INTRODUCTION:
Mechanical loading is necessary for proper development and maintenance of the musculoskeletal system. There is a great deal of research in small animal models investigating the musculoskeletal response to increased mechanical loading, as well as total unloading (e.g. tail suspension). However, there has been little investigation into the physiological effects of partial weight bearing (PWB) environments, which may serve as models for clinically relevant conditions such as space flight, bed rest, immobilization, stroke, cerebral palsy, muscular dystrophy, spinal cord injury, or reductions in physical activity.

To address this limitation, we have developed a novel model of titrated weight bearing that offers a unique capability for exploring the chronic effects of reduced quadrupedal loading in mice. The system allows studies with controlled exposure to 20-80% weight bearing compared to normally loaded controls [1]. To quantify changes to the mechanical environment of mice in our PWB system, we measured in vivo tibial strain during treadmill walking while supporting 20-80% body weight compared to full body weight locomotion. We hypothesized that mice supporting a lower percentage of their body weight would have reduced tibial bone strain during locomotion, but that this reduced bone strain would not be linearly related to the amount of weight bearing due to changes in gait.

METHODS:
During PWB, the mouse is supported by a forelimb vest and tail wrap. Suspension is provided by a coiled line spring with adjustable length; a laboratory scale is used to adjust the level of PWB (Fig 1).

Single element strain gages (UFLK-1-11-L, 120Ω, TML Gages, Japan) were placed on the anterior-medial surface of the left proximal tibia of 11 wk-old female BALB/c mice (n = 7) under isoflurane anesthesia (2-3%), and were affixed with cyanoacrylate (Fig. 1). After recovering from isoflurane anesthesia, in vivo strain data was recorded during treadmill locomotion at a constant speed (8.5 m/min) at PWB levels of 20, 40, 60, and 80% body weight (BW), as well as jacketed and unjacketed running (100% BW). Strain data were collected at 100 Hz using a quarter bridge configuration, and filtered in MATLAB using a 15 Hz 9th order low-pass Butterworth filter. Animals were acclimated to the treadmill and suspension jackets for several days prior to surgery. All studies were approved by the IACUC of BIDMC.

Video footage was used to determine “typical” footfalls, and mean peak-to-peak strain during locomotion was determined for each condition. In addition, loading frequency (steps/s) was measured for the gaged limb, and qualitative differences in gait due to PWB were noted. A minimum of 20 footfalls were averaged for each condition. Gage placement was verified by scanning with micro-computed tomography.

RESULTS:
Mean peak-to-peak tibial strain during jacketed and unjacketed treadmill running was similar: 116.9 (range: 79.7-161.1) and 115.0 (range: 89.3-159.2) microstrain (με), respectively. PWB did not significantly decrease peak-to-peak cortical strain, regardless of the percentage of body weight that was being supported (conditions were compared using paired t-tests). Mean peak-to-peak bone strain was 125.0 με (range: 76.9-152.6) at 80% BW, 99.4 με (range: 43.7-158.0) at 60% BW, 118.8 με (range: 65.4-195.2) at 40% BW, and 103.8 με (range: 67.6-134.6) at 20% BW.

Step frequency during locomotion decreased significantly as body weight percentage being supported by the mouse decreased. During 100% BW jacketed and unjacketed running, the gaged leg of the mouse took 2.8±0.18 and 2.7±0.23 steps/s, respectively. Step frequency was significantly reduced (p < 0.05) during treadmill walking at 80% BW (2.6±0.14 steps/s), 60% BW (2.5±0.14 steps/s), and 40% BW (2.5±0.14 steps/s), compared to other conditions, with the gaged leg taking only 2.2±0.17 steps/s.

Mice in PWB, especially when supporting a low percentage of body weight, tended to “gallop” or leap forward rather than normal quadrupedal walking, and were more likely to take long strides with greater extension of the hind limbs.

DISCUSSION:
Contrary to our original hypothesis, we did not observe a decrease in cortical bone strain during treadmill walking as a result of PWB suspension, regardless of the amount of body weight being supported. Although this finding warrants further investigation, observed differences in gait and behavior by mice in PWB may partially explain this observation. The decrease in the number of steps (while walking speed was kept constant), as well as qualitative differences in gait between walking at full body weight vs. high levels of PWB point to behavioral changes that may keep the magnitude of bone strain at a relatively consistent level during controlled locomotion.

Our observations regarding gait kinematics are also supported by a previous study of PWB of mice performed in our lab [1]. This study used a treadmill and force plate to compare gait patterns and ground reaction forces of jacketed, unjacketed, and PWB mice. It was found that jacketed mice had significantly shorter duration and length of gait cycle, and that PWB significantly lengthened the swing phase of gait in both the forelimbs and hindlimbs, although total duration and length of the cycle were not different than jacketed controls. Additionally, suspension caused mice to preferentially shift their weight towards the rear during locomotion. This study also found that peak vertical ground reaction forces were decreased 85.3% and 70.7% in the hindlimbs of 38% BW and 16% BW PWB suspended mice, respectively, although the transverse ground reaction forces were relatively greater in PWB mice than controls.

Altogether, these results indicate that changes in gait and behavior in mice subjected to PWB may preserve bone strain magnitudes during treadmill walking. It is possible that mechanical loading serves a proprioceptive function during gait, and that ground/muscle forces that cause these bone strain magnitudes may be instinctively sought by locomoting animals. This is a potentially important finding, as decreases in bone mass due to PWB (which we have observed in preliminary studies in our laboratory) may be due altered bone strain patterns or changes in animal behavior and activity level rather than bone strain magnitude.

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