Title: Repeated exposure to high-frequency low-amplitude vibration induces degeneration of murine intervertebral discs and knee joints

Running Title: Whole body vibration induces joint degeneration

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ABSTRACT (250 words)

Objective: High-frequency, low-amplitude whole-body vibration (WBV) is being used to treat a range of musculoskeletal disorders; however, there is surprisingly limited knowledge of its effect(s) on joint tissues. An in vivo mouse model was used to study the effects of repeated exposure to WBV on bone and joint tissues.

Methods: Ten-week-old male mice were subjected to vertical sinusoidal vibration (45 Hz, 0.3 g peak acceleration, 30 min/day, 5 days/week), conditions that mimic those used clinically in humans. Following WBV, skeletal tissues were examined by micro-computed tomography, histology and immunohistochemistry, and gene expression was quantified using real-time PCR.

Results: Following 4 weeks of WBV, intervertebral discs displayed histological hallmarks of degeneration in the annulus fibrosus, disruption of collagen organization and increased cell death. Mice exposed to WBV demonstrated greater Mmp3 expression in the intervertebral disc, accompanied by enhanced collagen and aggrecan degradation. Examination of the knee joints after 4 weeks of WBV revealed meniscal tears and focal damage to the articular cartilage, changes resembling osteoarthritis. Moreover, mice exposed to WBV demonstrated greater Mmp13 gene expression and enhanced MMP-mediated collagen and aggrecan degradation in the articular cartilage compared to controls. No changes in trabecular bone microarchitecture or density were detected in the proximal tibia.

Conclusions: These experiments reveal significant negative effects of WBV on joint tissues in a mouse model, findings that suggest the need for future studies examining the effects of WBV on joint health in humans.
INTRODUCTION

Whole-body vibration (WBV) platforms, designed to deliver low-amplitude, high-frequency mechanical stimulation in the form of sinusoidal vibrations, have emerged as a popular trend in the fitness industry. Within the last decade, the use of WBV as a clinical therapy for musculoskeletal disorders has expanded from its original use as an adjunctive therapy for osteoporosis(1, 2), and is now being used to treat a wide range of conditions including stroke(3), spinal cord injury(4), and multiple sclerosis(5). Furthermore, clinical trials have investigated WBV as an adjunctive or stand-alone therapy for non-specific back pain(6) and osteoarthritis(7). A recent review of WBV as a treatment for back pain raised concerns based on the authors’ view that integration of WBV into clinical practice has not been founded on rigorous research-based evidence of efficacy and safety(8). In fact, the widespread use of WBV in the fitness industry, as well as the marketing of these devices for home gyms, has raised concern within the scientific community(9). WBV was implemented in the clinical setting as a therapy for osteoporosis based on reports of bone-strengthening effects in post-menopausal women(1, 2). Within the last ten years, numerous studies have validated the use of mouse models to investigate the biological effects of WBV and recent studies demonstrated that WBV induces local effects in bone that differ based on the anatomical site (e.g. tibia, femur, vertebrae)(10).

Of concern is the fact that the effects of WBV on joint tissues have not been thoroughly evaluated. Vibration of the intervertebral disc (IVD) poses an intriguing dichotomy. At amplitudes and frequencies associated with operation of heavy machinery, vibration is an established risk factor for back pain(11, 12); however, WBV platforms are being used to treat back pain(13-15). In clinical trials, WBV (30 Hz, 10 min/day) was reported to reduce the incidence of self-reported back pain following prolonged bed rest, however conflicting results
were provided regarding measurable changes in disc morphology(16, 17). In a rat model of hindlimb unloading, WBV (15 min/day; 45 Hz, 0.3 g) did not alter IVD biochemistry or morphology but enhanced muscle volume(18); however, changes in IVD morphology resulting from hindlimb unloading were ameliorated when the frequency of vibration was increased to 90 Hz(19). Interestingly, a recent study reported increased neurotrophins in cervical IVDs following exposure of rats to WBV (15 Hz, 30 min/day)(20).

Similarly, there are few studies that directly assess the effects of WBV on synovial joints. Patient-reported pain in osteoarthritis was shown to decrease following WBV(7, 21); however these studies did not directly assess joint health. One study in humans reported the ability of WBV to prevent loss of cartilage thickness resulting from prolonged immobilization of healthy male subjects(22). The overall lack of research demonstrates the urgent need to investigate the specific effects of WBV on joint tissues, given the increasing use of vibration platforms in both clinical practice and the fitness industry.

Our recent study using mouse models demonstrated anabolic effects of a single exposure to WBV on the IVD(23). Therefore, we sought to determine whether the transient changes previously reported would result in long-term beneficial effects on joint health. The current study was designed to assess the effects of repeated exposure of mice to WBV, using protocols that model vibration training in humans. We examined the effects of WBV on multiple skeletal tissues, including IVD, knee and long bones. Our findings point to deleterious responses of joint tissues to WBV in mice, highlighting the critical need to determine the safety of WBV for joint health in humans.

METHODS
**In Vivo Vibration.** All procedures were approved by the Council on Animal Care at The University of Western Ontario, in accordance with the guidelines of the Canadian Council on Animal Care. Based on parameters of WBV used in clinical protocols, 10-week-old male CD-1 mice were subjected to vertical sinusoidal vibration at a frequency of 45 Hz, peak-to-peak amplitude of 74 µm, and 0.3 g peak acceleration for 30 min/day, 5 days/week for 2 or 4 weeks, using a previously described vibration platform(23). Age-matched controls were housed in identical chambers on a sham (non-vibrated) platform to replicate handling and environmental conditions. Following WBV, mice were returned to conventional housing and monitored daily. At specified time points, mice were euthanized by a lethal dose of sodium pentobarbital.

**Tissue Harvest and RNA Extraction.** Twenty-four hours after the final exposure to WBV, thoracic IVDs (T10-T15) from each mouse were isolated by microdissection. Articular cartilage was isolated from the tibial plateau and femoral condyles (1 knee/mouse). One femur/mouse was isolated and flushed with sterile PBS to remove bone marrow. Tissues were immediately placed in TRIzol® Reagent (Life Technologies) and homogenized using a PRO250 tissue homogenizer (PRO Scientific). RNA was extracted according to the manufacturer’s instructions, quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific) and 0.5 µg was reverse transcribed into cDNA (iScript, Bio-Rad Laboratories).

**Real-Time Quantitative Polymerase Chain Reaction.** Gene expression was assessed by real-time PCR using the BioRad CFX384. PCR reactions were run in triplicate, using 120 ng of cDNA per reaction and 310 nM forward and reverse primers with 2X SsoFast EvaGreen Supermix (Bio-Rad Laboratories). The PCR program was: initial 2 min at 95°C denaturing; 10 s
at 95°C denaturing; and 10 s annealing/elongation for a total of 40 cycles (primer details in Supplemental Table 1). Transcript levels were calculated using ΔΔCt, normalized for input based on hypoxanthine-guanine phosphoribosyltransferase (Hprt)(32), and expressed relative to non-vibrated sham controls at each time point.

**Histology.** Intact lumbar spinal segments (L1-L5) and right knee (with joint capsule intact) were isolated and fixed in 4% paraformaldehyde. Spinal columns were decalcified for 5 days in Shandon’s TBD-2 (Fisher Scientific) and knee joints were decalcified for 10 days in 5% EDTA in PBS (pH 7.0). Tissues were processed and embedded in paraffin. Spines were sectioned sagittally and knees sectioned coronally, at a thickness of 5 µm using a microtome (Leica Microsystems). Using established protocols(23, 24), serial sections were stained with either 0.1% Safranin O/0.02% fast green for sulfated glycosaminoglycans or 1.3% picric acid and 0.1% Direct Red 80 (Sigma) for collagen. Sections were imaged on a Leica DM1000 microscope (equipped with polarizing filter (#11505087) and analyzer (#11555045)), with Leica Application Suite (Leica Microsystems). Safranin O/fast green stained sections were scored based on the modified Thompson grading scheme(25), which is a five-category grading scale originally used to assess the gross morphological changes in sections of cadaveric lumbar spine segments. Each tissue within the IVD was scored independently, based on changes in morphological features. The initial scheme reported intra-observer and inter-observer agreement values greater than 85%, maintained when the scheme was applied across species(26) and has been used previously used by our group to evaluate histological changes in the mouse (27).

**Immunohistochemistry.** Antigen retrieval was performed with 0.1% Triton X-100 in PBS for 20 min at room temperature and tissue sections were blocked with 5% goat serum/5%
bovine serum albumin for 1 h at room temperature. Tissue sections were incubated with rabbit primary antibodies against the products of ADAMTS-mediated aggrecan cleavage (NITEGE), MMP-mediated aggrecan cleavage (DIPEN)(28), or MMP-mediated collagen cleavage (C1,2C)(29) overnight at 4ºC. Goat anti-rabbit secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology) was used in conjunction with DAB+ chromogen (Dako Canada) for colorimetric detection of antibody binding. Secondary antibody only controls were performed in parallel. Sections were counterstained using 0.5% methyl green and imaged on a Leica DM1000 microscope.

**Cell Death Assay.** Spinal sections were assessed for *in situ* cell death using the terminal nucleotidyl transferase–mediated nick end labeling (TUNEL) staining kit (Roche), according to the manufacturer’s instructions. Sections were mounted using VECTASHIELD Medium with DAPI (Vector Labs) and imaged with a Leica DMI6000B inverted microscope with Leica Application Suite. Images were scored by two independent blinded observers, and TUNEL-positive cells were counted within each IVD compartment (nucleus pulposus (NP) and annulus fibrosus (AF)) and expressed as a percentage of the total cells. Pretreatment with DNase I (Roche) and omission of labeling solution served as positive and negative controls, respectively.

**Micro-computed Tomography (micro-CT).** Right hindlimbs (mid-femur to ankle, with knee joint capsule intact) were isolated and fixed in 4% paraformaldehyde. To avoid motion during scanning, specimens were fixed in 50 mL tubes containing 1% agarose in PBS. Individual tubes were mounted onto the bed of a GE eXplore Locus micro-CT scanner (GE Healthcare Biosciences) with a calibrating phantom composed of air, water and synthetic bone-mimicking
epoxy having a bone mineral equivalent of 1.1 g/cm$^3$ (SB3, Gammex RMI). The scanning protocol included 900 projection images at a 0.4 degree angle increment, obtained over a 165 min gantry rotation. The X-ray tube potential was 80 kVp with 450 µA tube current, 4500 ms exposure and 2 frames per view angle averaged. Images were reconstructed into 3D volumes with 20 µm isotropic voxel size and linearly rescaled into Hounsfield Units (HU) using internal calibration standards.

Using MicroView (GE Healthcare Biosciences), full volumes were cropped to contain only the tibia and fibula, and reoriented to the same axes. To determine trabecular bone morphometry, a standardized region of interest (ROI) was selected in the proximal tibia using the largest dimensions possible to capture trabecular bone and to exclude cortical bone in all scans. The ROI was an elliptic cylinder (41 pixels (x-axis) × 42 (y-axis) × 48 (z-axis) positioned 50 µm distal to the growth plate, to measure the secondary spongiosa. Bone morphometry was quantified using the Bone Analysis tool in MicroView, as previously reported(30).

**Statistical Analyses.** Data are presented from experiments conducted in 2 independent trials, each with 4-6 mice per treatment group. Parameters from mice exposed to WBV were compared to those from non-vibrated sham controls at the same time point using a parametric, two-tailed, unpaired $t$-test with a Welch’s correction. $P < 0.05$ was considered significant.

**RESULTS**

To examine the effects of repeated exposure to WBV on joint tissues, wild-type mice (10 weeks of age) were exposed to vertical sinusoidal WBV for 2 or 4 weeks. Conditions were based on currently used clinical and exercise protocols (45 Hz, 0.3 g peak acceleration, 30 min/day, 5
days/week). Skeletal tissues, including a synovial joint (knee), cartilaginous joints (IVDs) and bone (tibia and femur) were assessed and compared to those of age-matched control mice.

We first assessed lumbar IVD health. No discernible differences were detected in the IVD following 2 weeks of WBV. However, in mice exposed to WBV for 4 weeks, Safranin-O/fast-green staining demonstrated i) loss of a distinct nucleus pulposus (NP) and annulus fibrosus (AF) boundary, ii) the accumulation of mucinous material leading to enhanced glycosaminoglycan staining in the interlamellar matrix of the AF, and iii) focal disruptions in the AF lamellae, when compared to sham controls (Figure 1A). Evaluation of the histological appearance using the modified Thompson scoring system(25) indicated significant degenerative changes in the AF of mice exposed to WBV compared to sham controls (Figure 1B). Collagen organization within the AF was examined using picrosirius-red staining and bright-field microscopy; no discernible differences were observed between mice exposed to 4 weeks of WBV and sham controls, in either the NP or the AF (Figure 1C). However, examination of sections by polarized light microscopy revealed disrupted collagen organization with breaks within the collagen lamellae in the inner AF, and widening of the interlamellar spaces in the AF of mice exposed to WBV, changes not observed in sham controls (Figure 1D, arrows). Interestingly, similar degenerative changes induced by WBV in lumbar IVDs were also detected in the caudal spine (Supplemental Figure 1).

To investigate the cellular response to repeated exposure to WBV, we performed real-time PCR on whole-disc RNA (Figure 1E). IVD tissues from mice exposed to WBV demonstrated a significant increase in the expression of Sox9 (1.4-fold increase at 2 weeks, 1.6-fold increase at 4 weeks). This was accompanied by increased expression of Acan (1.5-fold increase at 2 weeks, 1.8-fold increase at 4 weeks) and a 2.0-fold increase in Col1a1 expression.
after 2 weeks of WBV. In contrast, no significant change in Col2a1 expression was detected at either time point following WBV.

We further investigated WBV-induced disc degeneration by examining the accumulation of matrix degradation fragments. A 4-week exposure of mice to WBV appeared to increase MMP-mediated aggrecan cleavage in the outer AF, as immunohistochemical staining for the DIPEN cleavage product appeared more intense following WBV compared to sham controls (Figure 2A, arrows). In contrast, no changes in aggrecanase activity were detected following exposure of mice to WBV, as detected by the intensity of NITEGE staining (Figure 2B). We next labeled C1,2C neoepitopes, which correspond to type I and/or type II collagen cleavage by MMPs(29). Similar to aggrecan, we detected greater intensity of MMP-mediated collagen cleavage products in the outer AF of mice exposed to WBV compared to sham controls (Figure 2 C, arrows). No differences in the intensity of staining were detected in either the inner AF or NP between mice exposed to WBV and sham controls. Increased matrix cleavage in mice exposed to WBV was accompanied by a significant increase in the expression of Mmp3 (1.5-fold induction at 2 weeks, 2.1-fold induction at 4 weeks over sham controls) (Figure 2D). In contrast, exposure of mice to WBV did not alter the expression of Mmp-13, Adamts-4 or Adamts-5 in IVD tissues compared to non-vibrated sham controls.

To determine if WBV altered cell viability in the IVD, TUNEL staining was performed on lumbar IVDs following 4 weeks of WBV. Mice exposed to WBV demonstrated a greater percentage of TUNEL-positive cells when compared to sham controls (Figure 3A) in the NP and, to a greater extent, in the AF (Figure 3B).

We next sought to determine whether the deleterious changes detected within the IVD also occurred within a synovial joint, such as the knee. Histological examination revealed tears in
the medial meniscus of mice exposed to 2 weeks of WBV (3 of 4 mice; Figure 4A ii & iii, arrows), changes that were not detected in the sham controls. No alterations in the articular cartilage were detected following 2-week exposure to WBV. However, signs of early osteophyte formation were observed on the medial tibial plateau of one mouse following 2-week exposure to daily WBV (Figure 4Aiii, asterisk), the animal demonstrating the most severe meniscal damage at this time point.

Assessment of knee joint tissues after 4 weeks of WBV revealed meniscal tears in 4 out of the 5 mice exposed to WBV (Figure 4A v & vi, arrows), changes not detected in the sham controls. The extent of meniscal damage was greater following 4 weeks of WBV than that detected after 2 weeks, and was likewise accompanied by osteophyte formation in one mouse (Figure 4Avi, asterisk). Extensive articular cartilage damage was observed in 2 of the 5 mice exposed to 4 weeks of WBV, with loss of the superficial zone cartilage extending to the calcified cartilage zone of both the tibial and femoral heads (Figure 4Av, arrowheads). Notably, focal damage to the articular surface was limited to the medial joint compartment; no defects were detected in the articular cartilage of the lateral joint compartment (Supplemental Figure 2). No apparent changes in the organization of collagen fibres in either the meniscus or articular cartilage were detected in the medial joint following WBV (Supplemental Figure 3).

To investigate the cellular response of articular chondrocytes to WBV, real-time gene expression analysis was conducted following 2 or 4 weeks of WBV (Figure 4B). While no significant changes were detected following 2 weeks of WBV, significant induction of both Sox9 and Col2a1 gene expression was detected in articular chondrocytes following exposure to 4 weeks of WBV (2.1-fold and 1.9-fold increase, respectively). No significant change in the expression of Acan was detected following WBV.
To assess articular cartilage breakdown, we performed neoepitope staining to detect matrix breakdown fragments within the joint compartment. In contrast to non-vibrated sham controls, diffuse extracellular DIPEN staining was detected in the superficial articular cartilage fibrillations on the tibial plateau as well as in the meniscus (Figure 5A, arrows). DIPEN staining associated with the chondrocytes of the calcified cartilage appeared more intense in mice exposed to 4 weeks of WBV, compared to sham controls (Figure 5A, arrowheads). Due to the loss of the superficial articular cartilage in the medial joint compartment following 4 weeks of WBV, we examined the lateral joint compartments for matrix degradation. Pericellular DIPEN staining was detected more uniformly throughout the meniscus in mice exposed to WBV than in sham controls (Supplemental Figure 4). Furthermore, pericellular DIPEN staining appeared more intense within the superficial zone of the tibial and femoral articular cartilage in mice following 4 weeks of WBV compared to sham controls (Supplemental Figure 4A, arrows). In contrast, no overt change in NITEGE staining was detected in the articular cartilage of either the medial or lateral knee joint compartments (Figure 5B, Supplemental Figure 4B). Pericellular NITEGE staining appeared more intense in the meniscus within the lateral joint compartment of mice exposed to WBV than in sham controls (Supplemental Figure 4B).

The presence of collagen breakdown fragments was likewise assessed following 4 weeks of WBV. Greater diffuse C1,2C neoepitope staining was detected in the fibrillated articular cartilage of the tibial plateau as well as the meniscus of mice exposed to WBV, compared to sham controls (Figure 5C). The lateral joint compartments of the same mice demonstrated a similar increase in C1,2C staining in the ECM of the meniscus, as well as pericellular staining throughout the superficial zone of the articular cartilage (Supplemental Figure 4C). Real-time gene expression analysis of articular cartilage RNA demonstrated no significant changes in...
$Mmp3$ expression; however, exposure of mice to 4 weeks of WBV induced a significant increase in $Mmp13$ expression (1.5-fold increase over sham controls) (Figure 5D). Similar to the IVD, no change was detected in expression of $Adamts-4$ or $Adamts-5$ in articular cartilage following exposure of mice to WBV.

We employed high-resolution micro-CT to determine whether exposure of mice to 4 weeks of WBV altered bone microarchitecture. Volumetric analysis was conducted within a region of interest localized to the secondary spongiosa of the tibia (Figure 6A & B). No significant differences in bone mineral density (BMD), bone mineral content (BMC), bone volume fraction (BVF), trabecular thickness (Tb.Th), trabecular number (Tb.N) or trabecular separation (Tb.Sp) were observed between mice following 4 weeks of WBV and sham controls (Figure 6C). To investigate cellular responses within bone to WBV, we performed real-time PCR on RNA isolated from the femur. Following 2 weeks of WBV, significantly greater expression of $Dmp1$ (2.5 fold) and $Ibsp$ (2.0 fold) were detected compared to sham controls (Figure 6D). Similarly, exposure of mice to WBV increased the expression of $Wnt10b$ (1.9 fold over sham controls), which encodes a secreted signaling molecule implicated as an effector of bone mechanotransduction(31). No significant change was detected in expression of the osteoclast receptor $Rank$ following exposure of mice to WBV at either time point.

**DISCUSSION**

WBV has recently been incorporated as an adjuvant or stand-alone treatment for a wide range of diseases, ranging from stroke to chronic-obstructive pulmonary disease. Notably, WBV is used to treat musculoskeletal disorders including osteoporosis(2), back pain(13), and osteoarthritis(7). However, recent investigations report conflicting results regarding the ability of
WBV to alter bone mineral density or trabecular bone structure, both in mouse models and clinical studies(32, 33). Further confounding their interpretation, many clinical studies of WBV are based on patient-reported pain, and not quantitative assessment of tissue health(7, 13). Surprisingly, to our knowledge, no studies have examined the cellular changes in soft joint tissues following exposure to WBV, in either animal models or humans. As such, the current study was designed to simultaneously examine the effects of repeated exposure to WBV on musculoskeletal tissues including the IVD and knee joints, using an in vivo mouse model.

The parameters of WBV used in the current study were chosen to introduce specific values of peak acceleration and frequency. The combination of acceleration and frequency uniquely determines the amplitude of the platform displacement. These parameters were chosen to be comparable to those used in human studies in order to expose individual cells – the functional units responding to vibration – to similar mechanical stimuli. Importantly, the parameters of WBV used in the present study (45 Hz, peak-to-peak amplitude of 74 µm and 0.3 g peak acceleration) were selected based on previous studies which reported beneficial effects of WBV on muscle and bone(34). It should be noted that there is currently no consensus as to the relative importance of specific parameters of WBV (e.g., amplitude, frequency, acceleration) with regards to their influence on bone adaptation, as evidenced by recent reports of apparently contradictory results(35). Interestingly, previous clinical studies which reported beneficial effects of WBV on hip bone mineral density(36) used similar frequencies, but acceleration values an order of magnitude greater than was experienced by mice in the current study. Thus, it is possible that our findings may underestimate the potential for joint damage in humans if exposed to even greater accelerations during WBV therapy.
Our previous work used ex vivo and in vitro mouse models to examine the acute response of IVD tissues to WBV. These studies established that a single exposure to WBV resulted in a transient decrease in the expression of catabolic genes (Adamts4, Adamts5, Mmp3) and increase in the expression of anabolic genes (Acan, Bgn, Dcn, Coll1a1, Col2a1), with significant increases in matrix proteins detected in the IVD 6 hours post-vibration(23). Therefore, we sought to establish if repeated exposure to WBV would result in beneficial effects on the IVD and cartilaginous tissues of the knee joint. To our surprise, the current study demonstrates that 4-week exposure of mice to WBV leads to degeneration of the IVD, with changes detected predominantly in the AF. A recent study demonstrated that exposure of excised annular lamellae to vibration resulted in alterations in the deformation magnitude of the tissue, which the authors postulate was caused by damage to the intra-lamellar matrix(37). Our findings are in keeping with the characterized effects of dynamic compressive loading on the IVD. Although structural changes were reported to occur predominantly in the NP, in vivo dynamic compression was shown to increase aggrecan expression and apoptosis in the inner AF, changes associated with the progression of degeneration(38, 39). Interestingly, these studies reported differential effects of dynamic compression on specific tissue compartments in the IVD, which varied with the stress and frequency of loading(38). Further studies are required to determine if the response of NP and AF cells to WBV may also be modulated by the parameters of the vibrational stimulus.

In the present study, changes in the histological appearance of the IVD induced by WBV were accompanied by increased MMP activity, as evidenced by the accumulation of matrix degradation fragments and increased expression of Mmp3. Interestingly, single nucleotide polymorphism(s) of the MMP3 locus which result in increased MMP3 expression are associated with degenerative lumbar spinal stenosis(40), correlate with the radiographic progression of
lumbar disc degeneration(40), and confer an increased risk for disc degeneration following occupational exposure to vibration(41). Our previous work demonstrated no change in 
*Adams*4/5 expression yet a transient decrease in *Mmp3* expression in the IVD following acute exposure to vibration(23), in keeping with data demonstrating a similar decrease in *MMP3* expression by AF cells following acute exposure to cyclic tensile strain(42). Taken together, these findings suggest that MMPs are regulated by mechanosensitive pathways in IVD tissues, dependent upon parameters of mechanical stimulation such as the cumulative exposure to the applied load.

In the knee, we detected meniscal damage and focal articular cartilage erosion in the medial joint compartment after 4 weeks of WBV, damage that resembles osteoarthritis. These results were surprising given the young age of the mice, since spontaneous osteoarthritis has been reported to present between 17-20 months of age in mice(43). We noted increased MMP-mediated extracellular matrix degradation within the joint tissues following 4 weeks of WBV, accompanied by increased *Mmp13* expression. We also noted increased *Col2a1* expression in the articular cartilage after 4 weeks of WBV, a change that has been associated with the osteoarthritis phenotype in *vivo*(44). Although *SOX9* expression is typically downregulated in cartilage during late stage osteoarthritis(44), mechanical loading has been shown to induce both *SOX9* and type II collagen in cells of the meniscus(45). These increases in *Sox9* and *Col2a1* expression may represent cellular attempts to maintain cartilage integrity that are ultimately unsuccessful because of the overwhelming induction of catabolic factors.

As with the IVD, there are surprisingly few studies aimed at assessing the effect of WBV on synovial joints such as the knee. Recent studies in rodent models examined the effects of WBV on articular cartilage in a model of osteoporosis induced by prednisolone(46) and a
surgical model of osteoarthritis(47); however, in both studies, cartilage was not examined in animals subjected to WBV alone. Our findings are consistent with the later study, which reported increased cartilage degradation and functional deterioration of osteoarthritis-affected joints following WBV(47). Although the extensive damage to the knee joint caused by WBV in our mouse model was unexpected, several mechanisms may underlie the observed changes. First, we noted that WBV-induced meniscus damage preceded damage to the articular surface, suggesting that destabilization of the joint may contribute to cartilage degeneration. While mechanisms responsible for initiation of meniscal damage are yet unknown, removal or damage to the meniscus is known to increase focal point stress on the articular cartilage surface of the tibial plateau(48), which mirrors the focal damage induced by WBV in our study. In fact, surgical destabilization of the medical meniscus is routinely used to induce an osteoarthritis-like pathology in animal models(49). Alternatively, articular cartilage damage may result from changes to the subchondral bone resulting from repeated exposure to WBV. Increased mechanical load on the articular surface has been shown to increase subchondral bone thickness(50), thereby altering joint biomechanics leading to increased loading of the articular surface(51) and altering cartilage biology by regulating the activity and availability of growth factors and cytokines within the tissue, promoting osteoarthritis progression(52). The cellular pathways responsible for the initiation of tissue degradation in response to WBV in the meniscus and articular cartilage require further examination.

Although the current study reveals damaging effects of WBV on joint tissues, it is important to acknowledge that our studies only examined wild-type CD-1 male mice, which were 10 weeks of age at the initiation of the vibration protocol. Although the age and gender of mice used in the current study do not mirror the wide range of human participants that currently
use WBV platforms, our study points to potentially negative effects of WBV that warrant further examination. The use of male mice may also have influenced the response of bone to WBV in the current study, specifically the lack of changes in bone microarchitecture in the secondary spongiosa. A previous study reported that 12-week-old female rats exposed to WBV showed no significant changes in trabecular spacing, number and thickness compared to non-vibrated controls; whereas, ovariectomized female rats exposed to the same parameters of WBV showed a significant increase in bone mass\(^{(53)}\). These findings suggest that systemic factors, such as circulating hormone levels, may modulate the response of bone WBV. Furthermore, the current study used young mice in which degenerative changes are typically not detected in the IVD or knee joint tissues. Given that WBV is being used clinically to treat musculoskeletal disorders, follow up studies should explore the response of diseased joint tissues to WBV to better model its clinical use in humans. An additional limitation associated with the use of young mice is inherent differences between the composition of the IVD in humans and mice. Human IVD tissues show a loss of notochord cells within the first decade of life\(^{(54)}\) and the NP is instead populated by smaller cartilage-like cells; whereas, mice maintain notochord cells in the NP into adulthood\(^{(55)}\). Interestingly, rabbit notochord cells have been reported to be less resistant to mechanical stress than NP cells\(^{(56)}\), suggesting that the cellular composition of the NP may be an important regulator of the response of the tissue to mechanical stimuli such as WBV.

Taken together, the current study demonstrates that repeated exposure to WBV leads to degeneration of both the IVD and knee joints in a mouse model. These findings raise concern due to the clinical use of these platforms for the treatment of various conditions, as well as the use of vibration platforms by the general population in daily exercise regimens. Further studies...
are required to determine if WBV is safe and effective for the treatment of human conditions and to specifically address potential negative effects on joint health.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Drs. McCann and Séguin had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.


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REFERENCES


FIGURE LEGENDS

Figure 1. Repeated exposure to whole-body vibration negatively affects the lumbar intervertebral disc. A. Representative histological sections of the lumbar spine stained with Safranin O/fast green from mice exposed to WBV (30 min/day; 45 Hz, 0.3 g; 4 weeks vibration) and non-vibrated sham controls. Images are oriented with rostral on top and dorsal on the right. B. Evaluation of the morphologic grade of tissue degeneration (using the modified Thompson score) demonstrates a significant increase in tissue degeneration in the AF of mice subjected to WBV. * = P < 0.05; n= 3 IVDs/animal; 5 animals/group (n=15). C. Picrosirius red stain imaged by polarized light (presented in D.) revealed disrupted collagen organization (arrows) and widening of the interlamellar spaces between lamella in the AF. E. SYBR-based real-time PCR analysis of IVD gene expression. Expression of anabolic genes was determined relative to the expression of the housekeeping gene Hprt and normalized to non-vibrated (sham) controls at each time point. Data are presented as the mean ± SEM, from 5 pooled IVDs per mouse, 4-5 mice/group, run in technical triplicate; * = P < 0.05, ** = P < 0.01.

Figure 2: Matrix degradation in mouse intervertebral discs following whole-body vibration. A-C. Representative sagittal sections of IVDs from mice subjected to 4 weeks of WBV or non-vibrated sham controls, stained for DIPEN (MMP-cleaved aggrecan neoepitope), NITEGE (ADAMTS 4/5-cleaved aggrecan neoepitope) and C1,2C (MMP-cleaved collagen neoepitope) and counterstained with methyl green. D. SYBR-based real-time PCR analysis of IVD gene expression. Expression of catabolic genes was determined relative to the expression of the housekeeping gene Hprt and normalized to non-vibrated (sham) controls at each time point.
Data are presented as the mean ± SEM, from 5 pooled IVDs per mouse, 4-5 mice/group, run in technical triplicate; * = P < 0.05.

**Figure 3.** TUNEL staining of intervertebral discs after repeated exposure to whole-body vibration.  
A. Representative sections of lumbar IVDs stained with TUNEL to detect DNA fragmentation and counterstained with DAPI.  
B. Percentage of TUNEL-positive cells was determined for specific regions (NP, AF) within the IVD. Vibration induced a significant increase in cell death within IVD when compared to non-vibrated sham controls, specifically within the NP and AF. Data are presented as the mean ± SEM, based on 3 IVDs per mouse, 4-5 mice/group (n=15). * = P < 0.05, *** = P < 0.001. NP = Nucleus Pulposus, AF = Annulus Fibrosus.

**Figure 4.** Histological sections of mouse medial knee joint after exposure to whole-body vibration.  
A. Coronal sections of knee joints from 1 control and 2 mice exposed to WBV at each time point stained with Safranin O/fast green to detect sulfated glycosaminoglycans. Images are oriented with medial meniscus on the left, femoral condyle on top and tibial plateau beneath. Arrows indicate microfissures detected within the medial meniscus in 3 of 4 mice at 2-weeks (ii,iii) and 4 of 5 mice at 4 weeks (v,vi) subjected to WBV. No meniscal damage was detected in non-vibrated sham controls (i,iv). Arrowheads indicate severe degeneration of the articular cartilage on the medial quadrants of both the tibia and the femur (vi). Focal defects in the articular cartilage were detected in 2 of the 5 mice subjected to WBV as seen in (iv). No articular cartilage degeneration was detected in the non-vibrated sham controls. Asterisks depict...
osteoophytes. **B.** SYBR-based real-time PCR analysis of articular cartilage gene expression. Expression of anabolic genes was determined relative to the expression of the housekeeping gene Hprt and normalized to non-vibrated (sham) controls at each time point. Data are presented as the mean ± SEM, 4-5 animals/group, run in technical triplicate; * = \( P < 0.05 \), ** = \( P < 0.01 \).

**Figure 5.** Matrix degradation in mouse medial knee joint compartment following whole-body vibration. **A.** Representative sections of medial knee joint from mice subjected to 4 weeks of WBV or sham controls stained by immunohistochemistry for DIPEN (MMP-cleaved aggrecan neoepitope), NITEGE (ADAMTS-cleaved aggrecan neoepitope) or C1,2C (MMP-cleaved collagen neoepitope) and counterstained with methyl green. **B.** SYBR-based real-time PCR analysis of articular cartilage gene expression. Expression of catabolic genes was determined relative to the expression of the housekeeping gene Hprt and normalized to non-vibrated (sham) controls at each time point. Data are presented as the mean ± SEM, 4-5 animals/group, run in technical triplicate; * = \( P < 0.05 \).

**Figure 6.** Analysis of bone microarchitecture and gene expression following whole-body vibration. **A.** Hind limbs from mice following 4 weeks of WBV (VIB) and non-vibrated controls (SHAM) were isolated post-mortem and prepared for micro-CT scanning with 20 \( \mu \)m isotropic voxel size. Representative coronal plane micro-CT images of the proximal tibia are depicted. A standardized region of interest (ROI – indicated in yellow) was selected in the proximal tibia to assess trabecular bone and exclude cortical bone. The ROI was an elliptic cylinder (41 pixels (x-axis) \( \times \) 42 (y-axis) \( \times \) 48 (z-axis)) positioned 50 \( \mu \)m distal to the growth plate to measure the
secondary spongiosa. B. Isosurface image of ROI indicated in A. from SHAM and VIB specimens. C. Histograms illustrate bone mineral density (BMD), bone mineral content (BMC), bone volume fraction (BVF, ratio), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular spacing (Tb.Sp). No significant differences were observed in bone density, mineral content or microarchitectural parameters. Data are presented as the mean ± SEM, 4-6 animals/group. Differences between sham and vibration groups were assessed using unpaired two-tailed t-tests. D. SYBR-based real-time PCR analysis of femur gene expression. Gene expression was determined relative to levels of the housekeeping gene Hprt and normalized to non-vibrated (sham) controls at each time point. Data are presented as the mean ± SEM, from 6 animals/group, run in technical triplicate; * = P < 0.05.
Figure 1. Repeated exposure to whole-body vibration negatively affects the lumbar intervertebral disc. A. Representative histological sections of the lumbar spine stained with Safranin O/fast green from mice exposed to WBV (30 min/day; 45 Hz, 0.3 g; 4 weeks vibration) and non-vibrated sham controls. Images are oriented with rostral on top and dorsal on the right. B. Evaluation of the morphologic grade of tissue degeneration (using the Thompson score) demonstrates a significant increase in tissue degeneration in the annulus fibrosus of mice subjected to WBV. * = P < 0.05; n = 3 IVDs/animal; 5 animals/group (n=15). C. Picosirius red stain for collagen organization imaged by polarized light (presented in D.) revealed disrupted collagen organization (arrows) and widening of the interlamellar spaces between lamella in the AF. E. SYBR-based real-time PCR analysis of intervertebral disc gene expression. Expression of catabolic genes was determined relative to the expression of the housekeeping gene Hprt and normalized to non-vibrated (sham) controls at each time point. Data are presented as the mean ± SEM, from 5 pooled IVDs per mouse, 4-5 mice/group, run in technical triplicate; * = P < 0.05, ** = P < 0.01.
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