INTRODUCTION: Osteoporosis gives rise to fragile bones that have higher fracture risks due to diminished bone mass and altered bone microarchitecture [1]. Mechanical loading mediated bone adaptation has demonstrated promising potentials as a non-pharmacological alteration for both osteogenic response and attenuation of osteopenia [2]. Intramedullary pressure (ImP) has been proposed as a key factor for fluid flow initiation and mechanotransductive signal inductions in bone. It is also suggested that integration of strain signals over time allows low-level mechanical strain in the skeleton to trigger osteogenic activities [3]. The potential bone fluid flow induced by strain and ImP mediates adaptive responses in the skeleton [4]. Previous in vivo studies using oscillatory electrical stimulations showed that dynamic muscle contractions can generate ImP and bone strain to mitigate disuse osteopenia in a frequency-dependent manner. To apply ImP alteration as a means for bone fluid flow regulation, it may be necessary to develop a new method that could couple external loading with internal bone fluid flow. In order to further study the direct effect of ImP on bone adaptation, it was hypothesized that external dynamic hydraulic stimulation (DHS) can generate ImP with minimal strain in a frequency-dependent manner. The aim of this study was to evaluate the immediate effects on local and distant ImP and bone strain induced by a novel, non-invasive dynamic external pressure stimulus in response to a range of loading frequencies.

METHODS: All experimental procedures were approved by Stony Brook University IACUC. Direct and simultaneous ImP and bone strain measurements under DHS were performed on three 15-month old female Sprague-Dawley virgin rats with a mean body weight of 425±11g. For the ImP measurement, a 1mm hole was carefully drilled into both right tibial and femoral marrow cavity from the proximal tibia and distal femur respectively. Guided by a 16-gauge catheter, a microcardiovascular pressure transducer (Millar Instruments, TX) was the inserted into each of the tibial and femoral marrow cavities. The catheter and the pressure transducer apparatus sealed tightly within the drill holes. For bone strain measurement, a single element strain gauge (120Ω, factor 2.06) was firmly attached to the flat surfaces of the same tibia and femur at the mid-diaphyseal regions (Figure 1).

DHS was then applied to the operated tibia by placing an inflatable cuff around the mid-tibia. The pressure stimulation was achieved by 40mmHg static pressure + dynamic pressures driven by a function generator that was set at 1.5V over a range of frequencies from 1Hz to 4Hz over 0.5Hz intervals, then from 4Hz-10Hz over 1Hz intervals. For each animal, the entire frequency spectrum was repeated for at least three times.

RESULTS: Approximately 1mmHg tibial ImP and 5mmHg femoral ImP were generated by the normal heart beat. There were no significant differences detected at this stage. However, DHS showed noticeable effects on ImP induction in the tibia. The observed ImP (p-p) values were induced in a nonlinear fashion under DHS. The most increases of ImP (p-p) values were within 2-3Hz range and peaked at 11.9±5.2mmHg at 2Hz (Figure 2). The induced ImP values normalized to cuff pressure values were on the order of 0.22±0.11mmHg at 2Hz, 0.20±0.10mmHg at 2.5Hz, 0.18±0.10mmHg at 3Hz, 0.19±0.04mmHg at 6Hz, and 0.09±0.04mmHg at 10Hz (Figure 3). Maximal bone strain measured in all loading frequencies was less than 3µε. No detectable inductions in ImP or bone strain were observed in the femur.

DISCUSSION: This study first time measured ImP and bone strain in both tibia and femur simultaneously under DHP, demonstrating that these stimuli can induce ImP in neither the stimulated bone without detectable bone strain on the same bone, nor effect distant bone. Induced ImP by DHS indicated a frequency-sensitive manner. DHS provides an external dynamic pressure stimulus that may build up vessel pressure gradient, triggering blood flow into the bone that affects ImP. ImP-induced bone fluid flow is known as an important mechanism for bone adaptation. Such adaptive effects have recently been shown in our 4-week in vivo study using a rat disuse osteopenia model. These results together imply the potential mitigation effect of DHS in bone loss.

One observation that we found in this study was in contrast to previous studies, in which DHS was more effective within the lower frequency range. This may be due to the reason that the viscoelastic property of muscle and hydraulic loader may have filtered out high frequency components. A repeated experiment is undergoing currently, with a larger sample size aiming to achieve higher statistical significance. Further studies will investigate into the effects of DHS on the downstream mechanotransductive signaling pathways.

In conclusion, DHS effectively induce ImP with minimal bone strain in the stimulated bone, which implies its potential as an effectively, non-invasive intervention for osteopenia treatment.

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