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Varying whole body vibration amplitude differentially affects tendon and ligament structural and material properties

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ABSTRACT

Whole Body Vibration (WBV) is becoming increasingly popular for helping to maintain bone mass and strengthening muscle. Vibration regimens optimized for bone maintenance often operate at hypogravity levels (<1 G) and regimens for muscle strengthening often employ hypergravity (>1 G) vibrations. The effect of vibratory loads on tendon and ligament properties is unclear though excessive vibrations may be injurious. Our objective was to evaluate how tendon gene expression and the mechanical/histological properties of tendon and ligament were affected in response to WBV in the following groups: no vibration, low vibration (0.3 G peak-to-peak), and high vibration (2 G peak-to-peak). Rats were vibrated for 20 min a day, 5 days a week, for 5 weeks. Upon sacrifice, the medial collateral ligament (MCL), patellar tendon (PT), and the Achilles Tendon (AT) were isolated with insertion sites intact. All tissues were tensile tested to determine structural and material properties or used for histology. Patellar tendon was also subjected to quantitative RT-PCR to evaluate expression of anabolic and catabolic genes. No differences in biomechanical data between the control and the low vibration groups were found. There was evidence of significant weakness in the MCL with high vibration, but no significant effect on the PT or AT. Histology of the MCL and PT showed a hypercellular tissue response and some fiber disorganization with high vibration. High vibration caused an increase in collagen expression and a trend for an increase in IGF-1 expression suggesting a potential anabolic response to prevent tendon overuse injury.

1. Introduction

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 formation rate, bone density, and subsequently bone strength (Oxlund et al., 2003; Rubin et al., 2001). High and low vibration regimens have also elicited notable increases in muscle cross-sectional area and strength in animals as well as humans (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. Past work (Hansson et al., 1988) has subjected rats to 4 h of high-vibration (6.1 G, 81 Hz) for two consecutive days. The stimulus was found to be traumatic for the Achilles tendon. They reported a hypercellularity of large, plump fibroblasts that appeared to be in a prolonged synthesizing state which endured 10 days after vibration trauma. Other studies have investigated the response of rat Achilles tendons to 2 G WBV and found no effect on biomechanical properties (Legerlotz et al., 2007). However, they applied between 2–7 min of vibration a day which is significantly less than that used in other studies or in clinical applications. Recent studies have found that 20 min of 0.3 G vibration applied daily increased rat flexor tendon cross-sectional area as well as stiffness (Sandhu et al., 2011). It was suspected that the 41% increase in stiffness was a result of structural changes, suggested by 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 tendon gene expression and the biomechanical properties of intact ligaments and tendons. Loading of musculoskeletal tissues has been shown to be anabolic but depending on the loading intensity a loading stimulus could also be understimulating or excessive; resulting in ineffectiveness or overuse injury. Commercially available vibration platforms generally operate between 2–6 Gs but some platforms can generate

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vibrations as large as 16 Gs. We hypothesized that both low vibration (0.3 G) and high vibration (2 G) would strengthen ligament and tendon and that low vibration would be more effective because high vibration may be near the threshold to tissue overloading.

2. Materials and methods

2.1. Experimental setup

After approval by UNC's Institute of Animal Care and Use Committee, 36 retired-breeder female Sprague-Dawley rats were divided into 3 groups of 12: control group, low vibration (0.3 G acceleration peak-to-peak) and high vibration (2 G acceleration peak-to-peak). Rats were assigned to groups so that the average rat weight in each group was 330 g $\pm$ 2 g. Vibration regimens were applied 20 min a day, 5 days a week, for 5 weeks. Rats were placed in a 4 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. In the remaining 27 left limbs, the patellar tendon was removed and stored at −80 °C for later evaluation by quantitative real time reverse transcription polymerase chain reaction (RT-PCR) and the remaining knee was stored at −20° C for biomechanical testing of the medial collateral ligament (MCL). All 36 right limbs were stored at −20° C and designated for biomechanical testing of the patellar tendon (PT) and the Achilles tendon (AT). Preparation for biomechanical testing 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.

2.2. 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° and the femur was allowed to suspend freely. Minimum and maximum thickness images were taken 90° apart at the midsubstance of the MCL. The cross-sectional area was calculated as a rectangle given its ribbon-like geometry

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 45° knee flexion with femur in-line with actuator and tibia angled at 45° (Lechner and Dahners, 1991). The PT and AT 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 tendon at its insertion to the patella during PT testing and the gastrocnemius/soleus muscle-tendon units during AT testing. For the tendons, the gage length was measured from the tendon insertion site to the cryoclamp edge. For the MCL, the gage length was calculated from the bone insertion site on the femur to the bone insertion site on the tibia.

All samples were pretensioned and preconditioned before testing to failure. Ligaments (MCL) were pretensioned to 0.5 N whereas tendons (PT and AT) were pretensioned to 2 N. All tissue types were preconditioned at 2% strain for 10 cycles. Ligaments and tendons were tensioned to failure at a rate of 0.2 mm/s and 0.4 mm/s, respectively. As the test was being executed, load and actuator displacement data were recorded at 30 Hz. From the load-displacement curve several structural parameters were derived: ultimate load, stiffness, displacement to ultimate load, and energy to ultimate load. Individual stiffness values were calculated by fitting a linear regression equation to the slope of the load-displacement curves between 20% and 60% of the ultimate load. The corresponding material properties were also computed utilizing the cross-sectional area and gage length of the specimens (i.e. energy density = energy to ultimate load/(area × gauge length); stain at ultimate load = displacement at ultimate load/gauge length).

2.3. Histological examination

MCLs and PTs designated for histology were immediately placed in 10% neutral buffered formalin for 48h followed by storage in 70% ethanol. 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. Longitudinal sections were taken in the sagittal plane for the PT and in the coronal plane for the MCL. Both tissues were stained with H&E and viewed with an Olympus BX40 Microscope (Olympus America, Center Valley, PA).

The histology slides were assessed for cellularity and collagen organization. Cellularity assessment was conducted by 3 blinded graders using a 40x objective. Each specimen had two sections made, separated by 100 μm. Five regions per section were evaluated by counting the number of fibroblasts per high powered field.

Table 1
Targeted genes for qPCR of rat patellar tendon (PT). Forward (F) and reverse (R) primers for each gene are displayed.

Name	Primers
IL1-beta	F: CACCTCTCAAGCAGAGCACAG
	R: GGGTTCCATGGTGAAGTCAAC
Col1alpha1	F: GTTCTCGTGGTGCTGCTGGT
	R: CTCTTTCTCCTCTCTGACCGGGAA
IGF-1	F: ATCTCTTCTACCTGGCACTCTGCT
	R: GGGGCTGGGACTTCTGAGTCT
VEGFA	F: GGAAAGGGAAAGGGTCAAAAACGA
	R: TTCTGTCGACGGTGACGATGGT
MMP-13	F: CCCCAAAACACCAGAGAAGTGTGA
	R: CAGCACTGAGCCTTTTCACCTCT
COX-2	F: CGAAGACTACGTGCAACACCTGA
	R: ATGGAGGCCTTTGCCACTGCT
TNFalpha	F: AACCAACTGGTGGTACCAGCAGA
	R: CCAAAGTAGACCTGCCCGGACT
BMP-12	F: GCAAGCCACTGCATGTGGACT
	R: ACCCTCCCCAGACCTCATGCT
IL-6	F: ATGTTGTTGACAGCCACTGCCTT
	R: TCCAGGTAGAAACGGAACTCCAGA
GAPDH	F: CACCACCATGGAGAAGGC
	R: CCATCCACAGTCTTCTGA
18S	F: ACTGCGAATGGCTCATTAAA
	R: CGTCGGCATGTATTAGCTCT
FLAP	F: TCCTGCTCTCTGAAGGTGTC
	R: TACAGAAAAACCACCCCAAA

2.4. RT-PCR

RNA was extracted from patellar tendons using the TRIspin method as previously described (Reno et al., 1997) and reverse transcribed using iScript cDNA kit (Biorad, Hercules, CA). Q-PCR was completed using the MyiQ detection system (Biorad). Gene expression was normalized to 18S levels. Relative expressions of the following genes were determined: Col1α, IGF-1, BMP-12, VEGF, COX-2, IL-1β, FLAP, TNF-α, IL-6, and MMP-13 (Table 1).

2.5. Bone density

The third caudal vertebrae of the each rat's tail was scanned axially with an *in vivo* micro-CT system (eXplore speCZT system, GE Heathcare Inc., Waukesha, WI) at a voxel resolution of 50 μm. The relative bone density was evaluated by measuring the mean gray level of a 1.5 mm diameter by 1.5 mm tall cylindrical region of interest centered in the proximal cancellous bone (1 mm below endplate).

2.6. 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. Results

No significant differences in the biomechanical data occurred between the control group and the low vibration group in any of the examined tissues. The high vibration ultimate load values for