INTRODUCTION:
Osteoporosis and osteopenia are diseases, which affect a large percentage of the population, mostly in elderly. They are characterized by an increased risk of fracture at critical skeletal sites, e.g., hip and spine. Complications associated with healing include nonunion and decreased quality of life.

Low energy mechanical stimulation, e.g., using low intensity pulsed ultrasound (LIPUS), can accelerate remodeling and healing of fractures in bone. It is effective at resolving all types of nonunion, accelerates in vivo all stages of the fracture repair process: inflammation, soft callus formation, hard callus formation. [1-3]

The objective of this study was to evaluate the potential for LIPUS to accelerate fracture healing under disuse conditions using hindlimb suspension (HLS) rats.

METHODS:
The experimental protocol was approved by Stony Brook University IACUC. A transverse fracture was created at the mid-diaphysis of six-month old Sprague Dawley rats (275g ± 30g). A K-Wire was inserted into the femur from the distal femoral condyle [4]. Animals were divided into four groups:
1. Age match fracture (AMF), without HLS:
   a. Ultrasound treatment with signal output, AMF (n=5)
   b. SHAM ultrasound control, but no signal output (n=5)
2. Hind limb suspension with femur fracture:
   a. With ultrasound treatment, HLS+LIPUS (n=4)
   b. No ultrasound treatment, HLS only (n=4)

24 hours following fracture, LIPUS was applied to experimental groups, (1.5MHz, 1KHz pulsed, 20% duty cycle, 30mW/cm^2 intensity, SATA) 20 minutes a day, 5days a week, for a total of 3 weeks.

2% Isoflurane anaesthesia was administered to both experimental (ultrasound treated) and sham control groups. An X-ray was taken at the femoral fracture to track the healing once a week throughout the experiment.

After 3 weeks, all animals were euthanized. The bone samples were carefully harvested. After carefully removing the K-wire from the femur, the callus density and quality was examined using microCT (SCANCO uCT40) with a resolution of 18um, 5mm (278 slices), covering the callus region. The protocol is able to calculate newly mineralized callus within the contour lines.

RESULTS:
Callus mineralization distribution (Figure 1), shows that group 2a (suspended rats treated with ultrasound), had a small peak distribution located between 750 and 800 mg HA/ccm points. This indicates that its callus mineralization was much higher than that of the other three groups (see Figure 2).

There were no significant differences found between the remaining 3 groups, 1a, 1b and 2b. However, it is important to note that group 2b, (suspended rats without ultrasound treatment), had lower mineralization around 600 mg HA/ccm point, making it the lowest in the 400-600 mg HA/ccm, mineralization range (Figure2).

CT threshold parameters (0.8, 1, 250) were applied throughout the study to determine group average and standard deviations for BV/TV (Table 1). The % change is calculated relative to the SHAM control group and reflects the percent change after 3 weeks.

Group 2a (suspended with ultrasound treatment) had the largest bone volume fraction, BV/TV, at least 20% larger than other groups. Group 1a, AMF, (unsuspended but treated with LIPUS), had 4% more bone fraction than the SHAM control group (1b). At threshold 250, to bone fraction BV/TV data between group 2a and 2b(HLS with and without LIPUS), one-way ANOVA is applied, and the P-value is 0.235

ACKNOWLEDGMENT: This work is kindly supported by NIH and National Space Biomedical Research Institute through NASA contract, and US Army Medical Research.

REFERENCES: