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C57BL/6 mice are resistant to joint degeneration induced by whole-body vibration

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SUMMARY

Objective: Whole-body vibration (WBV) platforms are commercially available devices that are used clinically to treat numerous musculoskeletal conditions based on their reported ability to increase bone mineral density and muscle strength. Despite widespread use, there is an alarming lack of understanding of the direct effects of WBV on joint health. Previous work by our lab demonstrated that repeated exposure to WBV using protocols that model those used clinically, induces intervertebral disc (IVD) degeneration and osteoarthritis-like damage in the knee of skeletally mature, male mice of a single outbred strain (CD-1). The present study examined whether exposure to WBV induces similar deleterious effects in a genetically different strain of mouse (C57BL/6).

Design: Male 10-week-old C57BL/6 mice were exposed to vertical sinusoidal WBV for 30 min/day, 5 days/week, for 4 or 8 weeks using previously reported protocols (45 Hz, 0.3 *g* peak acceleration). Following WBV, joint tissues were examined using histological analysis and gene expression was quantified using real-time PCR (qPCR).

Results: Our analyses show a lack of WBV-induced degeneration in either the knee or IVDs of C57BL/6 mice exposed to WBV for 4 or 8 weeks, in direct contrast to the WBV-induced damage previously reported by our lab in CD-1 mice.

Conclusions: Together with previous studies from our group, the present study demonstrates that the effects of WBV on joint tissues vary in a strain-specific manner. These findings highlight the need to examine genetic or physiological differences that may underlie susceptibility to the deleterious effects of WBV on joint tissues.

Introduction

Platforms that deliver whole-body vibration (WBV) are currently used in the clinical setting based on their reported ability to increase bone density[1] and muscle strength[2]. Within the last decade, WBV has been integrated into physical therapy regimens for a variety of musculoskeletal disorders, including osteoporosis[1], back pain[3], and osteoarthritis[4]. In addition to their clinical use, WBV platforms are promoted in the health and wellness industries as "no-work workouts" equivalent to traditional resistance training. The proposed use of WBV as a treatment for musculoskeletal conditions appears contradictory to epidemiological studies establishing an association between workplace exposure to WBV and the development of several conditions, including back pain[5]. Recognizing the potential of WBV to induce tissue damage, the International Organization for Standardization (ISO) implemented guidelines defining the maximum daily limit of WBV that workers can be exposed to without risk for injury[6]. Conversely, commercially available WBV platforms are currently unregulated, with some able to deliver accelerations that exceed current ISO-2631 recommended guidelines[7].

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Raising further concern regarding the use of WBV, clinical trials show conflicting reports regarding the ability of WBV to increase bone mineral density[1,8]. Similarly, clinical trials investigating the effectiveness of WBV for knee or back pain have failed to show conclusive evidence, with only a small proportion of trials showing benefits based on assessments of patient self-reported pain[3,9]. A cause for concern is that the widespread use of WBV has not been validated with rigorous research-based evidence addressing the broad range of parameters used and potential harmful effects.

Investigating the effects of WBV on joint tissues, our research has demonstrated that exposure of mice to protocols of low-amplitude, high-frequency WBV that model those used clinically induced intervertebral disc (IVD) degeneration and osteoarthritis-like damage in the knee of skeletally mature, male mice, of a single outbred strain (CD-1)[10–12]. Given these striking deleterious effects of WBV, the present study aimed to determine if the response of joint tissues to WBV was consistent in age- and gender-matched C57BL/6 mice representing a distinct genetic background.

Materials and methods

Whole-body vibration

Based on WBV parameters used in clinical protocols and our previous studies[10–12], 10-week-old male C57BL/6 mice (Charles River) were subjected to vertical sinusoidal vibration (45 Hz, peak-to-peak amplitude 74 μm, peak acceleration 0.3 *g*) 30 min/day, 5 days/week for 4 or 8 weeks. Age- and gender-matched controls were housed in identical chambers on a non-vibrating sham platform to replicate handling and environmental conditions. Following WBV, mice were returned to conventional housing and monitored daily. No differences in total body mass were detected between groups. Mice were euthanized with sodium pentobarbital 24 h after final exposure to WBV. The Council on Animal Care at Western University approved all procedures, in accordance with the Canadian Council on Animal Care and the ARRIVE guidelines.

Gene expression analysis

Thoracic IVDs (T10–T15) were isolated from mice exposed to 8 weeks WBV and sham controls, placed in TRIzol (Life Technologies) and homogenized using a PRO250 tissue homogenizer (PRO Scientific). RNA was extracted according to manufacturer's instructions, quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific), and 0.5 μg was reverse transcribed into complementary DNA (cDNA) (iScript; Bio-Rad). Gene expression was assessed by real-time PCR (qPCR) using the Bio-Rad CFX384. PCR analyses were run in triplicate using 120 ng of cDNA per reaction and 310 nM forward and reverse primers with 2× SsoFast EvaGreen Supermix (Bio-Rad) using previously optimized PCR parameters and primers[10]. Transcript levels were calculated using ΔΔCt, with data normalized for input based on Ribosomal protein S29 (*Rps29*) and expressed relative to non-vibrated sham controls.

Histological analyses

Intact lumbar spine segments (L1–L5) and knees were isolated, fixed and paraffin embedded as previously described[10]. Spines were sectioned sagittally and knees were sectioned coronally, at a thickness of 5 μm using a microtome (Leica Microsystems). Spines sections were stained using 0.1% Safranin-O/0.05% fast green, and knee sections were stained with 0.04% Toluidine Blue. Sections were imaged on a Leica DM1000 microscope, with Leica Application Suite (Leica Microsystems). To evaluate IVD degeneration, mid-sagittal sections were scored using the modified Thompson grading scheme to assess IVD health based on criteria specific to each tissue compartment (scores 1–4, where a lower score corresponds to a more healthy tissue), as previously reported[10,12]. Knee joint health was assessed using the murine Osteoarthritis Research Society International (OARSI) histopathological scale[13]. For each mouse, the four quadrants of the knee joint were evaluated in 10 serial sections taken 75 μm apart to include most of the weight-bearing area of the femorotibial joint. For each quadrant of each section individual scores were averaged between three independent blinded observers and summed across the 10 sections for each mouse. The whole joint (WJ) score was calculated as the sum of the quadrant scores for each mouse.

Statistical analyses

Data are from experiments conducted with $n = 5–6$ mice per group at each time point. For qPCR analyses, data from mice exposed to WBV were compared to non-vibrated sham controls using a parametric Welch's *t*-test. At each time point, histopathological scores from the IVD or knee joint were compared using a non-parametric Mann–Whitney *U* test. Differences were accepted as statistically significant at $P < 0.05$.

Results

To examine the effects of repeated exposure to WBV on joint health, 10-week-old C57BL/6 mice were exposed to WBV for 30 min/day, 5 days/week for either 4 or 8 weeks, using parameters that model clinical use in humans (45 Hz, 0.3 *g*)[10]. Importantly, these parameters were previously found to induce degeneration of both the IVD and knee joint in age- and gender-matched CD-1 mice, marked by histological hallmarks of tissue damage including annulus fibrosus (AF) degeneration in the IVD as well as meniscal damage and focal defects in the articular surface of the knee[10–12].

In the C57BL/6 mice, we first assessed IVD health. No discernable differences were detected in the histological appearance of lumbar IVDs in mice exposed to 4 or 8 weeks of WBV compared to their respective sham controls [Fig. 1(A)]. Accordingly, evaluation of IVD structure using the modified Thompson score showed no significant differences in degeneration grade between mice exposed to 4 or 8 weeks of WBV and their respective sham controls [Fig. 1(B)]. As a more sensitive measure of early degenerative changes in IVD tissues, we evaluated the cellular response to WBV using qPCR. Similar to histological evaluation, this analysis revealed no change in the expression of anabolic (*Acan*, *Col1a1*, *Col2a1*, *Sox9*) or catabolic (*Mmp3*, *Mmp13*, *Adamts4*, *Adamts5*) factors between mice exposed to 8 weeks of WBV and non-vibrated sham controls [Fig. 1(C)].

We then assessed if exposure of C57BL/6 mice to WBV would induce changes to knee joint tissues. Consistent with findings in the IVD, no discernable differences were detected in the knee joint of C57BL/6 mice exposed to 4 or 8 weeks of WBV compared to their respective sham controls [Fig. 2(A)]. In contrast to our previous studies in CD-1 mice[10], exposure of C57BL/6 mice to WBV did not induce any observable meniscal damage, articular cartilage erosion or osteophyte formation. Evaluation of cartilage degeneration using the OARSI histopathological score supported these observations; no significant differences were found in the summed OARSI score between mice exposed to WBV and sham controls [Fig. 2(B)].

Discussion

Despite the widespread clinical and recreational use of WBV platforms, there is an alarming lack of understanding of the effects of WBV on overall joint health as well as the effects elicited in