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DEPENDENCE OF HIGHER FREQUENCY COMPONENTS ON BONE TISSUE ALTERATIONS IN THE RAT-TAIL MODEL

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ABSTRACT

Through a recently conducted rat-tail vibration experiment, we have been able to determine that the tested frequencies of vibration have a significant effect on biochemical damage signified by nitro-tyrosine (NT) staining on trabecular bone, while structural damage quantified through a Hematoxylin and Eosin (H&E) stain on cortical bone exhibited statistical significance only for the 250 Hz group compared to the control group. These results seem to indicate a relationship between the growing quantities of biochemical damage when increasing the excitation frequency, thus further experimentation for frequencies between 250 Hz and 400 Hz is recommended.

INTRODUCTION

Prolonged exposure to hand transmitted vibration (HTV) can cause vascular, neurological, and musculoskeletal abnormalities in the hand-arm system, which together is known as hand-arm vibration syndrome (HAVS) [1]. NIOSH estimates that over 2 million workers in the United States and United Kingdom alone are at risk of developing HAVS [2]. This type of exposure is prevalent in occupations utilizing powered hand-tools, such as but not limited to construction, carpentry, plumbing, mining, and assembly.

Within the classification of musculoskeletal disorders, there are several bone disorders that are suspected to be related to vibration exposure, such as low bone mineral

density, bone lesions and carpal bone abnormalities. However, direct factor causalities between vibration and bone disorders cannot be established in vivo with human subjects due to the improbability that afflicted workers were only affected by vibration damage and no other type of trauma [3].

Many tools used in the industry, such as grinders and chipping hammers, generate significant vibration components in the frequency range of 100-400 Hz [4]. However, ISO standard 5349 (2001) has positive frequency-weighting for the range between 32.5 Hz and 100 Hz, which may be underestimating the risk of damage caused by higher frequency components. Epidemiological data on the quantitative association between vibration and risks of bone degeneration are still inconclusive. All experimentation and analysis were made possible by funding from the NIOSH-PRP grant #: T42/OH008432-05.

METHODS

The caudal vertebrae bone from the rat tail was selected because it had been previously used to study changes in bone tissue morphology and responses of bone under mechanical loading [5]. Rats were restrained in plexiglass restrainers, isolated from transmitted vibration and heat, and their tails duct-taped to a circular steel excitation platform. This setup can be seen as follows.

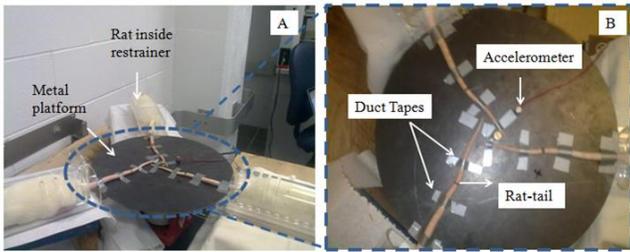


Figure 1. Experimental setup showing rats in restraints (A) and group tail arrangement (B)

The resonance frequency of the excitation platform (600 Hz) was determined to be much higher than the experimental excitation frequencies (125 and 250 Hz), utilizing an impact test. 24 male Sprague-Dawley rats (250 ± 15 gm) were used. Rat-tails were vibrated at 125 Hz and 250 Hz (49 m/s² rms) for 1, 5 and 10 days for 4 hours a day. Structural damage of bone was quantified by osteocyte count using H&E staining, whereas biochemical changes were assessed by nitrotyrosine (NT) staining. Examples of both of these stains can be seen in Figure 2 below. The results were analyzed using one-way mixed model ANOVA.

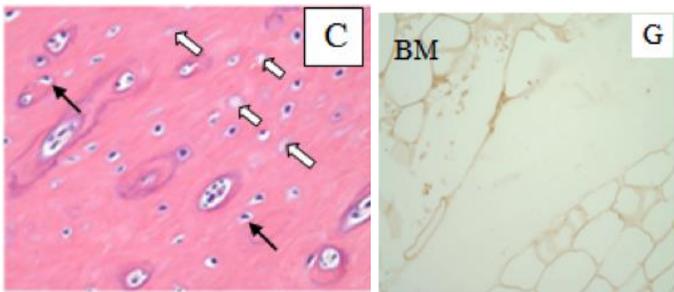


Figure 2. Slide images under X40 objective magnification. H&E with black arrows showing osteocytes and white arrows showing empty lacunae in cortical bone (A) and NT positive signal shown by brown hue in trabecular tissue (B)

RESULTS

The results of the frequency effect on biochemical changes that were quantified from the normalized amount of positive NT signal (NT signal intensity) are shown in Figure 3 below. Results are presented as mean ± standard deviation.

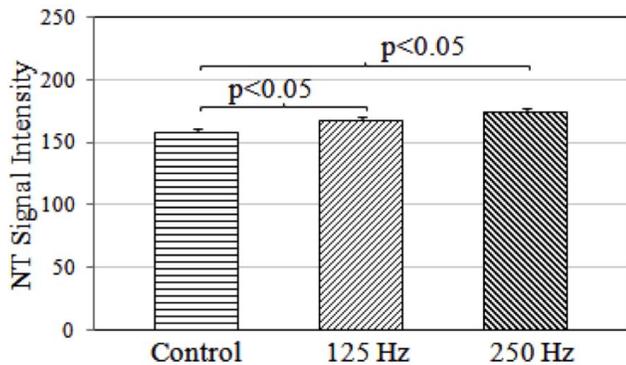


Figure 3. NT signal intensity in trabecular tissue

NT signal intensity in trabecular bone was found to be statistically significant from control (157.99 ± 3.47) for both of the 125 Hz (168.12 ± 2.74) and 250 Hz (174.09 ± 2.89) frequency groups.

The effect of frequency on structural changes was observed by manually counting the healthy osteocytes and empty lacunae locations, then computing a ratio of healthy osteocytes to total locations. These results are shown in Figure 4 below.

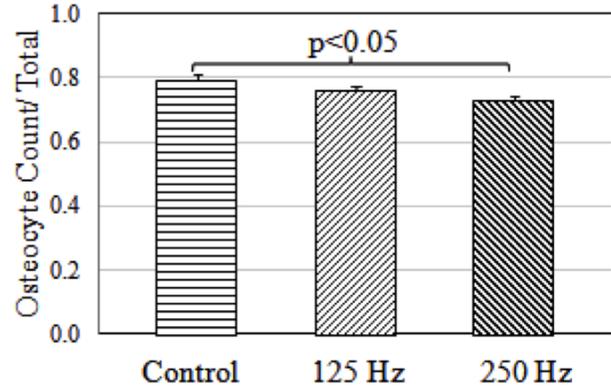


Figure 4. Osteocyte/Total count ratio from H&E stain

The osteocyte count/total was statistically significant only for the 250 Hz frequency group (0.73 ± 0.01) as compared to control group (0.8 ± 0.02).

CONCLUSION

While this was the first study in which our group had observed biochemical and structural changes in bone tissue morphology, the significance of both of the frequencies (125 and 250 Hz) when compared to the control group in the NT analysis is encouraging in our goal to explore a relationship between frequency and changes in bone tissue. Based on our result, we feel that exploring extended time duration effects and an extended range of frequencies from 100 to 400 Hz is necessary to elaborate on what we have found in this study.

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