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Effects of brief whole-body vibration on bone metabolic and immunological indices in rats

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ABSTRACT

Introduction. Numerous studies have focused on the musculo-skeletal effects on high-frequency vibration programs; however, bone tissue responses to brief sessions of intensive WBV stimulation have been less recognized.

Aim of Study. The study examined whether short-lasting whole-body vibration (WBV) was sufficient to induce bone metabolic and immunological response, and whether the increasing volume of vibration training modifies changes in bone and modulate immunological indices.

Material and Methods. Ten male Wistar rats, aged 6 months, were subjected to 5-week WBV (5 days/ /week, frequency 50 Hz, amplitude of oscillations 2.5 mm). Five animals underwent vibration training in a single session lasting 30 seconds (WBV30), while the other five were subjected to four vibration sessions of 30 seconds each, separated by 60-second rest intervals (WBV30x4). Results were compared with those of age-matched controls (n = 5).

Results. No significant vibration-related changes in bone mineral density or between-groups differences in serum concentrations of osteocalcin and C-terminal telopeptide of type I collagen after the vibration program were found in either group of animals. Significant differences were found in the soluble receptor activator of nuclear factor kappa-B ligand levels between animals in the WBV30 group and controls as well as in interleukin-10 concentrations between the WBV30x4 group and controls.

Conclusions. The brief vibration programs, especially with the higher WBV volume, were sufficient to induce metabolic response. The lack of vibration-related changes in bone mass and between-groups differences in concentrations of bone turnover markers does not permit drawing prospective conclusions with respect to bone tissue.

KEY WORDS whole-body vibration, bone turnover, inflammation, rat, BMD.

Introduction

Whole-body vibration (WBV) trainings are widely applied in exercise and medical practice. The desirability of this physical stimulus, defined by frequency, acceleration and magnitude, has been based on results of research reports published in last years. Experimental studies of humans [1, 2] as well as animals [3-5] present clear effects of WBV in view of markers of mineralization of bone tissue and its metabolism. Although bone tissue adapts to mechanical stress resulting from gravitational loading and musculoskeletal loads, studies on animal models have demonstrated that vibration applied at high frequency and low magnitude produces anabolic response even in non-weight bearing skeletal regions [3]. Thus, the cellular mechanism involved in responses to different vibration procedures remains unclear. Moreover, the occupational exposure to vibration led to musculoskeletal pain, ischemia or other syndromes [6, 7]. On the other hand, Broadbent et al. [8] described the vibration therapy as a method reducing plasma interleukin 6 (IL-6) and muscle soreness after downhill running.

Beneficial effects of vibratory programs affecting bone tissue, including increased bone mass and morphological changes, have been observed in the frequency range between 10-100 Hz [9]. However, there is less information about the efficiency of the vibration stress duration on bone metabolic effects. Animal studies have shown that a relatively few daily strain cycles are necessary to promote or

maintain the balance in bone turnover [10]. Several studies have indicated that the intermittent short vibration cycles followed by rest intervals are more efficient in stimulating bone formation than continuous vibration [11]. The WBV short cycles are frequently considered a form of strength training as vibrations stimulate muscle spindle afferents [12], which results in the Ia excitation of α-motoneurons and may initiate a muscle contraction on the same basis as the tonic vibration reflex [13, 14]. However, the influence of a brief whole-body vibration program on bone tissue remains unclear.

Aim of Study

The present study carried out on rats addressed the following questions: 1/ Is short-lasting vibration stress sufficient for inducing bone metabolic response? and 2/ Does WBV influence the level of standard immunological indices? To answer these questions we measured total bone mineral density and applied biochemical methods to determine the levels of bone turnover markers and pro- and anti-inflammatory cytokines. The results were compared between two WBV groups differing in applied vibration volume, and the controls.

Material and Methods

Ten male Wistar rats, aged 6 months, were subjected to a regular 5-week whole-body vibration program. The animals were acquired and treated in accordance with the European Union regulations, and all testing procedures were approved by the local Bioethics Committee, and were in compliance with the Polish Animal Protection Act.

The rats were kept in cages in room temperature following a 12-hour light/dark cycle. The WBV procedure was performed in a separate room at the place of housing. Over several days all rats gradually adapted to the vibration program. The animals were trained on a vibration platform (Power Plate®) that produced sinusoidal vertical vibrations, 5 days per week, for 5 weeks, at a frequency of 50 Hz and amplitude of oscillations of 2.5 mm. The highest acceleration of the platform measured by the accelerometer (ACL300, Biometrics LTD) amounted to 4.79 of gravitational acceleration. The rats were divided into two groups subjected to two different vibration procedures: one consisting of a single WBV session lasting 30 s (WBV30, n = 5); and the other of four WBV sessions of 30 s each, separated by 60-second rest intervals (WBV30x4, n = 5), respectively. The animals subjected to WBV were compared with age-matched controls (C, n = 5).

All animals were allowed *ad libitum* access to food and water. Before and after the 5-week WBV training dual-energy X-ray absorptiometric (DXA) measurements of total-body bone mineral density (BMD) were carried out in anesthetized animals (sodium pentobarbital, 60 mg/kg, i.p.) using a Lunar Prodigy Advance densitometer (Lunar Corp., Madison, WI, USA). All scans were taken in the same conditions and the Lunar device was calibrated daily. Quality control of the DXA scanner was undertaken following the manufacturer's instructions.

On completion of the training program and two days after the bone mineral density (BMD) measurement, the animals were anesthetized (sodium pentobarbital 60 mg/kg, i.p.) and a blood sample of approximately 8 ml was collected directly from the common carotid artery. After centrifugation at 5000 rpm at 4°C, the separated serum was stored at –70°C. An immunoenzymatic ELISA method was used to determine the concentration of bone turnover markers, including osteocalcin (OC) as a bone formation marker, and fragments of C-terminal telopeptide of type I collagen (CTX) as a bone resorption marker with Immunodiagnostic Systems tests (UK), as well as the levels of soluble receptor activator of nuclear factor kappa-B ligand (sRANKL) with an Immundiagnostik test (Germany). Furthermore, concentrations of cytokines: interleukin 6 (IL-6) and interleukin 10 (IL-10), were determined with R&D Systems tests (USA), and interleukin 1β (IL-β) with a Bender Med Systems test (Europe).

All data were expressed as mean values ± standard deviations (SD). A Wilcoxon test was used for statistical comparison of paired variables (BMD) between both terms of the study, and a Kruskall-Wallis test with post-hoc was applied to evaluate differences among the three investigated groups of animals ($p < 0.05$). All calculations were carried out with the use of Statistica 8.0 software package.

Results

There was no significant difference between two terms of the study in both groups of animals with respect to BMD values (Table I). The effects of WBV on biochemical indices of bone metabolism and immunological status of all studied rats are shown in Table II. There were no significant differences between the two groups of vibrated animals with respect to concentrations of measured indices. On the other hand, significant differences were found between controls and animals from the WBV30 group as well as WBV30x4 groups with respect to sRANKL and IL-10 concentrations, respectively (both $p < 0.05$).

We also observed that the concentration of pro-inflammatory cytokines IL-1β and IL-6 in blood serum revealed the tendency to increase, especially in the group that underwent a higher volume of vibration (WBV30x4), about two and three times, respectively, in comparison with the con-

Table I. Bone mineral density (BMD) in both groups of rats before and after two types of whole-body vibration (WBV30, WBV30x4)

Variable		before WBV	after WBV	*p*
BMD [g x cm^{-2}]	WBV30 group	0.371 ± 0.011	0.371 ± 0.006	0.8927
	WBV30x4 group	0.369 ± 0.003	0.375 ± 0.014	0.6858

*$p < 0.05$ – significant difference between measurements before and after the vibration program; data expressed as means ± SD

Table II. Results of biochemical measurements in both groups of rats after two types of whole-body vibration (WBV30, WBV30x4) as compared with the controls (C)

	C (n = 5)	WBV30 group (n = 5)	WBV30x4 group (n = 5)	Analysis of variance *p*-value
OC [ng/ml]	181.5 ± 45.52	172.2 ± 41.25	131.3 ± 33.14	0.1451
CTX [ng/ml]	19.7 ± 4.23	17.6 ± 3.17	16.7 ± 5.44	0.5326
sRANKL [pg/ml]	29.1 ± 9.35	71.2 ± 20.93*	66.2 ± 22.32	0.0178
IL-1β [pg/ml]	44.2 ± 18.98	49.2 ± 34.60	152.1 ± 211.81	0.6771
IL-6 [pg/ml]	105.7 ± 17.19	162.4 ± 83.22	196.6 ± 122.70	0.2276
IL-10 [pg/ml]	36.8 ± 6.84	13.9 ± 12.92	11.9 ± 5.83*	0.0263

*$p < 0.05$ – significantly different from controls; data expressed as mean ± SD; OC – osteocalcin, CTX – C-terminal telopeptide of type I collagen, sRANKL – receptor activator of nuclear factor kappa-B ligand, IL-1β – interleukin 1β, IL-6 – interleukin 6, IL-10 – interleukin 10

trol group. Interestingly, a contradictory trend was found for the level of the anti-inflammatory interleukin-10, i.e. it was considerably decreased and this difference between the WBV30x4 group and control animals was significant ($p < 0.05$).

Discussion

Numerous studies have focused on the musculoskeletal response on high-frequency vibration programs; however, the mechanism by which bone recognizes and converts the vibration signals is unclear. There are reports showing a large discrepancy between modes of applied vibration programs, e.g. various levels of vibration frequency [15], different acceleration magnitudes [16] as well as experimental duration [17]. Moreover, the explanation of physical and cellular mechanisms involved in the response to different vibration procedures is vague [9], and similarly the threshold of effective time of vibration stimulus is unknown.

The main goal of our study was to investigate the metabolic response to different modes of short-lasting WBV intervention, one or four 30-second sessions per day. We applied a relatively high magnitude of vibration stimulus (2.5 mm amplitude of oscillations, acceleration of 4.79 g). Vibration producing vertical plate displacements of less than 1 mm with accelerations lower than 1 g (1g = 9.81 m/s^2) were defined as low-level stimulations [9]. According to human safety recommendations, during short exposures (1-10 min) to WBV, higher magnitudes may be applied, while longer stimulation leads to potential health risks [18].

In spite of bone tissue benefits recognized in studies based on vibratory programs with high-frequency and low-magnitude of peak acceleration in humans [1] as well in animal models [4], vibrating plates used in fitness studios produce stimulations with a larger mechanical force (up to 15 g). There is no direct evidence whether the mechanism of the oscillatory signal is transferred from the vibrating plate to distal skeletal sites by increased muscle activity or other mechanisms, e.g. release of hormonal or cytokine factors. In the latter case the safety of this stimulation is questionable for humans. In our study significant differences were found between vibrated rats and controls with respect to the levels of interleukins, and the higher significance of these differences was associated with the longer (repeated) WBV stimulation (4 x 30 s). This points out evidently that the WBV procedure, even when applied only for a few minutes daily, is not neutral to a living organism.

The increase of cytokines concentration in our study is probably an outcome of mechanisms occurring in various tissues activated by vibratory stimuli. The tendency to the increased level of inflammatory factors, interleukins IL-1 and IL-6, especially marked for the WBV30x4 group, supports the possibility of sensitizing processes of the tissue and may affect pain. Dina et al. [6] provided evidence that longer vibration time (15-60 minutes) is an important factor in the process of muscle hyperalgesia. According to these authors an extended stimulation period leads even to a decrease of the nociceptive threshold of nerve fibres. Moreover, vibration stimuli are involved in high-frequency firing of muscle nociceptors followed by the IL-6 release in muscle tissue [19]. It is also known that systemically injected IL-1 stimulates bone resorption, indicating the involvement of cytokines in a systemic effect on bone [20].

Numerous studies have focused on the bone mass and strength benefits under the WBV interventions but the biochemical mechanisms by which mechanical signals become effective are less recognized. Therefore, we approached the bone biochemical response by measuring serum concentrations of bone turnover markers (OC and CTX). In our study no significant vibration-related changes in BMD values and between-groups differences in bone turnover markers concentrations after the vibration program were found. However, we noted the increased sRANKL concentrations in vibrated animals as compared with controls, especially in the WBV30x4 group. Although RANKL is a regulator of skeletal processes [21], this molecule plays also an important role in other systems, especially in immune and vascular [22, 23]. Various tissues or organs contribute to the production of this cytokine, among others endothelial cells or activated lymphocytes T may express RANKL [22, 24] and their activity may be regulated by various cytokines including IL-1 and IL-6 [25, 26]. Therefore, the influence of inflammatory factors on increased sRANKL levels in our study cannot be excluded.

We have found only few reports how WBV programs affect levels of RANKL in bone tissue [27, 28]. Lau et al. [28] revealed the decreased RANKL secretion and prostaglandin E_2 (PGE_2) release in conditions of low-magnitude (0.3 g) and high-frequencies (30, 60, 90 Hz) vibrations in osteocyte-like MLOY4 cells.

Although RANKL is a cytokine known as a key mediator of osteoclast differentiation, its function in promoting

vascular system calcifications has also been discussed. However, Panizo et al. [23], measuring the effect of RANKL in vascular smooth muscle cell calcification, suggested that changes in the circulating levels of RANKL did not reflect changes in vascular levels. Graham et al. [22], observed in an animal model that activated T lymphocytes may be one of the sources of RANKL serum levels, and that this mechanism may contribute to bone changes. Findlay et al. [29] documented a negative relationship between serum RANKL levels and RANKL mRNA expression in bone tissue in osteoarthritis patients. In our study, despite the increased sRANKL serum level in vibrated animals, no significant differences in the concentration of bone turnover markers in comparison to control animals were found.

Conclusions

The study demonstrated that brief vibration programs were sufficient for inducing the metabolic response, and changes were more strongly expressed at a higher volume of WBV. However, with respect to bone tissue, the lack of vibration-related changes in BMD and between-group differences in bone turnover markers concentrations does not allow us to draw prospective conclusions. Further studies are necessary to recognize the WBV-evoked effects of various magnitude and duration (also long-term) on skeletal and metabolic response.

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