# Evaluation of the multi-stimuli on osteoporosis in the mice

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## SUMMARY
The aim of this study is to evaluate the effects of the multi-stimuli on the rat tibia with morphological characteristics. Twenty female C57BL/6 mice (12 weeks old) were used for the experiment. The study was carried out on four groups of animals each consisting of five mice. Four groups of mice were ovariectomized. Animals were scanned at 0 and 2 weeks after ovariectomy by using micro computed tomography to estimate morphological characteristics of tibial trabecular bone. Morphological analysis showed that structural parameters of multi-stimuli group appear significantly better phase in BV/TV, BS/BV, Tb.Th, Tb.N, Tb.Sp, and Tb.pf than single stimulation groups. However, single stimulation groups didn't show significant effect on tibia with Sham group. This study suggests that multi-stimuli may restrain the change as the degenerate phase on osteoporosis in the mice tibia.

## INTRODUCTION
A lot of researches have been made an attempt to study the pharmacological or non-pharmacological treatment for osteoporosis.[1-3] Non-pharmacological treatment should be considered for an osteoporotic patient, because the pharmacological therapy over long periods can cause side effects.[3] Non-pharmacological therapy includes stimulation through physical and kinematics. Up until now, each single therapy in non-pharmacological treatment has been researched.[4-5] However, multiple therapies for osteoporosis were not researched. Thus, the aim of this study is to evaluate the multi-stimuli effects of multiple therapies in the mice tibia with morphological characteristics.

## METHODS
Twenty 12-week-old female C57BL/6 mice were used and allocated randomly into four groups; Sham (Sham, n=5), Vibration (Vib, n=5), Laser (Laser, n=5), and Laser plus Vibration (LV, n=5). All mice were ovariectomised (OVX) to induce osteoporosis for 2 weeks. Right tibiae of mice in Vib, Laser, and LV were stimulated for 2 weeks (3 days per week, Vib: 6Hz, 1500cycle, 2000 µstrain). Right tibiae in each mice were scanned by using in-vivo micro-CT (Skyscan 1076, Bruker AXS, Germany) at 0 week (before to stimulation) and after stimulation of 2 weeks. From acquiring images, structural parameters (BV/TV, bone volume/total volume, %; BS/BV, Bone surface/volume ratio, $mm^{-1}$; Tb.Th, trabecular thickness, mm; Tb.Sp, trabecular separation, mm; Tb.N, trabecular number, $mm^{-1}$; Tb.Pf, Trabecular pattern factor, $mm^{-1}$) were measured and calculated by CT-AN (Bruker AXS, Germany). All procedures were performed under a protocol approved by the Yonsei University Animal Care Committee (YWC-111221-1). All data were presented as mean and standard error and t-test used to statistical analysis ($p < 0.05$).

## RESULTS AND DISCUSSION
All structural parameters of LV at 2 weeks were greater than others (Table 1). The relative variations of structural parameters showed in Figure 1 (1 at 0 week). The relative changes in the BV/TV, Tb.Th, and Tb.N in the LV group were significantly higher than those in Sham group ($p < 0.05$). The relative changes in the BS/BV, Tb.Sp, and Tb.Pf in the LV group were significantly lower than those in the Sham, Vib, and Laser groups (except Tb.Pf of Sham group, $p < 0.05$). There were differences in the relative changes in the BV/TV and Tb.Th between single stimulation groups and multi-stimulation group ($p = 0.058$ and $0.051$, respectively). In addition, there were no significant changes in BV/TV, BS/BV, Tb.Sp, Tb.N, Tb.Pf between Sham and single stimulation groups. However, there were significantly changes in the BS/BV, Tb.Sp, Tb.N, and Tb.Pf between single stimulation groups and multi-stimulation group ($p < 0.05$).

Morphological characteristics are worsened in Sham group than single stimulation and multi-stimulation groups. Tibial trabecular bone quantity in single stimulation groups at 2 weeks was inclined to increase than that in sham group ($p > 0.05$). Furthermore, multi-stimulation for 2 weeks makes bone mass better than in single stimulation. These results suggested that multi-stimulation for 2 weeks would be better bone quantity to osteoporotic patient. In addition, single stimulation for 2 weeks might suppress a loss of bone quantity. We will continue to investigate to treat or prevent the osteoporosis through multi-stimuli research.

## CONCLUSIONS
In this study, we evaluated the effects of multi-stimuli for treatment or prevention of osteoporosis. Taken together, the results showed that multi-stimuli may suppress the continuous progress of bone deterioration, thinning and disconnectivity. Therefore, multi-stimuli may be effective for treat and prevention of bone loss.
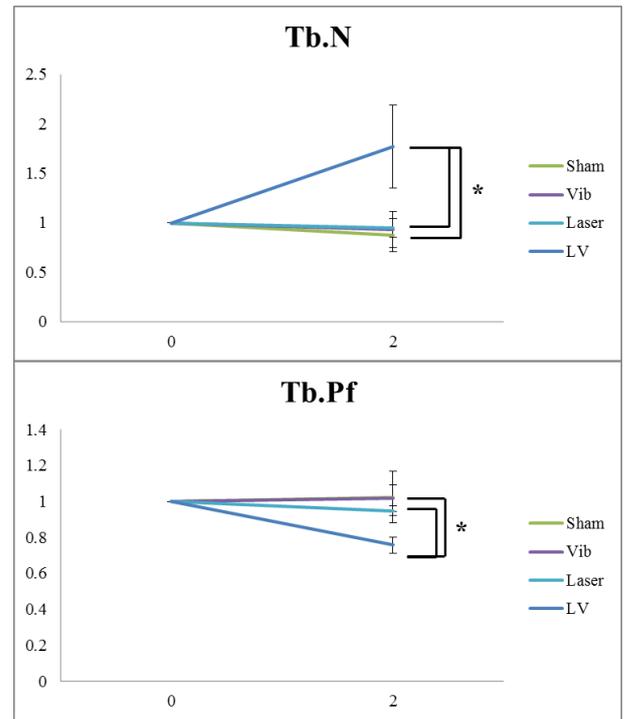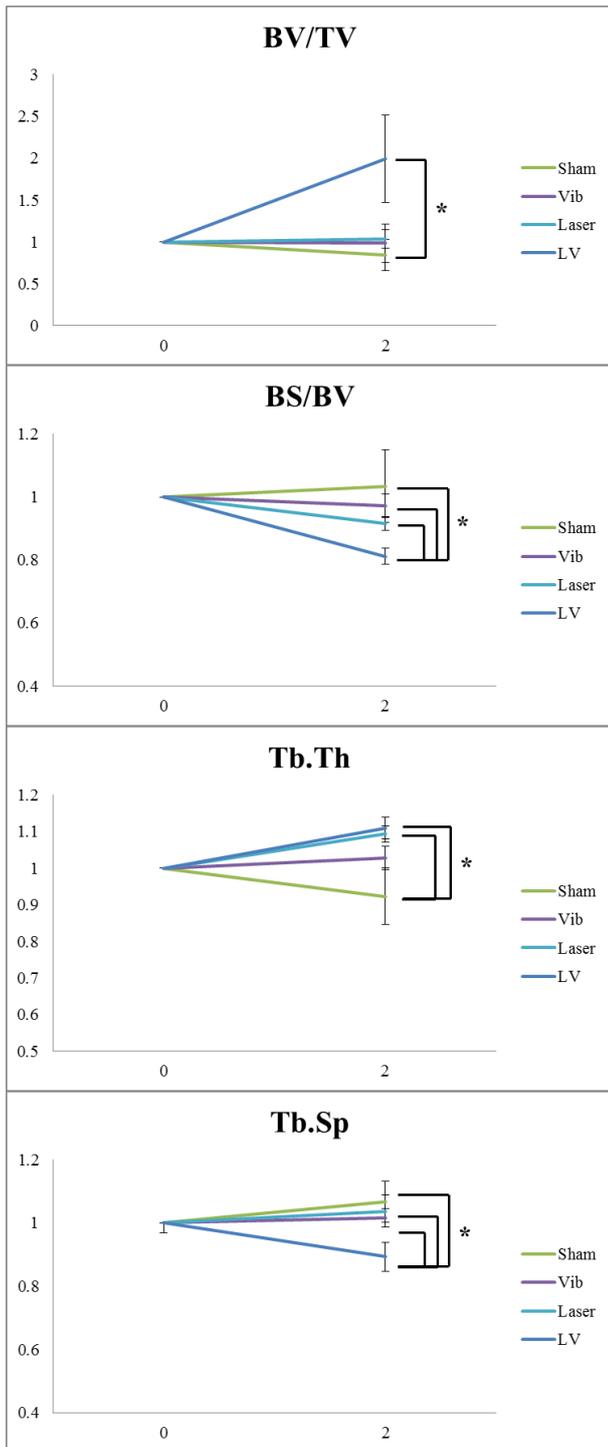
**Figure 1**: Relative variations of structural parameters on the trabecular bone (Mean ± SE), * p < 0.05.

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**Table 1:** Trabecular bone parameters in proximal tibial metaphysis on 2th week (mean ± SE).

| | BV/TV [%] | BS/BV [1/mm ] | Tb.Th [μm] | Tb.Sp [μm] | Tb.N [1/mm] | Tb.Pf [1/mm] |
|---|---|---|---|---|---|---|
| Sham | 3.3±0.47 | 67.63±1.79 | 75.53±0.08 | 603±0.02 | 0.43±0.05 | 26.22±1.42 |
| Vib | 3.03±0.22 | 68.47±1.51 | 78.56±0.01 | 610.55±0.01 | 0.38±0.03 | 28.14±1.22 |
| Laser | 3.64±0.47 | 68.57±2.98 | 78.11±0.01 | 549.85±0.03 | 0.46±0.05 | 27.12±1.11 |
| LV | 5.92±1.41 | 62.05±2.48 | 81.29±0.01 | 514.68±0.03 | 0.71±0.15 | 23.25±1.51 |