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Application of low intensity mechanical vibrations for bone tissue maintenance and regeneration

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Abstract: Physical exercise is beneficial for bone tissue health, yet its usage is limited for preventing osteoporosis. Even though natural for the bone tissue from development to homeostasis, mechanical loads present with a multitude of physical parameters, including amplitude, duration, frequency, and distribution. Utilizing the most beneficial parameters of mechanical loads may potentiate a nonpharmaceutical tool for biotechnology to prevent and treat bone loss related to aging, bedrest, sedentary lifestyles, weightlessness, and other diseases. Low intensity vibrations (LIVs) consist of mechanical loads with amplitudes smaller than loads prescribed by habitual activity, with a higher frequency. In this review, literature covering LIV signal application on bone tissue and cellular and molecular level is presented. Studies indicate that LIV signals are safe, anabolic, and anticatabolic for skeletal tissue and are of great significance in regenerative medicine applications.

Key words: Vibrations, osteoporosis, osteogenesis, cellular biomechanics, osteoblasts, mesenchymal stem cells

1. Introduction

Bone tissue serves vital functions in organisms by providing physical support/protection, contributing to mineral homeostasis, and housing bone marrow, an important source for mesenchymal and hematopoietic cells (Martini and Ober, 2006). Bone retains its capacity for those functions by being a very dynamic and adaptive organ. For example, changes in the serum calcitonin and PTH closely regulate Ca^{2+} binding or release to bone tissue, effectively regulating the calcium metabolism of the organism. Similarly, bone tissue readily senses the changes that occur in mechanical loads and adapts to them by changing its mass and morphology.

Mechanical loads are powerful determinants in bone biology, affecting bone tissue development, mass, morphology, repair, and aging (Ozcivici et al., 2010). Lack of mechanical loads is catabolic to bone and bone marrow tissue (Ozcivici et al., 2010; Ozcivici, 2013), though genetic factors determine the degree of catabolism an individual would experience (Judex et al., 2013; Ozcivici and Judex, 2014; Ozcivici et al., 2014). On the other hand, addition of mechanical loads to daily routine is anabolic to bone tissue. The most common case for anabolism is encountered in elite athletes from various sports disciplines, and the extent and localization of the adaptive response is activity dependent. For example, professional tennis players have significantly more bone mass in their dominant arm humerus compared to the contralateral (Jones et al., 1977). Similarly site specific effects of mechanical loads can be observed in bone mass of various elite trainees in different disciplines that require strenuous exercise, such as soccer (Karlsson et al., 2000), bodybuilding (Kelley et al., 2001), martial arts (Andreoli et al., 2001), ballet (Khan et al., 1998), and gymnastics (Nickols-Richardson et al., 2000). In order to account for the potential of self-selection in people that actively seek strenuous activities, several randomized longitudinal trials confirmed an accumulation of bone mass with additional physical activity in pediatric (McKay et al., 2000), adolescent (Kato et al., 2006) and geriatric populations (Maddalozzo and Snow, 2000; Snow et al., 2000; Bergström et al., 2008).

Increasing bone mass through mechanical loads is an important biomedical aim, as these loads are natural and omnipresent for biological structures and they are nonpharmaceutical in nature. For this aim to be achieved, however, individuals need to integrate planned physical activity into their daily routine. Unfortunately, that integration may not always be viable as individual compliance becomes an important hindrance (Mayoux-Benhamou et al., 2005; Kelley and Kelley, 2013).

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Furthermore, skeletal fragility in the elderly is another limiting factor for exercise to be employed for skeletal health. In order to trigger an adaptive response, mechanical loads should induce a level of strain (deformation normalized to geometry) on the bone structure. Magnitude of the strain is known to be a very important modulator for bone tissue (Robling et al., 2001; Frost, 2003), but these high magnitude loads may cause fractures in the already frail skeletons of the elderly. Overall, utilization of mechanical loads to improve bone mass and morphology should overcome the problem of compliance and be safe to be applied to individuals.

Other than the peak strain magnitude of mechanical loads, several studies have concentrated on the frequency (number of loading events in a second) dependence of the anabolic response, based on the natural occurrence of high frequency yet low magnitude mechanical signals in the adult skeleton (Fritton et al., 2000). Indeed high frequency loads can trigger adaptive response in the skeleton even though their magnitude is far below mechanical loads that are experienced by the skeleton during daily activities (Judex et al., 2007). In longitudinal studies, application of high frequency and low magnitude mechanical signals (referred to as low intensity vibrations (LIVs) hereafter) was found to be anabolic to bone tissue. LIVs provided significant benefits in bone mass by either increasing bone accrual or attenuating the bone loss in postmenopausal women (Rubin et al., 2004), adolescent women (Gilsanz et al., 2006), children with cerebral palsy (Ward et al., 2004; Reyes et al., 2011), adolescents with Down syndrome (Matute-Llorente et al., 2015), and individuals with adolescent idiopathic sclerosis related osteopenia (Lam et al., 2013). In contrast to strenuous physical activity or systemic pharmaceutical applications, application of LIVs is safe and natural for skeletal segments and triggering bone remodeling. On the other hand, application of LIVs may need to be optimized as its effectiveness may be limited as evidenced by other studies (Slatkovska et al., 2011; Lai et al., 2013; Kiel et al., 2015). Therefore, the biological mechanisms on how LIV signals are perceived by bone and marrow cells is still a subject of biomedical research studies.

This review article intends to reflect up-to-date information on LIV signals to be used in regenerative and restorative bone biology. For brevity, the article concentrates on bone tissue and decision making of bone cells in the presence of LIVs. Up-to-date review articles are available on the effect of LIVs on other skeletal tissues such as muscles and tendons (Edwards and Reilly, 2015) or on the effect of other mechanical conditions such as mechanical disuse on bone and bone marrow cells (Ozcivici, 2013).

2. Technical aspects of LIV signals

LIVs with characteristics of small magnitude and high frequencies (also called high frequency oscillations, low magnitude mechanical signals, etc. in the literature) are pressure waves that can be generated with a biomedical device in repetitive fashion. A surface or a platform then transmits these mechanical signals to biological systems, including the whole body, a local tissue, or an in vitro cell culture system. In the literature of mechanical vibrations for biomedical usage, the magnitude of the mechanical waves is often provided in terms of their proportion to the earth's gravitational field (*g*), where 1 *g* is equal to 9.81 m/s^2. The frequency of mechanical loads is generally depicted with number of repetitions in a given second (Hz). In this review, applied mechanical signals will be depicted with magnitude, frequency, duration, and study length as reported in the cited literature.

Mechanical vibrations that are the subject of this review are considered low magnitude and high frequency as they have lower magnitude and higher frequency compared to physiological loads that arise from daily activities. The general assumption is that when the magnitude of the mechanical signal is lower than 1 *g*, it is considered to carry low intensity (Chan et al., 2013). It is possible to use mechanical vibrations of higher magnitude for the augmentation of sports training, but the applications for regenerative medicine are comparatively limited. This difference perhaps arises from the subject of safety, because high intensity vibrations have safe exposure thresholds on the order of seconds, as defined by the International Standards Organization's "Human exposure to mechanical vibration and shock" (ISO-2631). Even though a healthy adult is recommended to be exposed to high intensity vibrations for very brief amounts of time, low intensity range vibratory signals can be received safely for hours (Muir et al., 2013). From a logical perspective, compliance to safety recommendations should be even firmer for an elderly or injured person who is seeking augmentation of rehabilitation from mechanical vibrations. Perhaps that is the reason for LIV signals' having broader range of applications in regenerative medicine (Ozcivici et al., 2010; Chan et al., 2013).

3. Tissue level response to LIV signals

The efficacy of LIV signals on skeletal segments was largely tested in in vivo animal models. These studies subjected not only healthy mature or adolescent models to LIV signals, but also tested chronic bone loss conditions such as mechanical disuse or estrogen deficiency and acute injury such as fracture healing or implant osseointegration. Overall, mechanical and time dependent characteristics of applied LIV signals vary greatly between studies, and measured outcomes span from molecular level to tissue level.

3.1. Effects of LIV signals on healthy subjects

Application of LIV signals (0.3 *g*, 30 Hz, 2 min/day – 1 year) to hindlimbs of mature female sheep induced new trabecular bone formation and increased trabecular bone mineralization compared to sham loaded controls (Rubin et al., 2002a, 2002b). Improved bone tissue indices were localized to hindlimbs of the sheep where the LIV signal was applied, and forelimbs were not affected. Moreover, trabecular bone mechanical stiffness and mechanical stress distribution values also indicated a stronger bone structure compared to sham controls (Judex et al., 2003).

3.2. LIV as a countermeasure for mechanical disuse

Mechanical disuse is the state of un- or underloading of skeletal tissue and it is an important catabolic stimulus for bone loss. Disuse exposure in individuals can be transient (such as spaceflight, bedrest, and fracture healing) or persistent (such as aging, stroke, and neuromuscular diseases). In translational studies, hindlimb unloading of rodents via tail suspension is a well-established simple model to simulate effects of mechanical disuse in the musculoskeleton (Morey-Holton et al., 2005). Adult female rats that were exposed to hindlimb unloading for 1 month showed significantly reduced bone formation rates compared to controls but application of LIV signals (0.25 *g*, 90 Hz, 10 min/day) normalized bone formation rates (Rubin et al., 2001). In a similar setup with mice rather than rats, application of LIV signals (0.2 *g*, 90 Hz, 15 min/day – 3 weeks) during hindlimb unloading prevented disuse induced retardation of bone marrow osteoprogenitor cells and during recovery significantly increased the number of osteoblasts, bone formation, and bone mass after the disuse, indicating a potential benefit for long-term recovery (Ozcivici et al., 2010). Unlike previously mentioned studies that applied LIV signals by interruption of hindlimb unloading by placing the rodent on a vibrating platform, applying LIV signals (0.6 *g*, 45 Hz, 20 min/day – 3 weeks) in the absence of weight bearing (by simple transmission of oscillatory motions) was reported to significantly increase trabecular bone formation rate, bone mass (Garman et al., 2007), and biomechanical properties (Ozcivici et al., 2007).

3.3. LIV as a countermeasure for estrogen related bone loss

Estrogen deficiency during female aging is another important modulator of bone loss. Similar to mechanical disuse, rodent models of ovariectomy (OVX) can simulate the absence of estrogen in the bloodstream. OVX rats that were exposed to LIVs (3 *g*, 45 Hz, 30 min/day – 90 days) showed significantly increased bone formation rates compared to sham controls (Oxlund et al., 2003). Similarly, trabecular and cortical bone quantity and quality and whole bone biomechanical properties were shown to be increased in a rat OVX model by daily application of LIV signals (3.9 *g*, 90 Hz, 15 min/days – 35 days), applied 3 months after the surgery at the vertebra (Sehmisch et al., 2009) and femur (Tezval et al., 2011). The importance of the LIV signal frequency was shown in another rat OVX study that used lower magnitude mechanical signals compared to others (0.15 *g*, 10 min/day – 28 days), indicating that LIV signals when applied at 90 Hz, but not 45 Hz, can induce significant increases in bone formation rate, and epiphyseal trabecular bone volume and thickness (Judex et al., 2007). The increase in whole bone strength and bone mineral density during the application of LIV (0.3 *g*, 30–35 Hz, 20 min/day – 6 weeks) in rat OVX models corroborated the decrease in RANKL and serotonin levels in circulation (Wei et al., 2014). In discordance with the studies that reported benefits from LIVs for bone mass and mechanical properties, some studies reported no benefit of LIV signals in rat OVX models (Brouwers et al., 2010; van der Jagt et al., 2012).

3.4. LIV application during fracture healing

The importance of bone formation and reformation can also be extended to cases where bone recovers from severe insults, such as fracture. The fracture healing process starts with an early immune response and formation of hematoma, depicting the initiation of the healing process (Park et al., 2002), followed by ossification that resembles development of skeletal segments (Colnot, 2009). Aging delays the fracture recovery for various reasons on the molecular, cellular, and tissue level (Gruber et al., 2006), extending morbidity and increasing healthcare costs for the individual. Daily application of LIVs (0.3 *g*, 35 Hz, 20 min/day – 4 weeks) significantly enhances fracture healing and improves callus strength in fracture models of young rats (Leung et al., 2009). Identical application of a LIV signal regime for up to 8 weeks in fracture healing models of OVX rats showed enhanced mineralization and remodeling (Shi et al., 2010) by inducing upregulation of genes related to chondrogenesis (Col-2), osteogenesis (Col-1), and remodeling (RANKL and OPG) (Chung et al., 2014). In a separate study with similar LIV application with bisphosphonates (bone remodeling suppressor antiosteoporotic drug) administration in OVX mice, LIV signals were found to alleviate bone remodeling suppression induced by the drug, increasing the fracture recovery time (Chow et al., 2011). Similar to studies with OVX model, discordant results were reported for application of LIVs (0.3 *g*, 35 or 45 Hz, 20 min/day – 10 or 21 days) on an adult female mice fracture model did not induce any benefit for healing for the 35 Hz signal and resulted in delayed bone formation for the 45 Hz signal, suggesting that required signals should be optimized further to prevent disruption in fracture healing (Wehrle et al., 2014).

3.5. Effect of LIV signals on implant osseointegration

Structural and functional integration of bone tissue with metal implants has great importance for the success of

reconstructive surgeries, as failure in osseointegration may result in loosening of the implant (Branemark et al., 2001). Application of varying frequencies of LIV signal (0.075–0.3 *g*, 12–150 Hz, 5 min/day – 1–4 weeks) had beneficial impact on bone recovery at the implant site as well as increased bone to implant contact (Ogawa et al., 2014). Not only effective for the osseointegration of implants during healthy conditions, but in an osteoporotic (OVX) rat model for osseointegration application of LIV signals (0.2 *g*, 45 Hz, 30 min/day – 4 weeks) improved the healing significantly (Liang et al., 2014). In a similar study, hydroxyapatite coated titanium screws in the tibia of rats were exposed to LIV (0.3 *g*, 40 Hz, 30 min/day – 12 weeks), and mechanical signals enhanced osteoblast differentiation, extracellular matrix synthesis, and mineralization together with an increase in bone mass and reduction in bone resorption (Zhou et al., 2015).

Overall, a large proportion of studies reported bone tissue level benefits of LIV signals, that daily application of signals induced preservation, improved healing/regeneration, and increased bone tissue mineralization. In addition to studies described above, proof-of-principle studies showed that LIV signals can induce structural benefits on other bone deteriorating conditions in animal models, such as osteogenesis imperfecta (Vanleene and Shefelbine, 2013), secondary osteoporosis caused by glucocorticoid treatment (de Oliveira, et al. 2010), lipopolysaccharide induced inflammation (Kim et al., 2014), obesity (Chan et al., 2012), and cancer (Pagnotti et al., 2012).

4. Cellular/molecular level response to LIV signals

The dynamic nature of bone tissue potentiates from the cooperative functioning of bone cells, the bone forming osteoblasts, and bone resorbing osteoclasts (Martini and Ober, 2006). Osteoblasts come from mesenchymal stem cell origin and once activated they attract calcium ions to form a mineralized matrix and transform into osteocytes within the same matrix they lay. Osteoclasts on the other hand, come from hematopoietic stem cell origin and they are responsible for releasing calcium ions from the mineralized matrix by dissolving the matrix with low pH secretions. Resorption of bone tissue is very important for both morphological adaptations and removal of accumulated cracks (matrix defects) in the tissue that occur from cyclic mechanical loading. As osteoclasts can induce a drastic effect on the bone tissue, their function is closely regulated by osteoblasts to remain local and transient (Asagiri and Takayanagi, 2007). This coupling (termed bone reformation) is realized via a functional assembly called a basic multicellular unit (Jilka, 2003). Being the primary regulator of this unit, it is not surprising that effects induced by mechanical loads were extensively studied in osteoblasts and their progenitors in the bone marrow in vitro to elucidate specific molecular pathways.

MC3T3-E1 murine osteoblastic cells respond to LIV signals of varying magnitude and frequency (acceleration not reported, maximum velocity 0.15–0.47 m/s, 5–100 Hz for 5 min) and induced frequency dependent increase in nitric oxide (NO) and decrease in prostaglandin E_2 (PGE_2) secretion from cells immediately after loading, indicating the activation of bone formation pathways (Bacabac et al., 2006). Over longer terms, MC3T3-E1 cells under LIVs (acceleration not reported, 400 Hz, 20 min/day – 1–7 days) increased NO secretion from cells, with mRNA upregulation of osteogenic genes such as fibronectin, bone sialoprotein, collagen type I, and osteopontin without changing the cell viability (Dumas et al., 2010).

Similar to data from cell lines, primary bone marrow stem cells respond to LIV signals (~0.5 *g*, 30 Hz, 45 min/day – 3–6 weeks) by increased osteogenic mRNA markers such as Runx2 and enhanced calcification (Prè et al., 2013). Similar improvement in osteogenesis was observed in a separate application of LIV (0.3 *g*, 30–40 Hz, 10 min/day – 1–3 weeks) not only improved calcification and osteogenic mRNAs such as osteocalcin, osteopontin, and bone sialoprotein but also induced a significant upregulation of vascular endothelial growth factor (VEGF) when applied to cells in 3D culture conditions (Kim et al., 2012). Other than the primary bone marrow cells, adipose tissue derived stem cells cultured under osteogenic culture conditions respond to LIV signals (acceleration not reported, 30 Hz, 45 min – 4 weeks) and significantly increased cellular calcification and transcription of osteogenic genes such as osteopontin, alkaline phosphatase, and collagen type I compared to controls (Pre et al., 2011).

Ultrastructural components of D1-ORL-UVA murine bone marrow stem cells show adaptive response to LIV signals (0.15 *g*, 90 Hz, 15 min/day – 7 days), implying the establishment of a stiffer physical form (Demiray and Özçivici, 2015). Fluid shear stresses on cells also induce cytoskeletal remodeling that results in stiffer structures (Yoshigi et al., 2005); however, the mechanism of LIV signals (0.15–2 *g*, 30–100 Hz, 30 min/day – 14 days) is shown to be distinct from fluid stresses (Uzer et al., 2012, 2013). Furthermore, LIV signals (0.7 *g*, 90 Hz, 20 min/day (×2 repeats)) appear to affect LINC (linker of nucleoskeleton and cytoskeleton) structures in primary bone marrow mesenchymal cells that provide mechanical coupling between the nuclear envelope and actin cytoskeleton (Uzer et al., 2015). These studies suggest that LIV signals, unlike high magnitude mechanical loads induced by substrate deformations, catalyze a cellular environment that can produce mechanical loads within the cell. The notion of cellular tuning to LIV signals by cytoskeletal rearrangement not only suggests that cells produce their own "internal forces" (Chan et al., 2013; Uzer et al., 2015) but also that

they have an ability to attract external forces better based on the increased stiffness. Together these notions diversify the utilization of LIV from musculoskeletal applications to cases where cells are more compliant (softer) than healthy cells, such as cancer (Guck et al., 2005; Xu et al., 2012; Olcum and Ozcivici, 2014).

Potential effects of LIV signals were also tested on osteocytes, the terminally differentiated osteoblasts that reside in the mineralized matrix. Osteocytes are important mediators of bone formation and resorption through secretion of paracrine factors and they can also sense and respond to mechanical signals (You et al., 2008; Bonewald, 2011). MLO-Y4 osteocytes once exposed to LIV (0.3 *g*, 30–90 Hz, 60 min) showed significant reduction in osteoclast activating RANKL expression, showing anticatabolic effect of LIV signals (Lau et al., 2010). Furthermore, application of LIVs (0.7 *g*, 90 Hz, 20 min/day (×2 repeats) – 17 days) to mesenchymal stem cell derived osteocytes significantly reduced their sclerostin expression, an antianabolic molecule that restricts the activation of osteoblasts (Thompson et al., 2015). These results highlight that LIV signals are not only effective mediators for commitment to bone formation in primitive stem cells and osteoblasts, but also continue to be sensed and responded to in mature osteocytes as well.

5. Conclusions

In this review, we presented studies related to the utilization of LIVs in bone regenerative medicine applications. Results indicate that LIV signals are anabolic and anticatabolic for bone tissue on a broad spectrum of medical conditions that induce bone deterioration.

Most of the studies presented here examined local effects of LIVs in the bone tissue and therefore effects of LIV on the immune, circulatory, neural, endocrine, and metabolic systems are not very clear. All these systems have inherent relationships with bone metabolism and conceivably they may act as determinants in the effectiveness of LIVs. Furthermore, how LIV signals affect and regulate other tissue types is also studied. For example, hematopoietic stem cells that determine the pool of immune cells as well as bone resorbing osteoclasts are known to be affected by various mechanical cues, including shear stress (Bianchi et al., 2013), cyclic stretch (Zhao et al., 2013), and substrate elasticity (Holst et al., 2010), making them a potential target for LIV therapy. Emerging studies show that LIV therapy can restore bone marrow hematopoietic cell populations during obesity (Chan et al., 2012), indicating that a wide range of medical conditions should be tested with LIVs in the future.

In summary, mechanical regulation of cellular processes has great potential to be used as a nonpharmaceutical means to improve bone mass and structure to protect individuals from debilitating effects of osteopenia. As a biotechnological tool, high frequency force generating devices that are tuned to the personalized requirements of individuals may inhibit accumulating osteoporosis related healthcare costs, especially in aging communities.

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