PARTIAL WEIGHTBEARING WITH A FORCE SPECTRUM TYPICAL OF NORMAL AMBULATION IMPROVES BONE STRENGTH IN HINDQUARTER SUSPENDED RATS

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Introduction: Previous work has demonstrated that episodes of dynamic loading prevent the relentless loss of mass in otherwise immobilized bone while static loading is ineffective. The best type of dynamic loading characterized by peak magnitude, repetitions and rate of administration, etc is not as well understood. These features of dynamic loading become critical when designing a mechanical countermeasure for long-term spaceflight as is anticipated for a mission to Mars. The combined effects of artificial gravity and prophylactic exercise are examined in this context using the hindquarter suspended rat as a ground-based model.

Methods: The most prevalent ground-based animal bone model for weightless spaceflight is the hindquarter suspended rat. We employ a modification of this model enabling titration of the loadbearing forces on the forelimb bones that we then analyze. Our hindquarter suspended rats used their front legs to walk on a computer controlled servo forceplate so that the dynamic forces could be managed in the frequency domain (Fourier spectrum). The foreleg weightbearing at rest was limited to 50% of the average load the free quadrupedal rat would experience. The forceplate is a glass platform supported by an 18” diameter speaker cone filled with expanding polyurethane foam. An infrared optical sensor attached to the speaker cone yields a voltage linearly related to vertical displacement of the glass platform. The speaker motor was driven in negative feedback so that minute displacements were proportional to the vertically applied force on the glass. The platform was sufficiently stiff that a 5 gm weight registered the same voltage regardless of where the weight was placed on the platform. The dynamic force on the paw was computed as a product of the apparent mass of the animal on the platform at rest and the acceleration of the platform determined from the second derivative of optical sensor output. The mass of the animal on the platform may be varied by adjusting tension on the tether suspending the animal. Because the platform displacement was small, the mass of the animal resting on the platform remained essentially constant.

Axial strain on the humeral cortex was measured using precalibrated single axis strain gauge implanted on the mid diaphyseal shaft at the level of the pectoral-humeral crest. The contralateral forelimb was tucked into a thoracic harness so that the platform supported the animal by a single foreleg. Simultaneous measurements of dynamic force on the rat’s paw were recorded with bone strain measurements for rats hopping on a single forelimb with the forceplate tuned to resonate at 3 Hz alternating with 16 Hz. In these experiments, the animal’s leg muscles actively resisted impact forces. Similar recordings were made with the forceplate passively oscillating the animal at 3, 16, 50, 100, 200 and 500 Hz. These studies were distinguished by continuous contact between the platform and the rat’s paw, while the leg muscles resisted the inertia of the rat’s body as the platform moved up and down. In all cases the peak force was constrained to be the same so that the relationship between paw force and bone strain could be compared as a function of the frequency spectrum of applied force.

The effect of 35 days of chronic exposure to specified dynamic loading on humeral strength was evaluated in rats suspended at 50% weightbearing on platforms that resonated at either 3 Hz or 16Hz or with every paw impact. Other animals, similarly suspended were exposed to passive oscillation at 3 Hz, 16 Hz or 500 Hz for 2 hours daily. Controls consisted of one group of rats suspended on nonmoving platforms at 50% weightbearing and one group of nonsuspended free roaming animals. Changes in humeral strength were assessed using pQCT measurement of diaphyseal cross-sectional moments of inertia on the first to the last day in each animal. Bone was double labeled with calcinein green in the final 10 days of the study for histomorphometric analysis of formation and resorption rates.

Data analysis reported using mean +/- SEM with statistical significance (p<0.05) was assessed by ANOVA, Student’s t, and Fisher’s PLSD.

Results:
1. Hindquarter suspended rats lose significant bone mass and strength from the humerus when the forelimbs bear 50% of the weight of their free roaming controls.
2. An impact on a surface resonating at 16 Hz resulted in a higher humeral strain than an impact on a surface resonating at 3 Hz. This finding is consistently observed when the peak force of impact is the same on the 16 and the 3 Hz surface.
3. Peak humeral bone strain was significantly lower (about 1/10th) when the floor surface was passively oscillated than when the rat’s paw made an impact on the floor resonating at the same frequency even though the displacement of the floor was the same in both cases.
4. The relationship between peak numeral bone strain and peak force on a passively oscillating surface was linear only for very small strains.
5. Peak humeral bone strain was significantly smaller when the floor was passively oscillated at high frequency (10-500 Hz) than when the floor was passively oscillated at low frequency (< 4 Hz).
6. Daily exposure of the forelimb to either a resonant floor or a passively oscillating floor operating about 3 Hz consistently resulted in stronger humeri (by pQCT moment analysis) than did exposure to 16 Hz resonance, passive oscillation or passive oscillation at 500 Hz. This finding was observed with constraints that the average peak force and mechanical work was titrated identically in platforms resonating at 3 and 16 Hz. Passive oscillation also produced the same peak force as the resonant platform with the exception that the 500 Hz oscillation produced higher forces than the 3 and 16 Hz oscillation. Histomorphometric analysis is pending.

Discussion:
1. Artificial gravity at 0.5G or prolonged exposure to Martian gravity 0.38G is unlikely to prevent significant bone loss.
2. Higher bone peak strains observed from impacts on a floor with resonant properties above the spectrum of normal ambulatory forces result from a stiffer floor (with the higher natural frequency).
3. Passive oscillation of the floor is less effective than active movement of the animal in producing bone strain because antigravity muscles stiffen the leg during impact as opposed to increasing compliance during passive movement. Muscles rather than loads on the paw impose the predominant bone strain.
4. Jointed construction of the leg with multiple muscle insertions prevents mathematical modeling of the leg as a simple array of springs and dashpots except for very small loads.
5. The leg behaves as a low pass filter with passively applied loads.
6. Dynamic mechanical loading activity offered significant protection of bone strength when applied in the normal ambulatory spectrum, but was inconsistently effective when applied at high frequencies. The inertia of the leg effectively limits muscle responses to very rapidly changing external loads. Because muscle forces dominate regional specific bone shaping processes, dynamic loads that fail to be directed through muscles are unlikely to offer physiologic protection in settings of reduced weightbearing such as spaceflight.

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