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# High-acceleration whole body vibration stimulates cortical bone accrual and increases bone mineral content in growing mice

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ABSTRACT

Whole body vibration (WBV) is a promising tool for counteracting bone loss. Most WBV studies on animals have been performed at acceleration $<1g$ and frequency between 30 and 90 Hz. Such WBV conditions trigger bone growth in osteopenia models, but not in healthy animals. In order to test the ability of WBV to promote osteogenesis in young animals, we exposed seven-week-old male mice to vibration at 90 Hz and 2*g* peak acceleration for 15 min/day, 5 days/week. We examined the effects on skeletal tissues with micro-computed tomography and histology. We also quantified bone vascularization and mechanosensitive osteocyte proteins, sclerostin and DMP1. Three weeks of WBV resulted in an increase of femur cortical thickness (+5%) and area (+6%), associated with a 25% decrease of sclerostin expression, and 35% increase of DMP1 expression in cortical osteocytes. Mass-structural parameters of trabecular bone were unaltered in femur or vertebra, while osteoclastic parameters and bone formation rate were increased at both sites. Three weeks of WBV resulted in higher blood vessel numbers (+23%) in the distal femoral metaphysis.

After 9-week WBV, we have not observed the difference in structural cortical or trabecular parameters. However, the tissue mineral density of cortical bone was increased by 2.5%.

Three or nine weeks of 2*g*/90 Hz WBV treatment did not affect longitudinal growth rate or body weight increase under our experimental conditions, indicating that these are safe to use.

These results validate a potential of 2*g*/90 Hz WBV to stimulate trabecular bone cellular activity, accelerate cortical bone growth, and increase bone mineral density.



## 1. Introduction

An increase of bone mass during the bone acquisition stage is an important osteoporosis prevention factor (Babatunde and Forsyth, 2014). This is why various physical exercises are widely used to stimulate bone formation during growth period (Parfitt, 1994). At the same time bone adaptation to physical activity depends on the intensity of loading and the number of loading cycles (Qin et al., 1998). Therefore, whole body vibration (WBV) can be an addition or even an alternative to physical exercise, as it produces thousands of low-impact strain events in a relatively short period of time, with no significant efforts from a recipient.

A pioneering study performed by Rubin et al. (2001) showed that one year of 30 Hz vibration at 0.3*g* leads to a 30% increase of the trabecular bone volume and density, as well as bone strength and stiffness in sheep (Rubin et al., 2001). Even though in recent years there have been numerous WBV studies on both animals and humans, it is still difficult, due to high variability of experimental procedures, to decide which WBV protocol is the most beneficial for bones (Prisby et al., 2008). Among published WBV animal studies, acceleration ranges between 0.3*g* and 3*g* ($1g=9.81\ m/s^2$) (Rubinacci et al., 2008), and frequencies between 8 Hz and 90 Hz (Judex et al., 2007; Pasqualini et al., 2013). The studies also vary in duration of exposure and resting times (Xie et al., 2006; Zhang et al., 2014), choice of studied species (rat, mouse, sheep), and age of the animals (Lynch et al., 2010; Rubinacci et al., 2008; Rubin et al., 2002). Even though various combinations were possible, most WBV experiments have been performed with magnitudes below 1*g*, and frequencies from 30 Hz to 50 Hz on physiologically challenged (ovariectomized, unloaded, or immobilized) animals. This has been done in order to test WBV ability to prevent an induced bone loss. Notably, effects of low-magnitude WBV were mostly

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beneficial on osteopenic models, but did not promote bone accrual in control animals.

The effects of high-acceleration WBV (above 1g) have not been much investigated yet. It could be expected that higher acceleration would result in higher strain, and therefore bone formation (Ozcivici et al., 2010), even in healthy animals. However, despite the high g-level, WBV at 2g/50 Hz and 3g/30 Hz appeared osteogenic for ovariectomized animals but not for controls (Flieger et al., 1998; Rubinacci et al., 2008). Such results were presumably informed by suboptimal frequencies. Indeed, strain rate appeared to be an important component of the skeleton response to mechanical stimulation (Turner et al., 1995). Additionally, a study performed in our laboratory comparing effects of WBV frequencies (8 Hz, 52 Hz and 90 Hz) at the same acceleration (0.7g) revealed that only a frequency of 90 Hz stimulated bone formation in healthy rats (Pasqualini et al., 2013).

In the present study, our goal was to establish a WBV regimen, which promotes bone gain in healthy young animals. We hypothesized that, due to an interrelationship between loading cycles and bone adaptation (Ozcivici et al., 2010), a combination of 90 Hz frequency and high acceleration (2g) would efficiently stimulate bone growth in healthy mice. Young animals were chosen to verify that (a) WBV at a high acceleration does not impair bone growth or increase in body weight and (b) WBV applied during the period of active skeletal growth accelerates bone growth and/or bone mineralization leading to a higher peak bone mass. Blood vessels are known to play an essential role (delivery of osteoclast and osteoblast precursors and endocrine factors) in basic multicellular units where bone remodeling takes place (Sims and Martin, 2014). Also, bone angiogenesis is indispensable for bone gain induced by physical exercises (Zao et al., 2004). Thus, we assessed vascularization parameters using a previously validated quantification technique (Roche et al., 2012).

Osteocyte proteins sclerostin and Dentin Matrix acidic Phosphoprotein 1 (DMP1) were selected for analysis because they are known for bone formation control (Lin et al., 2009, 2005) and mineralization (Feng et al., 2006; Ling et al., 2005; Maciejewska et al., 2009) respectively, and their expression is affected by mechanical signals (Gluhak-Heinrich et al., 2003; Harris et al., 2007; Moustafa et al., 2012; Robling et al., 2008). We showed that in a short run (three weeks) a high acceleration WBV regimen stimulated mouse bone vascularization and bone cellular activity, accelerated cortical bone size acquisition, and, in the long run (nine weeks), resulted in a net increase in bone matrix mineral content.

## 2. Materials and methods

### 2.1. Animal care

Seven-week-old C57BL/6J male mice (Charles River Laboratories, l'Arbresle, France) were housed in the PLEXAN facility (Platform for Experiments and Analysis,

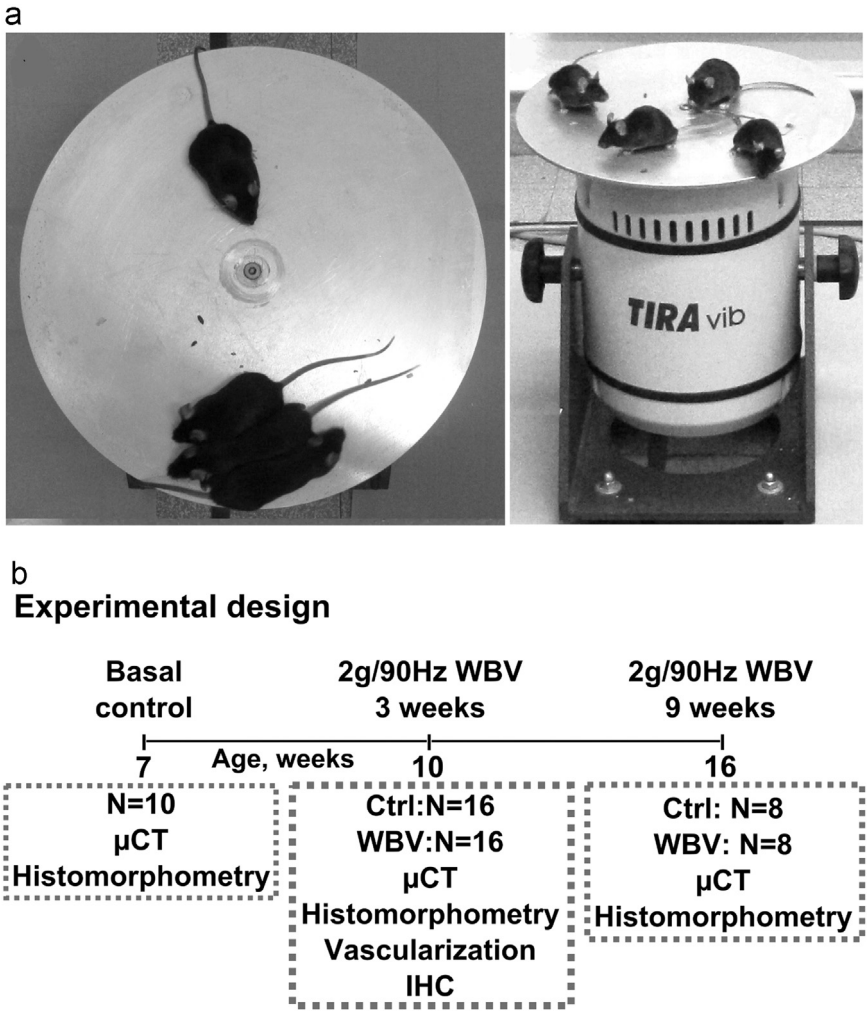


**Fig. 1.** Vibration device and a scheme of the experimental design. a – descriptive photo of the vibration device during a vibration session; b – diagram of the experimental design, number of animals and performed analyses are listed for each group/experiment. Ctrl = control, WBV = whole body vibration.

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