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Whole-body vibration of mice induces progressive degeneration of intervertebral discs associated with increased expression of *Il-1β* and multiple matrix degrading enzymes

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SUMMARY

Objective: Whole-body vibration (WBV) is a popular fitness trend based on claims of increased muscle mass, weight loss and reduced joint pain. Following its original implementation as a treatment to increase bone mass in patients with osteoporosis, WBV has been incorporated into clinical practice for musculoskeletal disorders, including back pain. However, our recent studies revealed damaging effects of WBV on joint health in a murine model. In this report, we examined potential mechanisms underlying disc degeneration following exposure of mice to WBV.

Methods: Ten-week-old male mice were exposed to WBV (45 Hz, 0.3 *g* peak acceleration, 30 min/day, 5 days/week) for 4 weeks, 8 weeks, or 4 weeks WBV followed by 4 weeks recovery. Micro-computed tomography (micro-CT), histological, and gene expression analyses were used to assess the effects of WBV on spinal tissues.

Results: Exposure of mice to 4 or 8 weeks of WBV did not alter total body composition or induce significant changes in vertebral bone density. On the other hand, WBV-induced intervertebral disc (IVD) degeneration, associated with decreased disc height and degenerative changes in the annulus fibrosus (AF) that did not recover within 4 weeks after cessation of WBV. Gene expression analysis showed that WBV for 8 weeks induced expression of *Mmp3*, *Mmp13*, and *Adamts5* in IVD tissues, changes preceded by increased expression of *Il-1β*.

Conclusions: Progressive IVD degeneration induced by WBV was associated with increased expression of *Il-1β* within the IVD that preceded *Mmp* and *Adamts* gene induction. Moreover, WBV-induced IVD degeneration is not reversed following cessation of vibration.

Introduction

The effects of vibration on spine health have been best studied in the context of occupational exposure to high-amplitude, low-frequency vibration which is an established risk factor for back pain[1] and disc degeneration[2]. In fact, an increased prevalence of low back pain has been reported in workers exposed to vibration[3]. Therefore, the recent adoption of low-amplitude, high-frequency whole-body vibration (WBV) as a therapeutic treatment for back pain[4] establishes an intriguing dichotomy. WBV therapy was originally reported to stimulate bone growth and subsequently incorporated as an adjunctive therapy for osteoporosis[5,6]. However, recent studies suggest that the effects of WBV on bone appear to vary depending on age, genetics, anatomical location, and the parameters of the applied

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load[7,8]. Moreover, assessment of transmissibility from commercially available platforms reported that some devices markedly exceed ISO guidelines for safety, delivering vibration levels up to seven times higher than what is considered a safe threshold for a single minute of daily exposure[9]. Nevertheless, the clinical use of WVB platforms continues to expand, targeting bone mass across a wide demographic ranging from children to the elderly[8,10], as well as numerous musculoskeletal disorders including back pain[11].

The tissues that form the intervertebral disc (IVD) – the cartilage end plates (CEP), annulus fibrosus (AF) and nucleus pulposus (NP) – function in concert to absorb mechanical loading and permit flexion, extension, lateral bending, and axial rotation of the spine. As with most skeletal tissues, mechanical loading contributes to IVD homeostasis. In animal models, physiological levels of dynamic compression promote extracellular matrix synthesis[12], whereas immobilization or over-loading promote matrix catabolism and degeneration[13]. Whereas numerous studies have characterized the biological responses of IVD tissues to compressive and tensile loading[14], there is comparatively little known as to the effects of vibration on IVD biology. Using a combination of *in vivo* and *ex vivo* models, our group demonstrated that IVD tissues respond directly to high-frequency low-amplitude vibration and that the cellular response varies according to the parameters of the applied load[15,16].

Clinical trials evaluating the utility of WBV for back pain reported improvements in patient self-reported pain and pain-related disability following 12 weeks of WBV[17,18]. However, these studies did not assess whether improvements were associated with increased muscle strength or alterations to IVD health. Studies assessing WBV to prevent IVD degeneration following prolonged bed rest reported a reduction in back pain with brief daily exposure to WBV; however, conflicting results were reported in WBV-induced changes in disc morphology[19,20]. Similarly, there have been limited studies using *in vivo* models to examine the effects of WBV on the spine. In a rat model of hind-limb unloading, WBV (15 min/day; 45 Hz, 0.3 *g*) did not alter IVD biochemistry, height, or volume, but enhanced muscle volume[21]. The same group subsequently reported that, at a frequency of 90 Hz, WBV countered changes in IVD morphology resulting from hind-limb unloading[22]. Importantly, a recent systematic review on the use of WBV to treat back pain concluded that there was no evidence for optimal treatment parameters, nor evidence for the long-term effectiveness of WBV[11]. These studies highlight the need to examine the relative contribution of WBV parameters on clinical outcome (including frequency, amplitude, duration, patient gender, age and physical condition), and a need for direct assessment of the effects of WBV on weight-bearing joint tissues.

Our initial study investigated the response of IVD tissues to a single acute exposure to WBV, and reported anabolic effects including increased gene expression and accumulation of proteoglycans and collagen within the ECM[15]. Using protocols that model vibration training in humans, our subsequent study demonstrated that 4 weeks of WBV (30 min/day, 5 days/week) induced significant damage to both the IVD and knee joint[16]. To better understand the effects of WBV on spinal tissues, the current study assessed: (1) if the detrimental effects of WBV on IVD tissues would progress with extended exposure to WBV; (2) if changes associated with IVD degeneration following WBV were reversible; and (3) if protocols of WBV associated with IVD degeneration altered vertebral bone mass. Our findings demonstrate progressive WBV-induced IVD degeneration associated with increased *Il-1β*, *Mmp3*, *Mmp13* and *Adamts5* gene expression. Moreover, we show that inclusion of a recovery period failed to potentiate the degenerative changes induced by WBV in the IVD. In this model, WBV did not alter vertebral trabecular bone microarchitecture.

Methods

WBV

Based on parameters of WBV used clinically[8] and our previous studies[16], 10-week-old male CD-1 mice were randomized and 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 weeks, 4 weeks, 8 weeks or 4 weeks WBV followed by 4 weeks recovery ($n = 5$–6 mice/group), using a previously described vibration platform. 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. Hind limbs were isolated to characterize effects of WBV on the knee joint, detailed in[23]. 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 and the ARRIVE guidelines[24].

Micro-computed tomography (micro-CT)

Twenty-four hours after the final exposure to WBV, mice were euthanized by a lethal dose of sodium pentobarbital, immediately scanned using a laboratory micro-CT scanner (eXplore Locus Ultra, GE Healthcare Biosciences) and analyzed as described previously[25,26]. Briefly, whole-body scans were acquired using 1000 projection images obtained over a single 16 s rotation (80 kVp, 55 mA tube current, 16 ms exposure). A calibrating phantom composed of air, water and cortical bone-mimicking epoxy (SB3; Gammex, Middleton WI, USA) was scanned together with each animal. Data were reconstructed into 3D volumes with an isotropic voxel spacing of 154 μm and scaled into Hounsfield units (HU). Using MicroView software (GE Healthcare Biosciences), three signal-intensity thresholds (−200, −30 and 190 HU) were used to classify each voxel as adipose, lean or skeletal tissue, respectively. Custom software was used to calculate tissue masses from assumed densities of 0.95 (adipose), 1.05 (lean) and 1.92 (skeletal) g/cm^3. Volumetric measures were averaged over voxel volume.

For high-resolution quantification of bone morphometry, intact lumbar spines (L1–S1) were isolated with surrounding muscular and ligaments intact, fixed in 4% paraformaldehyde and embedded in 1% agarose in PBS in 50 mL conical tubes. Spines were scanned using a laboratory micro-CT scanner (eXplore Locus, GE Healthcare Biosciences) with a calibrating phantom composed of air, water, and synthetic bone-mimicking epoxy (SB3; 2.8 × 3.4 × 8 mm) using established protocols[27]. Images were reconstructed into 3D volumes with 20 μm isotropic voxel size and linearly rescaled into HUs using the internal calibration standard. Using MicroView, full volumes were cropped to contain only the lumbar spine, and reoriented to the same axes. To determine trabecular bone morphometry, a region of interest (ROI) was defined within the L6 vertebrae to capture trabecular bone and to exclude cortical bone; ROI were manually outlined within a series of 2D planes and splined together to generate 3D ROIs. Bone morphometry was quantified using the Bone Analysis tool in MicroView, as previously reported[28].

Disc height index (DHI)

The IVD and vertebral bone heights were measured between L5 and L6 at the mid-sagittal plane in MicroView [Fig. 5(A)] and the DHI was calculated using the following equation[29].