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Low-magnitude mechanical signals that stimulate bone formation in the ovariectomized rat are dependent on the applied frequency but not on the strain magnitude

Stefan Judex*, Xin Lei, Daniel Han, Clinton Rubin

Department of Biomedical Engineering, State University of New York at Stony Brook, Stony Brook, NY, USA

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Abstract

There is growing evidence that extremely small mechanical signals, if applied at a sufficiently high frequency, can serve as anabolic signals to bone tissue. To determine if the responsiveness of bone to low-magnitude, high-frequency parameters is modulated by endocrine imbalance, ovariectomized (OVX) Sprague–Dawley rats were subjected to whole body vibrations (WBV, 0.15 g) at 45 Hz ($n = 6$) or 90 Hz ($n = 6$) for 10 min/day, and compared to OVX age-matched controls ($n = 6$). Five additional rats were used, in vivo, to establish the induced bone surface strain magnitudes (and strain rates). Following a 28 d protocol, bone formation rates in the metaphysis of the proximal tibia were 159% greater in 90 Hz rats when compared to age-matched controls, but 45 Hz rats were not significantly different from controls. Bone morphology of 90 Hz rats indicated significantly greater trabecular bone volume (22% and 25%) and thicker trabeculae (11% and 12%) over either controls or 45 Hz rats in the epiphysis of the distal femur, respectively. Despite the enhanced sensitivity of the skeleton towards the 90 Hz signal, the strain magnitudes and strain rates induced by this frequency were significantly lower than during 45 Hz vibration, suggesting that factors other than matrix strain are driving the anabolic response. Ideally, such mechanical signals represent a non-pharmacologic means of controlling bone mass and morphology in spite of systemic pressures for bone resorption.

Keywords: Trabecular bone; Ovariectomy; Strain frequency; Mechanical stimuli; Bone formation; Osteoporosis; Bone strain; Anabolic; Skeleton; Osteogenic; Strain magnitude

1. Introduction

Extremely low-magnitude (<10 microstrain (με)), high frequency (10–100 Hz) mechanical signals, introduced to the skeleton using whole body vibration (WBV) can be anabolic to bone tissue, contributing to a skeletal structure that is less prone to fracture (Judex et al., 2003; Rubin et al., 2001a; Ward et al., 2004; Jankovich, 1972; Flieger et al., 1998; Tanaka et al., 2003). These mechanical signals can be orders of magnitude below those more typically considered in conjunction with exercise or applied loading regimens (Rubin and Lanyon, 1985; Turner et al., 1994), and thus may present a non-pharmacologic means of preventing/reversing osteoporosis without putting the skeleton at risk of damage. Despite this promising potential, few studies have investigated whether vibrations can prevent the changes in bone formation/resorption and the deterioration of bone morphology induced by a catabolic stimulus.

In postmenopausal women, WBV applied at magnitudes exceeding 5 g were able to increase hip bone mineral density (BMD) (Verschueren et al., 2004) while a similar WBV intervention, but with peak (vibration) accelerations reduced by an order of magnitude, prevented the decline in BMD in regions of the femoral

*Corresponding author. Tel.: +1 631 632 1549; fax: +1 631 632 8577.
E-mail address: stefan.judex@sunysb.edu (S. Judex).

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neck and spine (Rubin et al., 2004a). Children suffering from cerebral palsy (Ward et al., 2004) and rodents subjected to disuse may also benefit from this extremely low-level mechanical countermeasure (Rubin et al., 2001b).

The degree by which variations in the parameters defining a WBV intervention, such as acceleration magnitude, frequency, or duration, alter the efficacy of the low-level mechanical signals is largely unknown. In the ovariectomized (OVX) rat, vibrations applied at either 17 Hz (0.5 g), 30 Hz (1.5 g), or 45 Hz (3 g) were all sensed in cortical bone but the signal at the highest frequency (and acceleration magnitude) was most effective in enhancing cellular activity and was the only one that prevented the loss of cortical bone strength (Oxlund et al., 2003). If bone indeed has a preference towards certain vibration frequencies and/or accelerations, the specific mechanical parameters modulating this different sensitivity have not been identified.

Considering the distinction between the relatively infrequent, but large magnitude locomotory strain signals and the omnipresent, but low-level mechanical signals that persist through actions such as standing, it is possible that the manner of the adaptive response to low-magnitude mechanical regimens does not follow the adaptive rules defined by factors such as longitudinal normal strain (Rubin and Lanyon, 1985; Turner et al., 1994), strain rate (O'Connor et al., 1982; Lamothe et al., 2005), or strain gradients (Judex et al., 1997; Gross et al., 1997), and that other loading characteristics, including the frequency of the signal or the number of loading cycles, play a more important role at these smaller magnitudes.

In an effort towards the development and optimization of WBV-based regimes that can effectively prevent and counteract bone loss, here, we tested the hypothesis that under hormonal challenges, a 90 Hz mechanical signal can be more effective in stimulating bone's anabolic activity than a signal half its frequency and that this differential sensitivity is independent of the induced strain magnitude.

2. Methods

2.1. Experimental design

All experimental procedures were approved by Stony Brook's Institutional Animal Care and Use Committee. OVX retired breeders (Sprague–Dawley) were purchased (Charles River Laboratories Inc., Wilmington, MA) and subsequently subjected to low amplitude (0.15 g peak acceleration) WBV at either 45 Hz ($n = 6$) or 90 Hz ($n = 6$) for 10 min/day (5 d/wk), or served as age-matched (long-term) OVX controls ($n = 6$). All rats were received in a single shipment and were 6–8mo old (female rats are retired according to the number of pregnancies and performance). Rats were maintained on a regular rodent chow with a calcium and vitamin D content of 1.0% and 2.4 IU/gm (LabDiet Prolab RMH 3000, Purina Mills LLC, St. Louis, MO) and commenced their 28 d experimental protocol 2 wk upon arrival (~5 wk post OVX). Demeclocycline (25 mg/kg, i.p.) and calcein (20 mg/kg, i.p.) were administered to all rats on days 1 and 18 to monitor static and dynamic indices of trabecular and cortical bone formation in the metaphyseal region of the proximal tibia. Body mass and femoral length of each rat were recorded. Trabecular bone and cortical bone morphology was assessed by micro-CT in the distal femur. An additional five age-matched rats from the same genetic strain were used for in vivo strain gaging.

2.2. Strain gage recordings

Under isoflurane anesthesia, single-element strain gages (UFLK-1-11-1L, 1 mm gage length, 120 Ω, TML Gages, Texas Measurements, College Station, TX) were attached (cyanoacrylate) to the anterior-medial surface of the proximal tibia of rats from the strain gage group. Upon recovery from surgery (1–3 h), strain data were collected for 5 s while the animals were standing on a plate vibrating (0.15 g) at 45 or 90 Hz. Strain gage signals were conditioned by a strain gage amplifier (Syminex Inc., Beacon Dynamics, Dover, NJ) with an excitation of 4 V and a 2000 × gain. To minimize noise, all cables connecting the components of the data acquisition system were carefully shielded and grounded and a Faraday cage isolated the vibration plate from the measurement devices. Strain signals were acquired at a sampling rate of 2000 Hz and 16-bit resolution. In this configuration (Fritton et al., 2000), the nominal strain resolution is below 0.1 με. For data analysis, all strain data were digitally filtered through a low-pass Fast Fourier Transform (FFT) filter with a cutoff frequency of 100 Hz. Five trials were collected per animal and frequency. FFT were also used to confirm the dominant frequency of the recorded signal. For each trial, the dynamic strain range was calculated as the mean difference in peak-to-peak strain magnitude across the strain oscillations. Average peak strain rates were determined as the first derivate of the strain signal. For both strain magnitude and strain rates, the average of the five trials was used for further analysis.

2.3. Histomorphometry and morphology

Histomorphometric analyses focused on trabecular and cortical bone of the proximal tibia because previous studies in rodents had found the largest effect of WBV on measures of bone formation at this site (Rubin et al., 2001b; Judex et al., 2002). It further allowed the direct relation of bone's response to the two mechanical regimes