Low Intensity Vibrations Attenuate the Age-Related Bone Loss and Immune System Damage Induced by Obesity
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Introduction:
Obesity is a pandemic condition associated clinically with both osteoporosis and immune dysfunction [1]. It is established that the immune and skeletal systems have significant interactions; particularly, cells in the osteoblastic lineage support B cells differentiation in the bone marrow niche [2]. Hence, it is plausible that a treatment effective for osteoporosis may also improve the compromised immune system in an obesity condition. Mechanical signals in the form of low intensity vibrations (LIV) have been shown to be anabolic to bone via modulating bone marrow cells development [3]. Hence, in this study, we tested the hypothesis that LIVs can simultaneously reduce the negative impact of obesity on both the bone quantity and immune system in adult.

Method:
This is a two-phase experiment with twenty 2mo old male C57BL/6J mice randomly assigned to four groups: (1) high fat diet (HF), (2) high fat diet and LIV (HFv), (3) regular diet (RD) and (4) regular diet and LIV (RDv) groups (n=5). All animal procedures were reviewed and approved by the University animal care and use committee. As shown in Fig. 1, the first phase of this experiment was only diet treatment for 3 months; obesity condition was induced with a high fat diet (45% kcal from fat) in HF and HFv groups. Following which the second phase was a 4-month LIV treatment (0.2g, 90Hz, 15min/d, 5d/wk; where g=gravitational force) along with the same diet treatment.

Before and after the LIV treatment (i.e., at 3 and 7 mo), tibia trabecular bone volume fraction (TB.BV/TV) and abdominal adipose tissue volume were assessed in vivo at 17μm and 76μm isotropic resolution using micro-computed tomography (μCT). The volume of visceral adipose tissue (VAT) in the lumbar region between L1 and L5, and the metaphyseal region of proximal tibia (960μm thickness and 480μm distal to the growth plate) were quantified in the 3D reconstructed μCT images using custom algorithms [4-5].

At sacrifice, both peripheral blood and bone marrow (BM) were harvested and analyzed with flow cytometry to quantify B cell population positive for B220 marker [6]. Additionally, total RNA was extracted from the BM for real time RT-PCR to evaluate the gene expression levels of B220 and TRAP, genes expressed during the immune B cell and bone-resorbing osteoclast development respectively. Statistical significance between groups was evaluated by one-way ANOVA, and correlation between adipose burden and B cell population was tested by Pearson correlation.

Fig. 1: Timeline showing a two-phase experiment in which all subjects were treated with the assigned diet condition before the LIV treatment begun for the RDv and HFv groups in the second phase.

Result:
At the end of the first phase, the high fat diet treatment increased the VAT volume by +14% in both HF groups compared to the mice fed a regular diet (Fig. 2A). However, at the end of the experiment, only HF group had significantly (+57%, p<0.05) higher VAT volume compared to the two RD groups. Over the second phase of the experiment, RD and RDv groups experienced similar age-related bone loss of -29%, while a high fat diet accelerated that bone loss to -56% in the HF group (p<0.05 vs RD groups) (Fig. 2B). Interestingly, the HFv group has only bone loss of -36% which is comparable to that experienced by RD groups (p>0.05).

Apart from the effects on obesity on bone and fat, Fig. 3A shows that the HF group suffered from significant reductions of B cell populations both in the BM (-47%, p=0.01) and blood (-36%, p=0.045). Interestingly, however, LIV significantly increases circulating B cells of HFv compared to HF (57%, p=0.018) and restored it to the level of RD (Fig. 3A). Additionally, Pearson’s correlation showed that both circulating (r=0.519, p=0.39) and BM B cells (r=-0.730, p=0.011) have significant negative correlations with VAT.

Compared to the RD groups, B220 and TRAP gene expression levels of HF was upregulated significantly by +62% (p<0.02) and +88% (p<0.03) respectively, but these levels of HFv were not significantly higher than the RD groups (p>0.05) (Fig. 3B).

Discussion:
Not only did the long-term high fat diet treatment induce significant increase in adipose burden, but it also negatively impacted the bone quantity, immune system and bone marrow cell development. Even though an obesity condition was induced before the 4-month LIV treatment began, LIV mitigated the adipose burden in the VAT of the HF group compared to the HF group. VAT is known to be more predictive of obesity-induced pathologies, such as type 2 diabetes and coronary heart disease, than either the total or subcutaneous adiposity tissue alone [7-9].

High fat diet suppressed the immune system as shown by the significant reduction of B cells [10]. Also, the Pearson’s correlation test showed that the increasing adipose burden was associated with the decline of the immune cells and hence immune surveillance. In other words, by restoring the B cell population and lowering the adipose burden, LIV may be beneficial to the immune system of the HF group. Furthermore, compared to the RD groups, B220 gene expression was upregulated significantly in the BM of HF group, but not in the HFv, suggesting the necessity of HF group to increase efforts in maintaining normal B-cell counts in the circulation.

Although, not surprisingly, all mice showed age-related trabecular bone loss over the second phase of the experiment (between the age of 5 to 9mo) [11], the high fat diet treatment significantly amplified the bone loss. A possible mechanism is a net increase in bone resorption in the bone remodeling process by increasing osteoclast number or activities as indicated by the increase in osteoclast-related TRAP gene expression.

Significance:
Even in the face of adverse effects of diet-induced obesity, mechanical signals in the form of low intensity vibrations may simultaneously be an effective treatment for both accelerated age-related bone loss and compromised immune system. This treatment may potentially be applicable to the less mobile and fragile patients who suffer from these similar pathologies.

References: