INTRODUCTION. Osteocytes are well believed to be the major mechanosensors in bone, responsible for sending signals to the effector cells (osteoblasts and osteoclasts) that carry out bone formation and resorption. Consistent with this hypothesis, it has been shown that osteocytes release various soluble factors (e.g., transforming growth factor-β, nitric oxide, and prostaglandins) that influence osteoblastic and osteoclastic activity when subjected to a variety of mechanical stimuli, including fluid flow, hydrostatic pressure, and stretching. Recently, low-magnitude (0.3 g), high-frequency (20 Hz) vibration has gained interest as studies show that such a mechanical signal can positively influence skeletal status in animals and humans. Although the anabolic and anti-resorptive potential of low-magnitude, high-frequency vibration is becoming apparent, the underlying cellular and molecular regulation of this mechanosensitivity is currently unknown. We hypothesize that osteocytes are the mechanosensors responsible for detecting the vibration stimulus and producing soluble factors that modulate the activity of effector cells. Here, we applied low-magnitude (0.3 g) vibrations at varying frequencies (30-90 Hz) to osteocyte-like MLO-Y4 cells, after which we studied differential changes in mRNA expression of several bone regulatory genes and explored the potential of conditioned media collected from vibrated MLO-Y4 cells to inhibit the formation of osteoclasts (OC).

METHODS. Cell culture: MLO-Y4 cells (gift from Dr. Lynda Bonewald, University of Missouri–Kansas City) were maintained in α-MEM supplemented with 2.5% FBS, 2.5% CS, and 1% PS on type I rat tail collagen-coated plates at 37 °C and 5% CO2. For vibration experiments, MLO-Y4 cells were plated in 12-well plates at 7.5x10^4 cells/well. After 48 h, wells were completely filled with supplemented media and tightly sealed with gas-permeable sealing film (Aeraseal, Scientific). Reverse transcription was performed on 0.5 µg RNA. The mRNA quantification Total RNA was isolated from MLO-Y4 cells 2 h after vibration and treated with DNase I (Fermentas). Reverse transcription was performed on 0.5 µg RNA. The resulting cDNA samples were subjected to quantitative PCR (qPCR) using gene-specific primers and SYBR Green I in LightCycler 480 (Roche). Standards and samples were run in triplicate. mRNA levels of each gene of interest were normalized to 18S levels.

RESULTS. There is a trend of increasing COX-2 and RANKL mRNA levels in MLO-Y4 cells from 0 to 60 Hz. At 90 Hz, mRNA expression dropped to a level comparable to no vibration condition. Cells that were subjected to 60 Hz vibration showed a significantly higher expression in COX-2 and RANKL (3.4 and 2.5-fold increase, respectively; p<0.05) as compared to non-vibrated controls. In addition, the effect of CM collected from vibrated MLO-Y4 cells on OC formation was evaluated. Cultures containing CM from vibration conditions had fewer OCs, but the decrease was not statistically significant.

DISCUSSION. Our data suggest that MLO-Y4 cells respond to the vibration signal at least at the transcript level, with vibration at 60 Hz being the most potent signal. This corresponds partially with animal studies, where vibrations at 90 Hz were more anabolic than a 45 Hz signal in ovariectomized rats. The seemingly contradictory observation that MLO-Y4 cells showed a lower response to the 90 Hz signal may be explained by the viscoelasticity of cells, where cell stiffness defines an upper limit of loading rate that causes cell deformation. Similar to previous studies in which bone cells were stimulated with fluid flow, vibration stimulation caused an increase in the expression of COX-2, an enzyme that is critical for the production of prostaglandins and has been implicated in promoting bone formation. Vibration also resulted in increased expression of RANKL, an important molecule for osteoclast formation. Interestingly, however, our preliminary data from the CM study indicate a decreased number of OCs in vibration conditions. While the preliminary data does not show a statistically significant difference, future experiments involving larger sample size and quantitative protein analysis may confirm whether osteocytes release soluble factors that inhibit OC formation in the presence of vibration stimulation. This study is the first to identify the regulatory biochemical agents associated with the vibration signal in osteocytes, which serve as potential targets for pharmacological interventions of osteoporosis.


ACKNOWLEDGEMENTS. This research is supported by Ontario Graduate Scholarship for Science and Technology, NSERC 315868, and CFI 14071.