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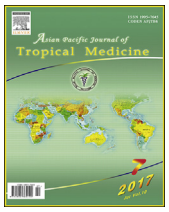

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Effect of axial vertical vibration on degeneration of lumbar intervertebral discs in modified bipedal rats: An *in-vivo* study

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ABSTRACT

Objective: To assess the effects of axial vibrations on gene expression and lumbar intervertebral disc degeneration *in vivo*.

Methods: A modified bipedal rat model was established using a brachial plexus rhizotomy approach to imitate human upright posture. The experimental animals were randomly divided into three groups: control, vertical vibration, and whole-body vibration. Gene expression in degeneration of the intervertebral discs was assessed by reverse transcription-quantitative polymerase chain reaction.

Results: The expression of aggrecan, Col1α1, Col2α1, and decorin were shown to be up-regulated in 14-week-old rats in the vertical vibration and whole-body vibration groups, whereas biglycan and versican expression was down-regulated in 14-week-old rats of the two experimental groups. Furthermore, biglycan and versican expression levels were shown to be lower in the whole-body vibration group than in the vertical vibration group ($P < 0.05$).

Conclusions: This *in-vivo* study demonstrated that vibrations can influence the expression of anabolic genes. Furthermore, whole-body vibrations seem to have a greater effect in this regard than vertical vibrations. A new method is expected to relieve the low back pain of the patients through our research.

1. Introduction

Chronic low back pain is a common clinical complaint from patients in modern society and often results from degeneration of the intervertebral discs (IVDs) [1]. Most epidemiological studies have suggested various reasons for disc-related back pain, including genetics, smoking, loading experience or occupation [2,3], and exposure to certain vibration types [4].

At present, there is no consensus on the effects of vibrations on disc-related low back pain, since multi-axis vibrations have been shown to have a range of effects on IVDs; adverse disc degenerative effects, no effect, and beneficial effects have been reported for multi-axis vibrations [4–6].

The IVDs transmit forces between adjacent vertebrae and prevent direct contact between vertebrae, thereby providing tolerance to a degree of instability. The vibration inputs experienced by vehicle drivers are primarily axial-spinal and we previously demonstrated that vertical vibration has a transient effect at the level of anabolic gene expression, with the greatest increases in expression occurring at 30 Hz and 0.49 g root mean square (RMS) *in vitro*. It remains unclear, however, whether axial vertical vibration has the same effect.

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The objective of this study was to investigate the effects of vibrations on degeneration of lumbar IVDs, using a modified bipedal rat model. The findings may help us understand the effects of vibrations on degeneration of lumbar IVDs of humans and discover a new way to prevent the degeneration of IVDs.

2. Materials and methods

2.1. Animal model and experimental design

All experiments were approved by the Ethics Committee of the First Affiliated Hospital of Soochow University and the Guidelines for Care and Use of Laboratory Animals were strictly followed. Four-week-old female Sprague–Dawley rats were obtained from the Experimental Animal Center of Soochow University. All animals were anesthetized by intraperitoneal injection of 4% chloral hydrate (1 mL/100 g body weight) before undergoing both brachial plexus rhizotomy and tail amputation procedures. After surgery, the animals were fed in ordinary cages for 3 d after which they were transferred to customized cages in which food and water were supplied and can be adjusted weekly according to each animal's vertical height. Oral antibiotics (Enrofloxacin [100 mg/mL]; GuideChem, Nanjing, China) were given in the animals' drinking water for 3 d post operation.

Six weeks post-operation (*i.e.*, at 10 weeks of age), four randomly selected rats were euthanized. The remaining 36 experimental rats were randomly assigned to three groups: 1) a sham control group of rats (SC, n = 12) that were housed in the custom-made cages without being subjected to vibration; 2) a second group of experimental rats (UP, n = 12) subjected to axial whole-body vibration at 30 Hz and 0.49 g RMS with upright posture for 15 min/d, achieved by using a vertical custom-made plastic cylinder (height, 40 cm; inner diameter, 10.2 cm); and 3) a third group of experimental rats (FP, n = 12) was interrupted by axial whole-body vibration at 30 Hz and 0.49 g RMS with free posture for 15 min/d. The vibration instrument (ES-3-150) was provided by Dongling Tech (Suzhou, China). The UP and FP groups were treated at the same time every day. Four randomly selected experimental rats from each group were euthanized at 11, 12, and 14 weeks of age (*i.e.*, 7, 8, and 10 weeks post surgery). Six lumber IVDs (L1-2, L2-3, L3-4, L4-5, L5-6, and L6-S1) per rat were dissected (24 in total). For every group, reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was carried out on 18 lumber IVD samples to assess the expression of extracellular matrix mRNAs.

2.2. Cell isolation

The intact IVDs (including NP, AF, and cartilage end plates) were separated from lumbar segments under aseptic conditions. The IVD tissues were immediately cut into small pieces (<1 mm^3) and digested with 150 U/mL collagenase I (VETEC, V900891) and collagenase II (VETEC, V900892) in DMEM-LG medium for 10 h in a humidified incubator at 37 °C with 5% CO_2. The resulting suspensions were filtered through 70-μm mesh before being centrifuged at 1000 r/min for 5 min to allow the lumbar IVD cells to be collected at the bottom of the tubes.

2.3. RT-qPCR

Total RNA was extracted from samples, using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol, and was quantified by spectrophotometry at 260 and 280 nm (A260/A280 ~1.6; A260 = 120 μg RNA/mL), using a NanoDrop (ND-8000) spectrophotometer (Thermo Fisher Scientific, Braunschweig, Germany). The isolated RNA was then reverse-transcribed to cDNA, using a RevertAid First Strand cDNA Synthesis kit (Thermo Scientific) according to the manufacturer's instructions, and the resulting cDNA was then combined with SsoFastTM EvaGreen® Supermix (Bio-Rad, Hercules, CA) and immediately analyzed. qPCR amplification was performed using a CFX96TM real-time PCR system (Bio-Rad) and the SYBR Green I fluorescent dye method was used to quantify cDNA. The PCR cycling conditions were as follows: 10 min at 95 °C (initial denaturing step); then 40 cycles of 30 s at 95 °C, followed by 30 s at 60 °C, followed by 40 s at 70 °C. A stable and reliable standard curve was established by plotting the threshold cycle (C_t) values. Following amplification, melting curve analysis was performed to verify the specificity of the amplified product by its specific melting temperature (T_m). The housekeeping gene *GAPDH* was used as an internal control, such that the relative levels of mRNA of the target genes (aggrecan, Col1α1, Col2α1, biglycan, decorin, and versican) could be determined, which in turn allowed for relative gene expression levels and trends of change to be determined. The specificity of each reaction was confirmed by melting curve analysis. A negative PCR control containing water instead of cDNA was prepared and the relative levels of mRNA were analyzed by the $2^{-\Delta\Delta Ct}$ method. qPCR was conducted in triplicate across three independent experiments. The sequences of the primers are presented in Table 1. The primers

Table 1
Sequences of primers used in the real-time qPCR.

Gene	Primer	Primer sequence (5′ to 3′)
Aggrecan	Forward	TTCGGCAAGCTACCTTCTGG
	Reverse	TTCTCCTGAACCACTGACGC
Col1α1	Forward	GTACATCAGCCCAAACCCCA
	Reverse	ACAAGCGTGCTGTAGGTGAA
Col2α1	Forward	GCCAGGATGCCCGAAAATTAG
	Reverse	CTCGTCAAATCCTCCAGCCA
Biglycan	Forward	GGCTGGGCTTAGGACACAAT
	Reverse	CACTTCCCAGTAGGGCACAG
Decorin	Forward	CGGTGGCAAATACCCGGATTA
	Reverse	AGGGGATTGTCAGGGTCGTA
Versican	Forward	AGACATGCTTCCCTCTCCCT
	Reverse	AGCTCTCTCGGGTACCATGT
GAPDH	Forward	TGCCACTCAGAAGACTGTGG
	Reverse	TTCAGCTCTGGGATGACCTT

used were either designed based on published primer sequences or were designed *de novo* (Table 1). All primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd (Shanghai, China).

2.4. Statistical analysis

Gene expression levels in IVD tissue exposed to axial whole-body vibration at varying frequencies or amplitudes were compared to those in non-vibrated sham controls by one-way analysis of variance (ANOVA) with Dunnett's post hoc test. Differences with $P < 0.05$ were considered statistically significant. Statistical analyses were carried out using SPSS software (SPS Inc., Chicago, IL).

3. Results

To assess and compare relative mRNA expression levels across the experimental groups, the qPCR data were grouped by rat age (10-, 11-, 12-, and 14-week-old rats). Aggrecan, Col1α1, Col2α1, and decorin were found to be up-regulated in the two experimental groups: vertical vibration and whole-body vibration, among which the whole-body vibration animals exhibited higher expression levels for these genes (Tables 1 and 2). Biglycan and versican were found to be down-regulated in the two experimental groups, where the whole-body vibration animals exhibited lower expression levels of the two genes than the animals in the vertical vibration group (Tables 1 and 2). The expression levels of aggrecan, versican, and Col1α1 were furthermore found to follow a downward trend over time in the control group but an upward trend in the two experimental groups. Biglycan expression showed a trend of up-regulation over time in all groups (Table 3).

4. Discussion

Degeneration of the intervertebral discs, considered an important pathogeny of low back pain [1], is gaining more and more attention. Many studies have demonstrated a relationship between multi-axis vibrations and degeneration of IVDs. Kumar *et al.* reported that whole-body vibrations were unrelated to low back pain by studying tractor-driving farmers in north India [7], whereas Jensen *et al.* showed that professional drivers experience a high risk of various disorders of the locomotor system [8], but that vehicle type and specific working conditions determine the health effects of driving on the locomotor system. Whether and how vibrations affect IVDs has therefore been shown to depend on the vibration type, frequency, and magnitude. Unfortunately, there is still no reliable explanation for how multi-axis vibrations affect the degeneration of IVDs.

In this study, the vibrations applied were carried out at 30 Hz and 0.49 g RMS for several reasons: firstly and most importantly, our preliminary research on the effects of axial vibration on degeneration of lumbar intervertebral disc of modified bipedal rats *in vitro* showed that anabolic genes were up-regulated at 30 Hz and that the most marked effect on up-regulation of all genes of interest was observed at a factor of amplification of 0.49 g RMS. Secondly, medical workers in clinical practice have found that mid-frequency vibration (18–30 Hz) may have a beneficial impact on low back pain in whole-body vibration exercise programs [9,10]. The choice of vibration parameters applied in this study is furthermore supported by a report by Kasra *et al.* on the effects of high-frequency vibration (20–300 Hz), which was suggested to promote protein and collagen biosynthesis in rabbit IVDs [11]. The findings reported by Kasra and colleagues are in agreement with our preliminary study data and accordingly, 30 Hz and 0.49 g RMS were selected as the experimental conditions in this study.

The qPCR data reported here show that vibrations do have a beneficial impact on the expression levels of several important genes including aggrecan, Col1α1, Col2α1, and decorin. Decorin, a small leucine-rich chondroitin-dermatan sulfate proteoglycan expressed by neurons and astrocytes in the central nervous system, is both anti-fibrotic and anti-inflammatory and attenuates the formation and partial dissolution of established and chronic scars [12]. Aggrecan is a large proteoglycan (>2800 kDa) with a largely mechanical function in the tissue

Table 2
qPCR results for 11-week-old rats.

Group	Aggrecan	Col1α1	Col2α1	Decorin	Biglycan	Versican
Control	1.00 ± 0.04	0.98 ± 0.01	1.01 ± 0.04	0.98 ± 0.04	1.09 ± 0.08	1.07 ± 0.05
Axial vibration	1.34 ± 0.07	1.39 ± 0.03	1.36 ± 0.02	1.22 ± 0.07	0.88 ± 0.05	0.87 ± 0.11
Whole-body vibration	1.57 ± 0.04	1.67 ± 0.05	1.67 ± 0.01	1.41 ± 0.06	0.56 ± 0.07	0.64 ± 0.03

Aggrecan, Col1α1, Col2α1, and decorin were up-regulated in 11-week-old rats from two experimental groups: vertical vibration and whole body vibration. Biglycan and versican were down-regulated in 11-week-old rats of the two experimental groups.

Table 3
qPCR results for rats of different ages.

Group	Age (weeks)	Aggrecan	Col1α1	Col2α1	Decorin	Biglycan	Versican
Control	12	1.10 ± 0.07	1.05 ± 0.05	0.96 ± 0.05	0.93 ± 0.08	0.92 ± 0.10	1.01 ± 0.03
	14	0.80 ± 0.20	1.03 ± 0.16	0.90 ± 0.14	0.87 ± 0.13	1.01 ± 0.05	0.99 ± 0.07
Axial vibration	12	1.24 ± 0.07	1.25 ± 0.04	1.21 ± 0.02	1.09 ± 0.04	0.75 ± 0.01	0.93 ± 0.05
	14	1.34 ± 0.04	1.31 ± 0.06	1.37 ± 0.08	1.16 ± 0.19	0.82 ± 0.08	0.97 ± 0.09
Whole-body vibration	12	1.39 ± 0.15	1.61 ± 0.10	1.85 ± 0.07	1.68 ± 0.26	0.55 ± 0.03	0.73 ± 0.04
	14	1.94 ± 0.02	1.66 ± 0.09	1.67 ± 0.18	1.31 ± 0.06	0.59 ± 0.08	0.81 ± 0.10

Aggrecan, versican, and Col1a1 show a trend of down-regulation over time in the control group, while a trend of up-regulation was observed over time in the two experimental groups. Biglycan showed a trend of up-regulation over time in all groups.

matrix [13]. The effects on expression reported here are in agreement with those reported previously: Wang *et al.* observed a similar up-regulation of Col1α1, Col2α1, and aggrecan expression following dynamic compression of bovine IVDs and vibration frequency significantly affects the expression of collagen type II, decorin, and versican mRNA and that vibration amplitude significantly affects biglycan, collagen type I, collagen type II, decorin, and versican mRNA expression [14].

Biglycan and versican were shown to be down-regulated in 14-week-old rats in both experimental groups in this study. Biglycan is a small molecule which participates in the osmotic swelling pressure system [15,16] and versican is a large proteoglycan (~1000 kDa) which functions in cell adhesion and cell signaling [17]. Aggrecan, versican, and Col1α1 showed a downward trend of expression over time in the control group, whereas an upward trend in expression was observed over time in the two experimental groups. These findings suggest that vibrations of 30 Hz and 0.49 g RMS may stimulate the expression of anabolic genes and suppress the degeneration of IVDs *in vivo*.

During this study, it was noted that vibrations of the same frequency and amplitude impact the expression levels of different genes to varying degrees. Some genes are sensitive to vibration frequency while some are sensitive to amplitude, which may explain our observations in this regard. On the other hand, IVDs consist of various components including nucleus pulposus and annulus fibrosus, each of which has a different composition: the annulus fibrosus is composed of concentric lamellae formed by parallel bundles of type I collagen fibers, which provide tensile strength, while the nucleus pulposus is composed of an irregular network of type II collagen, large aggregating proteoglycans(aggrecan), versican, and small proteoglycans (decorin and biglycan) [18]. Owing to the different elements and structures of these two IVD components, vibration is likely to have varied effects in terms of accelerating fatigue failure mechanisms within the various components of the IVDs.

This *in-vivo* study showed that vibrations affect the expression of anabolic genes. The findings reported here indicate that vibrations (30 Hz and 0.49 g RMS) enhance the expression of anabolic genes. Whole-body vibrations were furthermore shown to have a greater effect than vertical vibrations. The mechanisms underlying the effects of vibrations on the degeneration of intervertebral discs of humans, however, remain unknown and future studies in this research area will therefore further assess the effects of vibration on the degeneration of intervertebral disc *in vivo*. We look forward to find a new method to relieve the low back pain of the patients through our research.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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References

[1] Baliga S, Treon K, Craig NJA. Low back pain: current surgical approaches. *Asian Spine J* 2015; **9**(4): 645-657.
[2] Hemanta D, Jiang X-X, Feng Z-Z, Chen Z-X, Cao Y-W. Etiology for degenerative disc disease. *Chin Med Sci J Chung-Kuo Hsueh Ko Hsueh Tsa Chih* 2016; **31**(3): 185-191.
[3] Wong AY, Karppinen J, Samartzis D. Low back pain in older adults: risk factors, management options and future directions. *Scoliosis Spinal Disord* 2017; **12**: 14.
[4] Burström L, Nilsson T, Wahlström J. Whole-body vibration and the risk of low back pain and sciatica: a systematic review and meta-analysis. *Int Arch Occup Environ Health* 2015; **88**(4): 403-418.
[5] Virtanen IM, Karppinen J, Taimela S, Ott J, Barral S, Kaikkonen K, et al. Occupational and genetic risk factors associated with intervertebral disc disease. *Spine* 2007; **32**(10): 1129-1134.
[6] Fritz M, Fischer S, Bröde P. Vibration induced low back disorders–comparison of the vibration evaluation according to ISO 2631 with a force-related evaluation. *Appl Ergon* 2005; **36**(4): 481-488.
[7] Kumar A, Varghese M, Mohan D, Mahajan P, Gulati P, Kale S. Effect of whole-body vibration on the low back. A study of tractor-driving farmers in north India. *Spine* 1999; **24**(23): 2506-2515.
[8] Jensen A, Kaerlev L, Tüchsen F, Hannerz H, Dahl S, Nielsen PS, et al. Locomotor diseases among male long-haul truck drivers and other professional drivers. *Int Arch Occup Environ Health* 2008; **81**(7): 821-827.
[9] Kaeding TS, Karch A, Schwarz R, Flor T, Wittke T-C, Kück M, et al. Whole-body vibration training as a workplace-based sports activity for employees with chronic low-back pain. *Scand J Med Sci Sports* 2017; http://dx.doi.org/10.1111/sms.
[10] Maddalozzo GF, Kuo B, Maddalozzo WA, Maddalozzo CD, Galver JW. Comparison of 2 multimodal interventions with and without whole body vibration therapy plus traction on pain and disability in patients with nonspecific chronic low back pain. *J Chiropr Med* 2016; **15**(4): 243-251.
[11] Kasra M, Goel V, Martin J, Wang S-T, Choi W, Buckwalter J. Effect of dynamic hydrostatic pressure on rabbit intervertebral disc cells. *J Orthop Res Off Publ Orthop Res Soc* 2003; **21**(4): 597-603.
[12] Singh K, Masuda K, Thonar EJ-MA, An HS, Cs-Szabo G. Age-related changes in the extracellular matrix of nucleus pulposus and anulus fibrosus of human intervertebral disc. *Spine* 2009; **34**(1): 10-16.
[13] Liu Z, Li C, Meng X, Bai Y, Qi J, Wang J, et al. Hypoxia-inducible factor-lα mediates aggrecan and collagen Π expression via NOTCH1 signaling in nucleus pulposus cells during intervertebral disc degeneration. *Biochem Biophys Res Commun* 2017; **488**(3): 554-561.
[14] Wang D-L, Jiang S-D, Dai L-Y. Biologic response of the intervertebral disc to static and dynamic compression in vitro. *Spine* 2007; **32**(23): 2521-2528.
[15] Marfia G, Campanella R, Navone SE, Zucca I, Scotti A, Figini M, et al. Potential use of human adipose mesenchymal stromal cells for intervertebral disc regeneration: a preliminary study on biglycan-deficient murine model of chronic disc degeneration. *Arthritis Res Ther* 2014; **16**(5): 457.
[16] Shi P, Chen E-Y, Cs-Szabo G, Chee A, Tannoury C, Qin L, et al. Biglycan inhibits capsaicin-induced substance P release by cultured dorsal root ganglion neurons. *Am J Phys Med Rehabil* 2016; **95**(9): 656-6662.
[17] de Vries SAH, Potier E, van Doeselaar M, Meij BP, Tryfonidou MA, Ito K. Conditioned medium derived from notochordal cell-rich nucleus pulposus tissue stimulates matrix production by canine nucleus pulposus cells and bone marrow-derived stromal cells. *Tissue Eng Part A* 2015; **21**(5–6): 1077-1084.
[18] Humzah MD, Soames RW. Human intervertebral disc: structure and function. *Anat Rec* 1988; **220**(4): 337-356.