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The etiology of the stress fracture syndrome is incompetely understood but it is clear that skeletons with high peak bone quantity and quality will be better pretected against incurring this crippling condition. In our one-year report, the ability of low-level whole body vibrations to decrease the levels of osteoclastic resorption and to enhance osteoblastic activity in the adolescent mouse skeleton, in the absence of detrimental side-effects on growth or the chemical composition of the matrix, was described. In this second annual report, data are presented that indicate that the efficacy of extremely low-level whole-body mechanical vibrations can be enhanced by altering the number of daily loading sessions or the magnitude of the acceleration. In summary, an extremely low-level mechanical stimulus, inducing deformations orders of magnitude below those that can actually damage bone, is capable of decreasing bone resorption and enhancing bone morphology – effects that may help to reduce the incidence of stress fractures and to avoid skeletal pathologies later in life.

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Table of Contents

Introduction

The stress fracture syndrome is associated with mechanical environments involving very large numbers of loading cycles, yet its etiology is insufficiently understood. It is clear, however, that those recruits with low bone quantity (and bone quality) are not only at greater risk of incurring stress fractures, but are also more likely to suffer from other bone diseases later in life such as osteoporosis. Therefore, interventions which effectively increase bone quantity, and ideally quality, during growth could in turn reduce the incidence of these crippling conditions. FDA approved pharmaceutical interventions of low bone mass may have side-effects and are primarily anti-catabolic in nature. Physical exercise may be capable of positively influencing peak bone mass reached in young adulthood but a principal hurdle has been the identification of safe anabolic and/or anti-catabolic mechanical interventions with high compliance. Mechanical stimuli induced by such interventions would have to be small in magnitude but effective in enhancing peak bone mass and strength to ultimately reduce the incidence of stress fractures and the associated substantial socio-economical costs to the DoD and recruits. In our one-year report, the ability of low-level whole body vibrations to decrease the levels of osteoclastic resorption and to enhance osteoblastic activity in the adolescent skeleton, in the absence of detrimental side-effects on growth or the chemical composition of the matrix, was described. In this second annual report, data are presented that indicate that the efficacy of extremely low-level whole-body mechanical vibrations can be enhanced by altering the number of daily loading sessions or the magnitude of the acceleration.

Body

Increasing peak bone mass during young adulthood is central to optimizing skeletal health as bone mass is inversely related to the risk of incurring a number of bone pathologies, including osteoporotic and stress fractures. Variability in peak bone mass is modulated by genetics, life-style, and functional load bearing. Physical activity plays an important role in achieving high peak bone mass; however, activity levels in children and young adults are declining rapidly and alternative means of stimulating bone mass non-pharmacologically are required. For bone tissue's sensitivity to mechanical signals to be used as a means of enhancing bone quantity and/or quality during skeletal growth, it will be necessary to employ strategies that are safe, effective, short in duration, and can achieve high compliance. In the *adult* skeleton, previous studies have indicated high frequency (> 20Hz), low magnitude ($0.3g=2.9m\cdot s^{-2}$) mechanical signals can promote bone formation, enhance bone morphology, and increase bone strength in rodents and larger mammals. Recent data described in our last annual report suggest that high frequency (45Hz), low magnitude (0.3g) whole body vibrations (WBV) applied for 3wk to the adolescent mouse skeleton favorable influences bone resorption and formation [1], but the experimental duration had to be extended to 6wk before a significant enhancement of bone morphology could be detected [2]. The magnitude of the vibration frequency, acceleration, and duration were chosen somewhat arbitrarily and it is possible that the signal can be optimized by adjusting these parameters. In an effort to provide morphological benefits to the skeleton in a shorter period of time, here, we hypothesized that increasing the number of daily bouts to two and/or doubling the magnitude of the acceleration may increase the efficacy of low-level vibrations in the growing skeleton.

Methods

Eight-week old male BALB/cByJ mice were divided into six groups, baseline control (BC, n=8), long-term age-matched controls (AC, n=12), whole body vibration (WBV) at 45Hz (0.3 g) for 15 min once per day (0.3g, n=12), WBV at 45Hz (0.3g) for 15 min twice per day (6h between the two bouts, 0.3*2, n=11), WBV at 45Hz (0.6g) for 15 min once per day (0.6, n=12), and WBV at 45Hz (0.6 g) for 15 min twice per day (6h between the two bouts, 0.6*2, n=11). After the 3-wk protocol, the right proximal tibiae were scanned (microCT 40, Scanco Medical, 12μm resolution). The epiphysis (240μm in length) and metaphysis (600μm in length) were defined according to precise landmarks that attempted to maximize trabecular volume within each region. Bone volume fraction ($BV\cdot TV^{-1}$), trabecular separation (Tb.Sp), trabecular thickness (Tb.Th), trabecular number (Tb.N), connectivity density (Conn.D), geometrical degree of anisotropy (DA), and the structural model index (SMI) were determined. 5-μm undecalcified frontal sections of proximal right tibia were analyzed for indices of bone formation and in situ chemical composition (synchrotron infrared microspectroscopy). 7-μm decalcified frontal sections of proximal left tibia were analyzed for indices of bone resorption by Tartrate Resistant Acid Phosphatase (TRAP) staining.

Results

Normal growth over the 3wk period increased total bone volume (BV) by 11.4% ($p<0.05$), trabecular BV by 27.1% ($p<0.05$), trabecular bone volume fraction ($BV\cdot TV^{-1}$) by 30.5% ($p<0.002$), trabecular thickness (Tb.Th) by 18.5% ($p<0.001$), mineral density by 2.9% ($p<0.004$) in the metaphysis of the tibia (**Fig.1**), while bone formation rates ($BFR\cdot BS^{-1}$) decreased by 67% ($p<0.001$) (**Fig.2**). Vibration applied once a day at 0.3g for 3wk increased $BFR\cdot BS^{-1}$ by 67% ($p<0.001$), but had no significant influence on bone morphology. Increasing the number of bouts to two (0.3*2) significantly increased $BFR\cdot BS^{-1}$ by 109% (**Fig.1**), metaphyseal trabecular BV by 17.3%, $BV\cdot TV^{-1}$ by 12.6%, Conn.D by 27.8%, and Tb.N by 8.9% while Tb.Sp was significantly decreased by 9.0% (**Fig.2**). Chemical analyses indicated no significant difference in newly formed bone (2-7d old) or old bone between the 0.3*2 group and AC group.

Increasing the magnitude of the vibration to 0.6g, without increasing the number of loading bouts, also significantly increased trabecular $BFR\cdot BS^{-1}$ by 104% and $BV\cdot TV^{-1}$ by 12.6%. Two bouts of 0.6g WBV (0.6*2) vibration, however, negated the beneficial morphological effect, but significantly increased $BFR\cdot BS^{-1}$ by 65%. Neither normal growth nor any WBV regime significantly altered the levels of trabecular bone resorption in the metaphysis of the tibia.

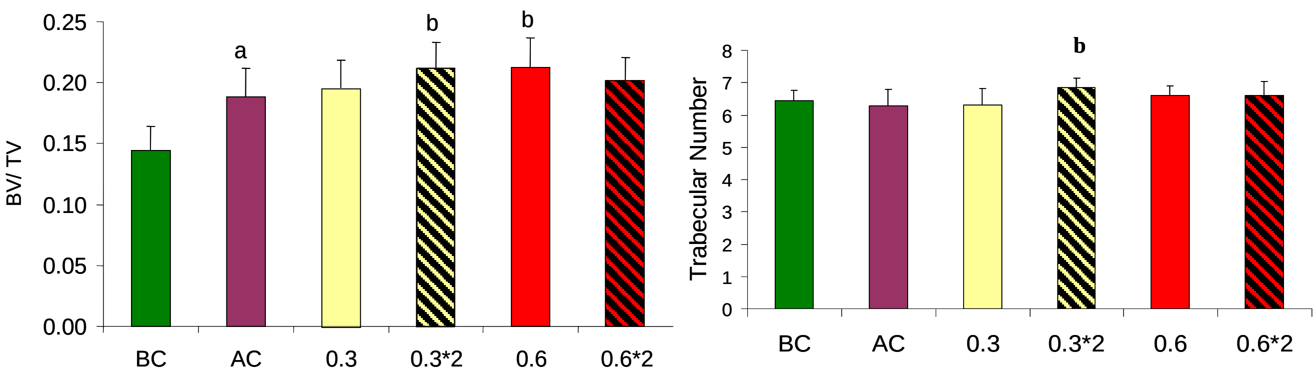

Figure 1. *Trabecular bone volume fraction (BV/TV) (left panel) and trabecular number (right panel) in the tibial metaphysis of mice that were subjected to different combinations of loading bouts and acceleration magnitudes. . a: p<0.05 between AC and BC. b: p<0.05 between WBV and AC.*

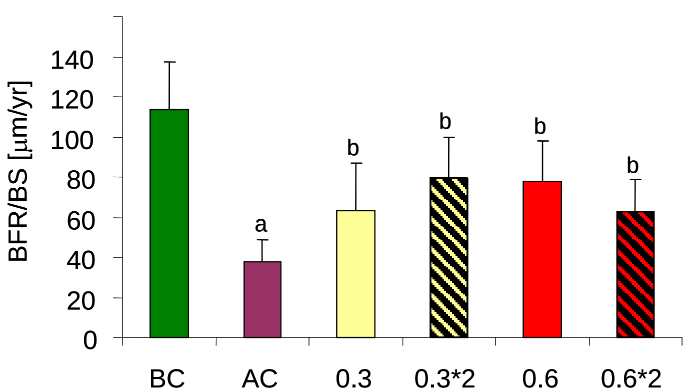

Figure 2. *Bone formation rates (BFR/BS) in trabecular bone of the tibial metaphysis in mice that were subjected to different combinations of loading bouts and acceleration magnitudes.* ***a:*** *p<0.05 between AC and BC.* ***b:*** *p<0.05 between WBV and AC*

Discussion

Consistent with studies on the female BALB skeleton [1], the application of one short bout of WBV to the growing male skeleton did not produce changes in bone morphology within the 3wk loading protocol. In stark contrast, increasing the acceleration magnitude to 0.6g, or doubling the number of daily loading bouts to two, revealed anabolic effects in trabecular bone quantity and micro-architecture without loss of bone chemical quality. These alterations in bone morphology appear to be the result of enhanced osteoblastic activity and not the suppressed osteoclastic activity. These data strongly suggest that the efficacy of whole body vibrations can be substantially modified by altering aspects related to the number of loading bouts, acceleration magnitude, and perhaps loading cycles.

Studies using the same model have indicated that the inclusion of a short rest period (10-15sec) after every or after every 15 seconds of WBV renders the low-level high-frequency mechanical stimulus less effective [1], perhaps suggesting that the efficacy of extremely low-magnitude WBV may be dependent on the temporal continuity of a large number of loading cycles. Considering that, here, the addition of another daily bout of 0.3g WBV significantly increased the efficacy of WBV may indicate that the rest period chosen previously was too short or that the total number of daily loading cycling plays an important role in defining bone response to WBV. This hypothesis is currently being tested. Interestingly, the addition of another bout of WBV at 0.6g decreased the efficacy of the single-bout 0.6g signal. While the mechanism by which this reduction occurred is unclear, it may imply that a dose of two bouts of 0.6 WBV is too high for the growing mouse skeleton.

In summary, these data indicate that the design of whole body vibration regimes with optimal efficacy afford the optimization of the signal and the number of daily bouts. Whether an optimized design may provide the basis for a non-pharmacological and safe means to increase peak bone mass and ultimately, reduce the incidence of osteoporosis or stress fractures later in life, remains to be determined.

Key Research Accomplishments

- Low-level (0.3g) whole body vibrations applied for only 15 min/d can be anabolic to trabecular bone, structurally enhance bone morphology, and are not detrimental to bone growth or cartilage.
- The efficacy of extremely low-level mechanical vibrations can be enhanced by altering the number of loading sessions or by increasing the magnitude of the stimulus.
- The increase in bone formation induced by WBV is accomplished without negative effects on bone's chemical matrix properties.

Reportable Outcomes

1. Peer-Reviewed Manuscripts Directly Supported by this Grant (2005-2006)

Judex, S., Zhong, N., Squire, M., Ye, K., Donahue, L.R., Hadjiargyrou, M., Rubin, C.T. (2005) Mechanical modulation of molecular signals which regulate anabolic and catabolic activity in bone tissue. *Journal of Cellular Biochemistry* **94**(5), 982-94.

Busa, B., Miller, L.M., Rubin, C.T., Qin, Y.X., Judex, S. (2005) Rapid establishment of chemical and mechanical properties during lamellar bone formation. *Calcified Tissue International* **77,** 386-394.

Murfee, W.L., Hammett, L.A., Evans, C., Xie, L., Squire, M., Rubin, C., Judex, S., Skalak, T.C. (2005) High-frequency low-magnitude vibrations suppress the number of blood vessels per muscle fiber in mouse soleus muscle. *Journal of Applied Physiology* **98,** 2376 - 2380.

Xie, L.Q., Jacobson, J., Choi, E., Busa, B., Donahue, L.R, Rubin, C.T., Miller, L.M., Judex, S. (in press). Low-level mechanical vibrations may influence bone resorption and bone formation in the growing skeleton. *Bone*.

2. Conference Proceedings and Abstracts Directly Supported by this Grant (2005-2006)

Busa, B., Miller, L.M., Rubin, C.T., Qin, Y.X., Judex, S. (2005) Rapid establishment of chemical and mechanical properties during lamellar bone formation. *Calcified Tissue International* **77,** 386-394.

Judex, S., Little, W., Rubin, C., Donahue, L., Miller, L. (2005) Accretion of bone quantity and quality in the developing murine skeleton. 27th Annual Meeting of the American Society of Bone and Mineral Research, Nashville, TN. *Journal of Bone and Mineral Research* ***20*** *(S1), p. 318.*

Xie, L.Q., Busa, B., Donahue, L.R, Rubin, C., Miller, L.M., Judex, S. (2005). Low-level mechanical vibrations enhance bone formation without loss of bone quality in the growing skeleton. 27th Annual Meeting of the American Society of Bone and Mineral Research, Nashville, TN. *Journal of Bone and Mineral Research* ***20*** *(S1),* p. 349.

Xie, L.Q., Donahue, L.R, Rubin, C., Judex, S. (2006). Doubling the bouts or acceleration magnitude may increase the efficacy of low level vibrations in the growing skeleton. *Transactions of the Orthopaedic Research Society*, Chicago, IL.

Conclusions

The anabolic effect of extremely low-level whole body vibrations has been previously demonstrated in the adult skeleton of a variety of species. Our data indicate that these signals may not only be anabolic but may also be anti-resorptive in the growing skeleton. Further, they suggest that the signal can be optimized by altering the number of loading cycles as well as the magnitude of the acceleration. The extremely small deformation magnitudes induced in the bone matrix, together with the short duration at which these signals become effective (15 min/d) suggest that vibrations may be a safe and effective intervention to increase peak bone mass – thereby, perhaps, reducing the incidence of stress fractures in army recruits and preventing the occurrence of bone diseases (e.g., osteoporosis) later in life.

References

[1] Xie, LQ**,** Jacobson, J**,** Choi, E**,** Busa, B**,** Donahue, LR**,** Rubin, CT**,** Miller, LM, Judex, S. (in press) Low-level mechanical vibrations may influence bone resorption and bone formation in the growing skeleton. Bone.