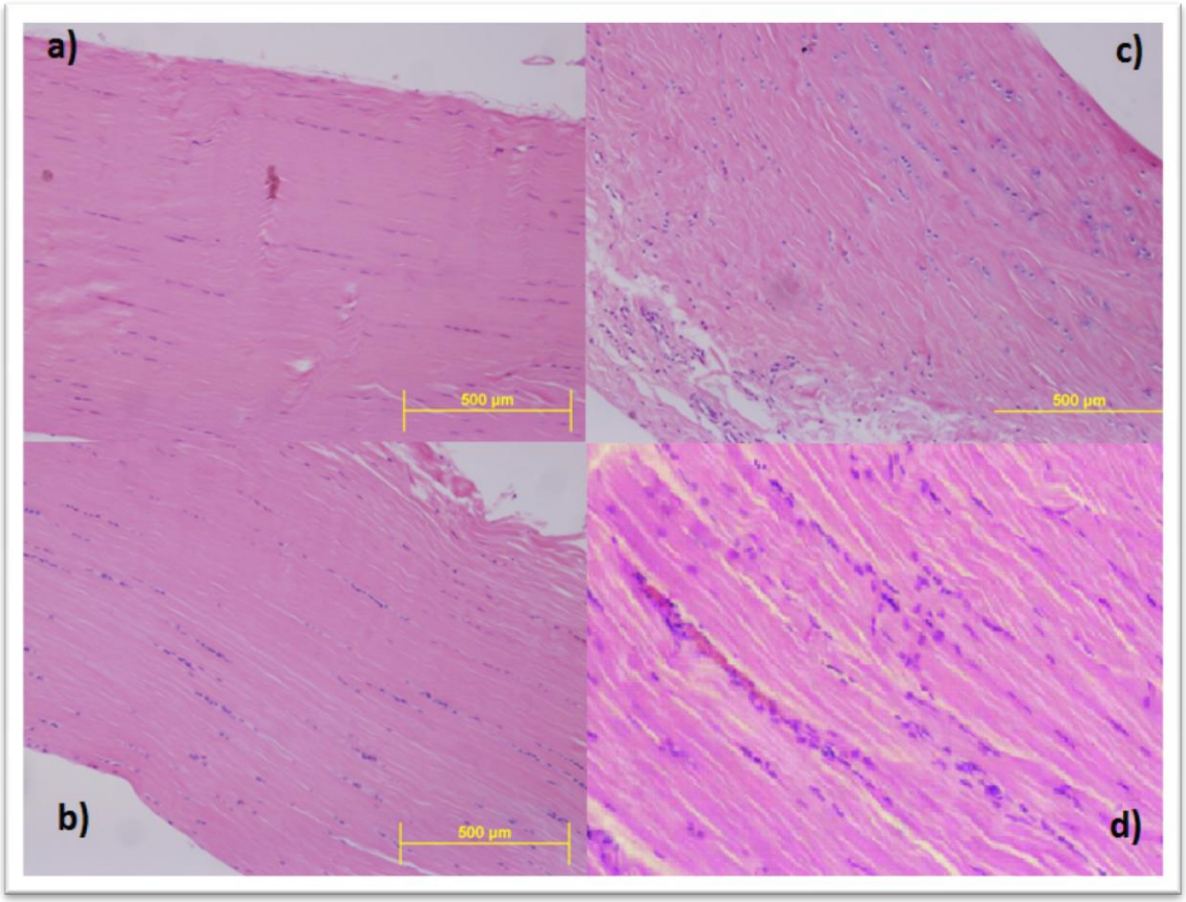


Figure 11: Histology of a) Control Tissue, b) Low Vibration, c) High Vibration, d) Tendinopathy (Borrowed from Rees et al. 2009.

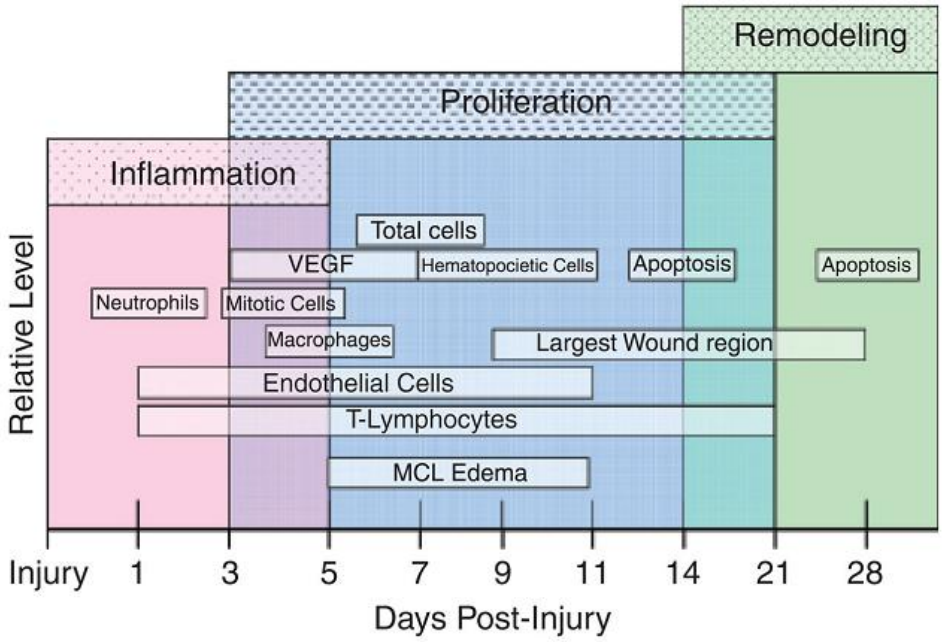


Figure 12: Healing response time course for a rat. Borrowed from Chamberlain et al. 2009.

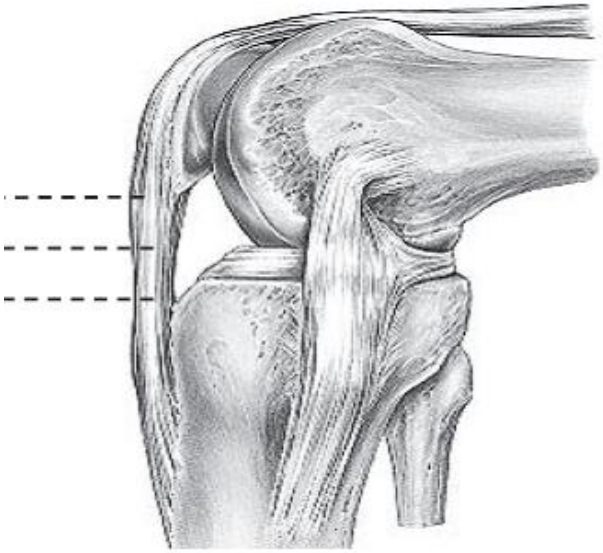


Figure 13: Regional hypertrophy of the human patellar tendon (PT) discovered by Couppe et al. 2008. The study found no significant changes in cross-sectional area of midsubstance (middle line) and significant changes in cross-sectional area of proximal (top line) and distal (bottom line) regions.

APPENDIX B

Low Magnitude, High Frequency Vibration to Accelerate Ligament Healing

Introduction:

Noninvasive therapies to accelerate or improve ligament and tendon healing are needed. Low magnitude, high frequency vibration (LMHFV) is a form of vibration operating at *hypogravity* accelerations (<1G). LMHFV has recently been found to attenuate osteoporosis [Fritton et al. 1997, Qin et al. 1998, Rubin et al. 2004] as well as accelerate fracture healing [Leung et al. 2009]. The mechanism by which LMHFV accelerates musculoskeletal tissue healing and strengthening remains speculative. LMHFV is thought to stretch the internal mechanosensitive cells which are responsible for regulating collagen production and activating extracellular pathways. Other evidence suggests that WBV could be improving blood flow to the injury site [Stewart et al. 2005, Sun et al. 2011] or eliciting hormonal responses [Bosco et al. 2000, Kvorning et al. 2006]. Although the mechanism remains unclear, recent work has shown that LMHFV exposure can increase the stiffness and cross-sectional area of intact tendon suggesting anabolic effects [Sandhu et al. 2011]. We hypothesized that LMHFV might also accelerate medial collateral ligament (MCL) healing after acute injury. Our objective was to evaluate the influence of LMHFV on the biomechanical properties of the healing MCL.

Methods:

After approval by the institutional animal care and use committee (IACUC), 32 Sprague Dawley rats were divided into two groups of 16 animals so that the average weight in each group was 300 ± 2 g. The study consisted of one control group and one experimental group, receiving the whole-body vibration stimulation (0.3 G acceleration peak-to-peak). Under inhalation isoflurane anesthesia all the rats had their left medial collateral ligament (MCL) surgically transected at the midsubstance, just distal to the knee joint and their wound was closed using a wound clip. The vibration regimen began two days post-operation. The WBV regimen consisted of 30 minutes of vibration a day, 7 days a week, for 12 days. Rats were placed in a four chamber vibration platform that was coupled with an electromagnetic shaker (Model N-300, Agac-Derritron Inc.). A function generator was used to send a 30 Hz amplified sine wave signal to the shaker. Rats in the control group were placed in a similar chamber but were not stimulated. At 14 days post-operation, the rats were sacrificed and their hind limbs were removed.

Two limbs from each group were designated for histology and fixed in 10% neutral buffered formalin. These specimens were embedded in paraffin, sectioned and stained with hematoxylin and eosin. The remaining limbs (injured and contralateral) were wrapped in saline soaked gauze, placed in individual sealed bags, and stored in a -20˚C freezer until biomechanical testing. The proximal portion of the femur and the distal end of the tibia were potted in PVC tubes with bone cement. A Dino-Lite Digital Microscope Pro (BigC, Torrance, CA) was used to take triplicate measurements of the MCL's width and thickness and the cross-sectional area was estimated as an elliptical geometry. The tibia and femur were mounted in grips on a material testing apparatus with the knee flexed at 90˚and the

MCL aligned with the loading axis. All samples were preconditioned to 2% strain and pretensioned to 0.5N before being tensioned to failure at a rate of 0.2 mm/sec. The load and grip displacement data of the tensile test was used to compute the stiffness, ultimate load/strength, and energy to ultimate load for each sample. The raw structural biomechanical properties and structural properties, normalized by body weight, were statistically analyzed. A one-way ANOVA or Kruskal-Wallis ANOVA on ranks was run to determine group differences in biomechanical properties. An alpha level of 0.05 was used to determine significance.

Results:

Neither of the groups experienced any significant weight change over the course of the study. None of the structural parameters, including ultimate load (Fig. 1), stiffness, and energy, showed any significant differences between groups for either the injured or intact limbs. Also, the structural parameters normalized by body weight for both injured and intact limbs did not show significant differences between groups. Furthermore, there were no significant differences between the groups for the cross-sectional area or ultimate tensile strength of the injured MCLs. Histological sections revealed no changes in tissue cellularity or organization.

Discussion:

Our findings of no differences in the structural properties of the healing MCL between groups did not support our hypothesis that LMHFV could accelerate healing. It is possible that our injury model may have limited the potential of LMHFV to stimulate

healing. Our midsubstance transection injury of the ligament removed tension from the MCL and this may have limited transmission of the vibrational energy to the injury site. A partial transection model would have maintained tension, which may be needed for the resident tendon cells (tenocytes) to sense the vibration, but production of a consistent partial injury is problematic.

This study had similar WBV parameters (30 Hz, 0.3 G) as two other studies investigating the effect of WBV on tendon and ligament [Sandhu et al. 2011, Keller et al. 2012] which exhibited mixed results. The previous studies examined the effects of WBV (30 Hz, 0.3 G) on intact tendons and ligaments after five weeks of exposure. Sandhu et al. found a 41% increase in stiffness and 32% increase in cross-sectional area mean values of the vibrated rat flexor carpi ulnaris tendon of the forelimb compared to the controls. Since the rats employed in Sandhu et al. were part of a hindlimb fracture model they may have relatively overloaded their forelimbs. Greater than normal loading with the addition of LMHFV could explain the strengthening effect seen in of the flexor carpi ulnaris tendon of the wrist as well as the closer proximity of the tendon insertion to the vibration source. Keller et al. examined the response to 0.3 G vibration in the MCL, ACL (anterior cruciate ligament), patellar tendon, and Achilles tendon and found no statistically significant differences in any biomechanical properties. It is possible that 0.3 G vibration may be below some critical loading threshold needed to cause an anabolic tissue response. Neither the intact MCLs in this study nor the MCLs from Keller et al. had a tissue hypertrophy response as seen in Sandhu et al.

It is unclear whether WBV acts more like endurance exercise or low-intensity pulsed ultrasonics (LIPUS). The microstrain loads experienced during our 0.3 G LMHFV vibration

are thought to directly deform the cell membrane of mechanosensitive cells. This triggers anabolic and catabolic pathways, resulting in increased collagen turnover. However since the microstrains generated by 0.3 G WBV are quite small [Qin et al. 1998] it is possible that another mechanism may be occurring. It is also thought that vibration might enhance the early stages of ligament healing by improving blood flow and angiogenesis, similar to low-intensity pulsed ultrasound on ligament healing [Fu et al. 2008]. However, the results from the current study suggest that LMHFV may have little effect on the inflammation and proliferation stages of healing. The rat MCL healing response transitions from the proliferation stage to the remodeling stage around fourteen days after injury occurrence. It is possible that LMHFV could be more effective in stimulating ligament healing if applied during the final stage of healing, the remodeling phase.

Future research should further explore how WBV's parameters may impact tendon and ligament healing. We suggest applying higher magnitude vibrational loads by raising the acceleration level from our 0.3 G level may be necessary to observe a stimulatory effect of WBV on ligament or tendon healing. However, greater load intensities often results in greater collagen turnover, which suggests a longer recovery period between WBV sessions will be needed [Magnusson et al. 2010, Keller et al. 2012]. Vibration was delivered at 30 Hz in our study and this frequency has been proven to transmit effectively in other studies [Qin et al. 1998, Rubin et al. 2004, Rubinacci et al. 2008]. A 30 Hz stimulus seems near optimal for transmission to the knee when operating at larger magnitude levels, but 35 Hz may have been better for our lower magnitude level (0.3 G) [Kiiski et al. 2008]. Furthermore, transmission of vibration to the healing MCLs may not have been limited by the frequency but by the complete transection of the MCL midsubstance. Either a partial transection model

or an insertional injury model may be more appropriate for future studies. An insertional model may be clinically more relevant because MCLs most often fail at their osseoligamentous junction. However, such a model has been infrequently used to study MCL injuries. Recent studies have also shown that intact tendons and ligaments structurally adapt to mechanical stimuli near their bony insertions rather than at the midsubstance [Magnusson & Kjaer et al. 2003, Kongsgaard et al. 2007, Couppe et al. 2008]. Therefore an insertional injury model may be able to exhibit differences of ligament healing caused by LMHFV.

In conclusion, twelve days of low-magnitude high-frequency vibration (LMHFV) did not appear to influence the initial healing response of injured rat MCLs. Neither the intact nor injured MCLs that were exposed to LMHFV differed from controls when examining biomechanical and histological responses. LMHFV operates at *hypogravity* accelerations (<1 G) which have been shown to be useful for bone maintenance and to accelerate fracture healing. It is possible that the 0.3 G level may not be high enough to stimulate a healing response in ligament tissue. It is also possible that vibration has greater importance during the remodeling phase and does not affect the initial stages of the healing process. Further work needs to be done to understand WBV's effects on intact and healing tendons.

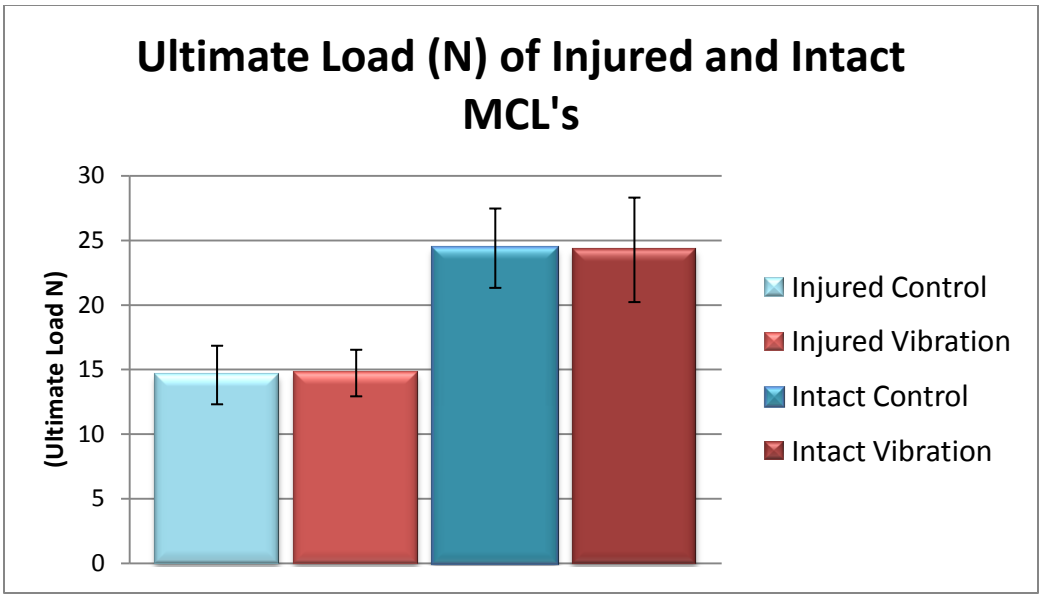


Fig. 14: Ultimate load of intact and injured MCL did not differ between the vibration and control groups.

Significance:

LMHFV is being investigated as a potential therapy for accelerating healing after musculoskeletal injury. While LMHFV did not improve early ligament healing after midsubtance injury, the stimulus also did not demonstrate an inhibitory effect on the biomechanical properties of the healing ligament.

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