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INTRODUCTION

Ameliorating bone loss with prolonged exposure to weightlessness remains a top priority and is rated a Tier I risk in the Critical Path Roadmap. LeBlanc et al. (1996) have aptly documented site-specific decreases in bone mineral density (BMD) ranging from 0.4% to 1.7% per month in cosmonauts flying missions of 4–14 months in duration. This rate of loss is some 10 times more rapid than that observed in the typical post-menopausal woman. Given the strong relationship between BMD and resistance to fracture, these data lead to serious concern that a fracture event is highly likely during a prolonged Lunar or Mars mission. Such an event would critically impact on crew ability to perform required work tasks; unknown is how well that fracture might heal in a microgravity or partial-gravity environment. Intertwined with the bone loss issue is that of muscle atrophy and impaired muscle function both during flight and upon return to normal or partial g environments. Significant muscle atrophy ranging from 4-10% occurs even over the first 8 days of flight, particularly in the anti-gravity muscles of the leg and back (LeBlanc et al., 1995). Decrements in muscle strength as well as integrated functions like upper limb motor control and gait coordination also impact in significant ways on mission operations. Successful countermeasures for changes in muscle function could contribute significantly to minimizing loss of bone mineral density; the reverse may also be true. We offer a brief summary of investigators’ presentations during this year’s Bioastronautics Workshop, concluding with a discussion of gaps in knowledge and future directions the participating investigators believe are of highest priority.

SUMMARY OF PRESENTATIONS

Bone and Soft Connective Tissue

In the area of bone biomechanics, Whalen et al. (2001) have quantified the role of reduced daily loading histories on bone geometry and focal BMD changes in the calcaneus and the distal tibia. The daily loading history of lower limb bone can be modeled by the vertical component of the ground reaction force, which includes both body weight and a dynamic force component, as measured by the NASA GRF Activity Monitor. This model has been validated in a population of young and elderly women runners and non-runners, in which BMD within focal regions of the calcaneus can be predicted. Integration of pQCT methodology with correction for beam-hardening errors will enable improved precision in this predictive model. Cavanagh et al. (2001) will monitor ground reaction forces, joint angles and muscle activity (via surface electromyography (EMG) in three International Space Station crew members in-flight and in 1 G conditions on Earth using the Lower Extremity Monitoring Suit (LEMS). These data will be collected over four 12-hour working days in each environment and should provide invaluable data on mechanical loads imposed on the skeleton in microgravity as well as actual muscle activity in the lower limbs. The LEMS has been validated in community-dwelling older men; preliminary data indicate trends differentiating high and low BMD subjects by daily mechanical loading and net neural drive (from EMG’s). Wootten et al. (2001) are investigating the usefulness of the mechanical response tissue analyzer (MRTA) in estimating changes in vivo in the bending stiffness of long bones. Early results from young women engaging in a strenuous 6-week running and resistance training program indicate that MRTA may not be sensitive enough to detect transient changes in bone stiffness. However, in longer duration studies it may prove a useful adjunct to bone mineral densitometry. Finally, Cavanagh and Sharkey (2001) will be using the Zero Gravity Locomotion Simulator (ZLS) to acquire estimates of ground reaction forces and muscle activity for humans during walking and running. These estimates will then be input data for a second simulator, the Dynamic Gait Simulator, to directly measure bone strains on cadaver limb bone while reproducing the kinematics used on the ZGL. These experiments will provide key data on the relationship between external loading and bone strain during treadmill exercise in orbit.

A number of studies investigated mechanical and/or pharmacological countermeasures. Judex et al. (2001) delivered a 90-Hz whole body vibration treatment for 10 min/d to hindlimb unloaded (HU) rats. This intervention significantly attenuated the decrease in trabecular bone formation rate normally seen in the proximal tibia, while simultaneously abolishing the usual increase in gene expression for osteoclast differentiation factor seen.
with disuse. These data imply that the anabolic effect of this mechanical intervention is augmented by its effect on expression of a gene important in maturation of active bone-resorbing osteoclasts. Schultheis et al. (2001) have developed a unique HU rat model that can provide for 50% weightbearing on the forelimbs as a simulation of the partial G forces on the Martian surface. Importantly, they have documented that trabecular bone loss in the humerus does occur even with this partial weightbearing. A mechanical loading intervention simulating normal impact forces of ambulation on the humerus could not prevent this decrease in trabecular BMD, but successfully stimulated an increase in cortical bone mechanical properties by increasing cross-sectional moment of inertia and bone area. Pretreatment with the anti-resorptive agent ibandronate prevented decreases in bone formation rate, geometric and mechanical properties in cortical bone of the 50% weightbearing humerus; it also effectively attenuated loss of trabecular BMD with partial weightbearing.

Shackelford et al. (2001) are testing the efficacy of alendronate, another anti-resorptive agent, and high-intensity resistance exercise training in preventing the bone loss observed with 17 weeks of bed rest in adult humans. Both treatments produced a positive calcium balance in their bed rest subjects. The combination of alendronate at 10 mg/d and resistance exercise appears, from preliminary results, to be the most effective approach in attenuating losses of BMD at most skeletal sites. These results echo those of Schultheis et al. in that the combination of an anti-resorptive agent with the anabolic effects of increased mechanical loading may prove to be the most effective approach. A comprehensive project utilizing a different ground-based model will examine patterns of bone loss in spinal cord injured (SCI) patients, with and without the use of zolendronate, a third-generation bisphosphonate (Shapiro et al., 2001). Femoral CT scans will allow for further applications of finite element analysis modeling for estimating fracture risk with changes in BMD and bone geometry. Concurrent changes in muscle in these patients will be tracked using CT measures of muscle mass and muscle biopsies to study altered fiber histology and protein synthesis/proteolysis.

An alternative to using anti-resorptive agents is described in studies by Smith (2001), which are testing the utility of agonists for estrogen and vitamin D receptors (VDR) as countermeasures for microgravity-induced bone loss. EB1089, a VDR agonist, is able to stimulate osteoblast activity without simultaneously increasing osteoclast activity in vitro. Preliminary results suggest that EB1089 prevents loss of trabecular BMD at the proximal tibia during HU in rats. Raloxifene, an FDA-approved selective estrogen receptor modulator, appears to attenuate trabecular BMD loss with HU in ovariec-tomized rats.

Several studies focus on the recovery of bone from simulated microgravity or disuse. Using cast immobilization of a forelimb in adult dogs for up to 6 months, Schaffler et al. (2001) demonstrated that cortical bone mass does not recover to baseline levels after 12 months of remobilization. However, a compensatory periosteal expansion during recovery thereby increased total bone area by 10-18%, presumably returning bone mechanical properties to baseline values. These data underscore the importance of considering bone geometry in addition to traditional measures of bone mass (e.g. BMD). A new study by Bloomfield et al. (2001) will define the time course of recovery of bone and muscle simultaneously after HU in adult rats, focusing on a hypothesized mismatch in bone and muscle strength early in recovery. Several mechanical loading interventions and two pharmacological countermeasures (parathyroid hormone and growth hormone) will be tested for their effectiveness in promoting bone recovery to minimize that functional mismatch between bone and muscle strength.

Calcium kinetics were addressed by Smith et al. (2001) using stable isotope tracer kinetic studies before, during, and after a Mir flight in three men. These investigators confirmed that the negative calcium balance commonly observed in-flight is due to a very consistent increase in bone resorption (assessed by collagen crosslink excretion) and perhaps also to a decrease in bone formation. Another contributing factor might be reduced calcium absorption at the gut during spaceflight, which appeared consistently suppressed. Additional data from upcoming shuttle and ISS flights will add substantially to the interpretation of these findings. A related study by Rianon et al. (2001) verified that determinations of calcium balance in bed rest subjects is not significantly affected by the amount of calcium in sweat, even if the subjects perform an hour of exercise in a cool environment.

Mechanisms of mechanotransduction in bone cells were explored in elegant in vitro studies exposing osteoblast-like MC3T3-E1 monolayers to fluid shear stress, thought to be an important mediator of increased mechanical loading on whole bones. Duncan et al. (2001) have demonstrated that different intracellular signaling pathways that respond to shear stress are activated depending on the source of increased intracellular calcium: via release of intracellular calcium stores or on rapid entry of extracellular calcium via channels at the cell membrane. These intracellular calcium signaling pathways are also affected by variations in cell attachment to different extracellular matrix proteins. Ryder et al. (2001) utilized the same in vitro model to determine that parathyroid hormone (PTH) reduces the mechanical threshold for these bone cell monolayers to elicit activation of intracellular calcium signaling pathways; i.e., after PTH application these bone cells demonstrate an enhanced response to the same level of shear stress. PTH appears to mediate this effect by activating both voltage-sensitive and
mechanosensitive calcium channels. Delp et al. (2001) described unique studies of alterations in bone blood flow to the unloaded hindlimb in adult rats. Within 10 minutes of the shift to the head-down tilt position of tail suspension, blood flow to most regions of the femur and to marrow decreases significantly. There are further decreases in these blood flow rates after 7 and 28 days of unloading, along with increases in blood flow to bones in the head and forelimb. Decreases in blood flow to unloaded bone precede declines in bone formation rate, and appear to be mediated by both increased vascular resistance and decreased perfusion pressure. Altered blood flow to bone may provide an alternate mechanism for altered patterns of bone remodeling with exposure to weightlessness.

Martinez et al. (2001) described studies in wound healing of knee ligaments during hindlimb unloading. Preliminary data indicate that strength of the femur-ligament-tendon unit during healing is lower in rats subjected to hindlimb unloading than in ambulatory controls. Future experiments will determine if growth hormone injections during hindlimb unloading will have a positive impact on ligament healing. These studies promise to yield valuable data in an area that has had little attention to date.

Lastly, several projects focused on providing in-flight imaging of bone. Seely et al. (2001) are developing a portable, pulsed x-ray source for 2-D dual energy x-ray absorptiometry. The ultimate size of this unit will be under 16 kg with a precision of ± 2%; it will be able to assess BMD at the spine or hip with a low mRem exposure (5 mRem). An alternate technology, the Advanced Multiple Projection DEXA, will provide for 3-D imaging to assess bone geometry changes as well as alterations in BMD (Charles et al., 2001). Its ultimate goal weight is 46 kg; its current precision is ± 1%. It will also be capable of soft tissue analysis, allowing for tracking of skeletal muscle mass changes. Future refinements in software algorithms will allow for radiographic quality images, broadening the operational usefulness of this equipment.

**Skeletal Muscle**

The summaries of presentations in the areas of skeletal muscle are grouped according to the techniques of study that were used. These include molecular and cell biology, magnetic resonance spectroscopy, ultrasound, electromyography, joint position analysis, and resistance exercise.

A number of projects used various molecular and cell biology techniques. Goldberg et al. (2001) employed food deprivation in mice to induce muscle atrophy. They then used cDNA micro arrays to establish the profile of changes in gene expression during muscle atrophy. Under these conditions protein synthesis is decreased and proteolysis increases. 98% of gene transcriptions were not different from controls, and up-regulation of ubiquitin, as well as a generalized decrease in mRNA for many myofibrillar genes and enzymes of glycolytic and oxidative metabolism, was found. The authors located previously unidentified groups of genes, in particular one that they termed “atrophin 1”, whose expression increased nine-fold specifically in skeletal muscle but not in other tissues of food-deprived mice. Goldberg et al. hypothesize that this gene may be found in the degradation of critical regulatory proteins in the atrophying muscles.

Baldwin et al. (2001) used rodent models of functional overload (OL) (by surgical removal of synergist muscles); resistance training (T); or continuous hind limb suspension (HS). Western blot or RT-PCR based mRNA analyses were used to assess regulatory markers of protein translational processes. OL induced an ~4-fold increase in the level of IGF-1 peptide in the first 24 hours of overload and this response was maintained throughout an 8-day period. Additional analyses revealed that the mRNA for both IGF-1 and a muscle-specific variant was increased in both OL and T treated rats. Both OL and T induced increased phosphorylation of markers of protein translation. The authors believe that resistance training augments the potential of muscle growth, whereas unloading induces the opposite based on their respective effects on protein translation/degradation processes.

Vailas and colleagues (2001) focused on musculoskeletal connective tissue metabolism in rhesus monkeys before and after 14 days of space flight using daily urine samples and portions of small muscle biopsies obtained from lower leg extensor and flexor muscles. Connective tissue markers in urine were also studied during and after chronic exposure to hypergravity (2G). These investigators found high daily variability in the urine concentrations of collagen metabolic markers. Collagen crosslink content in the postflight urines were significantly greater in the flight group compared to controls. The muscle biopsy data showed decreased expression of major muscle collagen in the tibialis anterior but no change in the medial gastrocnemius, indicating that muscle collagen adaptation may be dependent on muscle function and/or muscle duty cycle during spaceflight. 2G exposure resulted in significant elevation of markers of bone connective issue degradation midway through the exposure, followed by decline towards the end of the exposure period. The markers were elevated postflight and were still elevated above resting levels in the second week after 2G exposure. These experiments suggest that intermittent exposure to hypergravity as a countermeasure to spaceflight-induced bone loss may itself result in tissue damage.

Tidball et al. (2001) used in vivo methods (rat or mouse hind limb suspension followed by reloading) and in vitro (measurement of muscle cell lysis in co-cultures of muscle cells and selected myeloid cell populations)
assess the contribution of inflammatory cells to muscle injury that occurs during periods of reloading following periods of unloading. They found that the course of muscle injury and repair is determined by interactions between myeloid and muscle cells. They also indicated that muscle unloading may compromise the free radical defense mechanisms of muscle by reducing nNOS expression. They identified increased expression of Leukemia Inhibitory Factor (LIF) in macrophages during reloading after a period of inactivity. The authors suggested that the modulation of LIF may be a future target of gene therapy in order to prevent muscle atrophy.

Kusmerick (2001) proposed an ambitious integrated model of human energetics and mechanics. With a combination approach of \( ^{31}P \text{NMR spectroscopy, ultrasound functional images, biomechanical analyses, and multilevel modeling, he hopes to show that the tissues of the limb have ideal properties and components that render hierarchical modeling feasible and will expand a mathematical model for intracellular energetics to include mechanics and blood flow.}

De Luca and Erim (2001) are using electromyography to understand the effects of microgravity on the control of motor units in human muscle and to determine the time course of recovery after re-exposure to Earth’s gravity. In this pre- and postflight experiment, intramuscular EMG of the first dorsal interosseous (FDI) of the hand and a knee extensor will be collected, as well as surface EMG from all four components of the quadriceps. The authors intend to use signal processing techniques to extract individual motor unit time histories from complex interference patterns.

Edgerton et al. (2001) monitored surface electromyograms from muscles crossing the elbow and ankle joints from four astronauts during a shuttle mission. Two or three 24-hour recording sessions before, during, and after flight were completed. The results showed that activation of tibialis anterior increased substantially during spaceflight while the ankle extensors were maintained at approximately normal activation levels. Activity of the elbow flexor and extensors was significantly greater during flight than either pre- or postflight. These results indicate that space flight is not necessarily a model of reduced or even low levels of activation for many muscles in humans.

As part of the same experiment, McCall, et al. (2001) studied limb proprioception during movements of the elbow using analyses of joint position. Subjects were asked to estimate joint angles during passive (relaxed) and active (10% maximal agonist effort) isokinetic extension and flexion of the elbow and plantarflexion and dorsiflexion of the ankle. Subjects were asked to identify when the joint passed through certain specific angles during flexion and extension in a dynamometer. During isotonic trials several tests resulted in underestimation of joint angles (generally, subjects identified a posture which was more flexed than the target posture) than during pre- or postflight measurements. These errors were rapidly restored upon return to 1G. The results indicate that the proprioception is altered by microgravity, but rapidly readapts to preflight control values after return to 1G. These changes were apparently independent of visual or vestibular sensorimotor changes.

Tesch et al. (2001) used a unilateral lower limb suspension model to achieve unloading in ambulatory human subjects, who walked on crutches for 5 weeks wearing an elevated shoe on the loaded side. One group performed no exercise while another group performed resistance exercise with their unloaded limb using a flywheel device. An ambulatory control group also performed flywheel exercise. The unloaded exercise group showed an increase in muscle cross sectional area and maintained their strength in the knee and ankle extensors. The results indicate that the potential for exercise-induced muscle hypertrophy is not reduced in unloaded muscle, and that resistance exercise can be a successful countermeasure to muscle atrophy during unloading.

### IMPLICATIONS FOR FUTURE RESEARCH

During the conference, an open session was held in which investigators expressed their views on space life science experimentation in the area of bone and muscle. This section of the paper summarizes the views expressed during that meeting.

The group reiterated the fact that there have been no on-orbit controlled trials of countermeasures for alterations in bone and muscle mass; further, the systematic addition of 1 G-like loads to on-orbit activity profiles has never been examined experimentally for its effect on bone and muscle. There was widespread support for the conduct of controlled experiments to examine countermeasure effectiveness. This includes the use of models in which only one limb is exercised and the contralateral limb serves as the control, and the use of non-exercising control subjects. It is realized that such protocols will need the support of the flight medicine community, but this should not be an insurmountable obstacle since the benefit/risk ratio of these approaches are high. Human space experimentation has traditionally depended on small numbers of subjects in each experimental protocol, but there are potential opportunities in the next few years to change this situation. It was recommended that consideration be given to flying the same experiment on multiple shuttle missions so that the number of subjects can be expanded to a
level where statistical testing of hypotheses is possible. It would also be possible under such circumstances for repeat flyers to act as their own controls.

In the area of animal studies, considerable support was expressed for the mouse model, particularly since the mouse genome is on the verge of being defined. The value of large animal studies (e.g. dogs) in the examination of the loading response of cortical bone was also emphasized, given the lack of intracortical remodeling in rodent models. In all animal studies, the group felt strongly that mature animals should be studied because the target human population for the application of these studies is adult men and women.

Several new experimental approaches were presented at the meeting and these deserve further use and examination. For example, although grant protocol reviewers have been critical of the unilateral human lower limb suspension model proposed by Tesch (2001), many researchers commented that this model has significant advantages compared to the more conventional bedrest model: the subject can be mobile, there is a contralateral limb available for control, and the cost of such a protocol is much lower than that of bed rest. Another technique of interest is the vibration model of Judex et al. (2001) mentioned earlier. Experimental examination of the anabolic effect of vibration during spaceflight seems warranted. Researchers in the bone area also emphasized the need to consider bone geometry variables as well as the more traditional bone mineral density.

A number of areas were identified in which there are gaps in present knowledge. Among these are detailed information on the endocrine changes that occur during spaceflight. Muscle researchers, in particular, felt, that this made interpretation of some of their findings difficult. This group also indicated that muscle size, per se, is not always the major determinant of muscle function and that the effects of a treatment or intervention on muscle function per se should always be studied if possible. In the bone area, the need for more studies of fracture healing was emphasized; it was felt that there should be more studies on anabolic agents in addition to those presently being conducted on anti-resorptive agents. The appearance of several promising pharmacological interventions for minimizing bone loss was noted and these should eventually lead to human flight experiments. The effects of muscle on bone have not received the attention that they deserve and studies that examine both of these tissues and their interactions should be encouraged. Support was also expressed for finite element modeling studies, which have the potential to examine many different conditions without long and costly experimental protocols.

Finally, the role of the National Space Biomedical Research Institute (NSBRI) was debated. Although the mandate of the NSBRI is to identify countermeasures that can prevent undesirable changes to humans during long-term spaceflight, the group supported the notion that mechanistic research to explain why particular countermeasures are effective should also be conducted where possible.
BIBLIOGRAPHY


Cavanagh PR, and Sharkey N. The biomechanics of exercise countermeasures.

Cavanagh PR, Ochiai RS, and Sneedeker JG. Indirect measures of bone loading from crew members on the International Space Station (ISS). In Bioastronautics Investigators’ Workshop, Abstract Volume 2001; USRA, Houston: p. 78.


Edgerton VR, Gotto J, McCall GE. Long term activity levels of flexor and extensor pools of the ankle and elbow of humans before, during and after spaceflight. In Bioastronautics Investigators’ Workshop, Abstract Volume 2001; USRA, Houston: p. 112.


Smith CL. Receptor countermeasures to microgravity induced bone loss. In Bioastronautics Investigators’ Workshop, Abstract Volume 2001; USRA, Houston: p. 84.


Changes in Bone Mineral Density During 17 weeks Bed Rest with Countermeasures
L Shackelford, A LeBlanc, S Smith, A Feiveson

Changes in bone density and biochemical markers of bone turnover were used to assess the efficacy of resistive exercise (5 men and 4 women subjects) or daily alendronate 10 mg (9 men) in preventing the bone loss associated with 17 weeks bed rest with no countermeasures (5 men and 3 women). Preliminary results indicate that both alendronate and resistive exercise result in a positive calcium balance. Alendronate results in a lowered metabolic rate in which bone resorption decreases more than formation decreases. Resistive exercise increases bone metabolism with greater percent increases in formation markers than percent increase in resorption markers. Individual regions of bone exhibited losses despite the overall positive calcium balance of individuals in both treatment groups. The calcaneus appeared more prone to bone loss in those taking alendronate. Exercisers appeared to have losses in the hip that were specific to the exercise biomechanics.
CALCIUM KINETICS DURING LONG-DURATION SPACE FLIGHT

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INTRODUCTION

Bone loss represents one of the most significant effects of space flight on the human body. Understanding the mechanisms underlying this loss is critical for maintaining crew health and safety during and after flight. This investigation documents the changes in bone metabolism and calcium kinetics during and after space flight. We previously reported calcium studies on three subjects during and after a 115-d stay on the Russian space station Mir (Am J Physiol, 277:R1-R10, 1999). We report here data on an additional three subjects, whose stays on Mir were approximately 4 (n=1) and 6 (n=2) mos. Previously published data are included for comparison.

CURRENT STATUS OF RESEARCH

Methods

Subjects were three men, aged 44±3 years and weighing 82.7±5.4 kg. Calcium kinetic studies were performed before, during (at 2-3 months of flight), and after space flight. Stable isotope tracer kinetic studies were performed before, during, and after flight. Following administration of isotopes (⁴⁴Ca orally, ⁴²Ca intravenously), blood, urine, saliva, and fecal (pre- and post-flight only) samples were collected for 21 days. Isotope enrichments were determined using Thermal Ionization Mass Spectrometry, and the data analyzed using the SAAM (Simulation, Analysis and Modeling) software program and multi-compartmental mathematical modeling techniques. In addition to the kinetic studies, endocrine and biochemical markers of bone and calcium homeostasis were determined using standard analytical techniques.

Results

Biochemical and endocrine data are reported as percent differences from individual preflight data. Similar to previous reports, ionized calcium was unchanged (2.8±2.1%) during flight, calcium absorption was variable in flight, but was decreased after landing, and vitamin D stores were decreased in flight by 35±24%. By contrast, serum PTH was decreased more (59±9%) during flight than previously reported, while 1,25(OH)₂-vitamin D was decreased during flight in only 2 of the 3 subjects. Markers of bone resorption (e.g., collagen crosslinks) were increased in all subjects during flight. Bone-specific alkaline phosphatase, a bone formation marker, was decreased (n=1) or unchanged (n=2), while osteocalcin was decreased 34±23%.

Results from the recent Mir calcium kinetic studies confirm previous observations (Fig. 1) of increased bone resorption during space flight. These data demonstrate that the loss of bone during space flight is associated with increased resorption and either unchanged/decreased formation. Bone balance was negative during space flight (-232 ± 150 mg/d) in the recent crewmembers studied, very similar to previously published results (Fig. 1).
Preliminary Conclusions
The data reported here support earlier reports, and demonstrate that the bone loss of space flight is clearly related to increased bone resorption, while the role of bone formation in this process is less clear. The calcium kinetic studies conducted to date (with one exception) have only been performed once per subject during flight - at approximately the same time (i.e., in the flight day 90-110 range). Further investigation is warranted in order to understand the time-course of bone-related changes during space flight, including both the immediate changes during the first days-weeks of flight, as well as the long-term adaptation.

FUTURE PLANS
In addition to the Mir flight data shown here, this research was also selected for International Space Station and Shuttle flights. Currently, this study is manifested on a 2001 Space Shuttle flight (STS-107) and potentially will fly on an additional Shuttle flight the following year. These short-term data, in addition to the long-duration Mir and ISS studies, will provide a better understanding of the time-course and nature of changes in bone and calcium metabolism during space flight. This research will aid in the understanding and treatment of both space-induced bone loss and Earth-based bone diseases (e.g. osteoporosis, paralysis).

INDEX TERMS
Bone, calcium, calcium kinetics, osteoporosis, bone loss, calcium metabolism
DUAL-ENERGY X-RAY SYSTEM FOR BONE MINERAL DENSITOMETRY 
AND DIAGNOSTIC RADIOGRAPHY

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INTRODUCTION
A portable, pulsed x-ray source with dual energy properties has been developed for implementation of the dual-energy x-ray absorptiometry (DEXA) technique for determining bone mineral density. The x-ray source has been integrated with two flat-panel amorphous silicon detection panels that are positioned in tandem for dual-energy detection.

CURRENT STATUS OF RESEARCH
Methods
The x-ray source is powered by a 30-capacitor Marx generator with charging voltage up to 200 kVp. The field emission tube produces an intense x-ray pulse with 60 nanosecond duration. The x-ray energy is relatively hard early in the pulse and soft late in the pulse. Two flat-panel amorphous silicon detectors are positioned in tandem and record two radiograms with differential energies. Image processing and separation algorithms are implemented to determine the bone mineral and soft tissue areal densities.

Results
The development of the pulsed dual-energy x-ray source has been completed. The x-ray source is compact, portable, and battery powered. Using single x-ray pulses, a number of x-ray images were recorded that indicates the utility of the x-ray source for diagnostic radiography in remote locations. The x-ray source was integrated with two detection panels into a operational system. Useful digital radiograms for implementation of the DEXA technique have been produced using single x-ray pulses.

Conclusion
The utility of the pulsed x-ray source for diagnostic radiography in remote locations was studied. The x-ray source and digital detection system is being evaluated for bone mineral density measurements.

FUTURE PLANS
Bone mineral density measurements will be performed using surrogate phantom test objects.

INDEX TERMS
Bone mineral density, DEXA, diagnostic radiography, x-ray source, amorphous silicon x-ray detection panel.
INDIRECT MEASURES OF BONE LOADING FROM CREW MEMBERS ON THE INTERNATIONAL SPACE STATION (ISS)

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INTRODUCTION
The loss of bone mineral in the lower extremities is widely viewed as the critical factor that may limit long-term human habitation of space. Decrements in muscle function as a result of prolonged exposure to microgravity also have important implications for performance and safety during space missions (LeBlanc et al. 1996). This project has the potential to shed significant new light on solutions to these problems since, although load reduction and reduced muscle activity are believed to play major roles in the loss of bone mineral and muscle strength, neither of these quantities has ever been measured before over any extended duration during space flight. Nor have comparisons been made of the loading levels of the same individuals on the ground and in orbit.

CURRENT STATUS OF RESEARCH
Methods
In this project, presently scheduled for ISS Increment 4, we will characterize the load on the lower extremities together with joint excursion and upper and lower extremity muscle activity in three crew members during entire days of working on Earth and on the Space Station. Pre- and post-flight estimates of bone mineral density (via Dual Energy X-Ray Absorptiometry), muscle cross sectional area (via Magnetic Resonance Imaging), and joint torques (via CYBEX) will provide a perspective against which the consequences of changes in activity profiles can be judged.

In this study, four 12 hour days will be monitored on earth and on the International Space Station using instrumentation from the Human Research Facility (HRF). During the 12-hour data collection periods, load will be continuously sampled using the Foot-Ground Interface (FGI) device, joint angles will be measured using Joint Excursion Sensors (JES), and muscle activity will be determined by surface electromyograms (EMGs). All data sets will be recorded on the Ambulatory Data Acquisition System (ADAS).

A Lower Extremity Monitoring Suit (LEMS) has been developed to monitor the above quantities while crew members continue with their daily activities (Figure 1). The suit consists of an instrumented pair of tights, an instrumented armband, and a waist belt for carrying the ADAS. Five muscles in the leg (vastus medialis, rectus femoris, biceps femoris, gastrocnemius, and tibialis anteriors) and two muscles in the right arm (biceps brachii and triceps brachii) will be monitored for EMG activity. Joint angles will be measured at the right hip, knee and ankle with electrogoniometers. The foot-ground interactions will be measured with capacitive insoles placed inside the shoes.

Calibration and verification of each piece of equipment will be performed at the start of the test day. The crewmember will then continue with his daily activities. Test days will be scheduled such that the LEMS will not interfere with scheduled work, e.g. extra vehicular activities.

From the data collected in 0g and 1g, the daily mechanical load stimulus (DMLS), which is an estimate of skeletal mechanical stress, will be calculated from the integrated ground reaction forces (GRF) (Beaupré et al. 1990, Whalen et al. 1988). In addition, net neural drive (NND) will be determined from the integrated EMG data. NND combined with the goniometric data will be used to calculate the concentric, eccentric and isometric actions of the muscles. This will indicate any change in muscle usage in the microgravity environment (Winter et al. 1991).
Results

A preliminary study was conducted using all of the instrumentation in the LEMS to determine the daily mechanical load stimulus and net neural drive in a small sample of community dwelling older men (Snedeker 2000). The men were divided into two groups (high and low) based on bone mineral density (BMD). Examples of the GRFs and net neural drive from one muscle during the 10-hour test periods are illustrated in Figures 2 and 3. Although there were no significant differences between the two groups for DMLS and NND, trends in the data indicate that people with greater DMLS and NND had higher BMD in the proximal femur.

CONCLUSIONS

The results show that the described methodology can be used for long-term quantification of daily activities. These pilot data also indicate that there are trends relating BMD with daily mechanical load stimulus and net neural drive.

FUTURE PLANS

The LEMS is presently in the final stages of development and flight certification. Each crew member will be personally fitted with their own suit to ensure comfort and wearability. Crew members will be trained in the set-up, calibration, and operation of the instrumentation. Monitoring daily activities in microgravity will provide a unique opportunity for comparison with ground values and to quantify the changes in muscle activity and ground reaction forces that occur over time during the mission. The comparison of 0g and 1g NND in the upper and lower extremity muscles will be particularly informative since BMD in the upper extremities appears to be preserved in long duration space flight (LeBlanc et al. 1996). This project will help lay the foundation for the continuing development of countermeasures to muscle and bone loss for future long-term missions.

INDEXING TERMS

Long-term space flight, bone loss, muscle atrophy, countermeasures, daily mechanical load stimulus, net neural drive, electromyography, joint angle, ground reaction forces, HRF

REFERENCES

ACKNOWLEDGEMENTS

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NON-INVASIVE INVESTIGATION OF BONE ADAPTATION IN HUMANS TO MECHANICAL LOADING

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INTRODUCTION
The objective of our research is to investigate the functional relationship between cumulative daily skeletal loading generated by daily activity and the regulation of bone density and bone structure. Applied to space flight, our working hypothesis is that musculoskeletal tissues can be maintained during long duration space flight with the appropriate type and level of exercise. The idea is certainly not new. Russian and US investigators understood early on the importance of gravitational and inertial (dynamic) loading in maintaining the musculoskeletal and cardiovascular systems on Earth. Treadmills in both space programs were developed with the specific purpose of generating similar lower body musculoskeletal and vascular tissue loading profiles in space (Stepantsov et al., 1974; Thornton and Rummel, 1977; Kozlavskaya et al., 1981; Thornton, 1986). Investigators also recognized the need to quantify daily activity on Earth in terms of cumulative musculoskeletal loading in order to put space flight exercise duration and intensity into proper perspective (Thornton, 1986; Cavanagh, 1986).

Our research is similar in approach to these earlier efforts. We have proposed the calcaneus and tibia as useful model bone sites loaded by internal forces in equilibrium with the ground reaction force during gait. By monitoring daily ground reaction forces (vertical component) with the NASA GRF Activity Monitor, we obtain a quantitative measure of daily activity level in terms of duration (number of daily loading cycles) and intensity (peak cyclic force levels). The daily history of the ground reaction force is also a good relative measure of daily tibial and calcaneal loading that can be compared to bone density of the calcaneus and cross-sectional geometry of the tibia.

CURRENT STATUS OF RESEARCH
Bowley (2000) applied these methods to investigate the relationship between daily activity level and bone density in young and elderly women runners and non-runners. Another objective of this study was to estimate a best-fit value of the “stress exponent” parameter in a mathematical model of bone adaptation using daily histories of the ground reaction force and calcaneal bone density. Tibia and fibula cross-sectional structural parameters were measured in a subset of the same population and compared (Cleek and Whalen, 2000).

Methods
Forty-five female subjects in two age groups (25-36 and >60 yo) and two “load intensity” levels (runners vs. non-runners) were recruited with NASA Ames IRB approval. Three subjects were eventually eliminated for reasons of incomplete data. Ground reaction forces were collected for five continuous days using the NASA GRF Activity Monitor. Calcaneal bone density was measured with the OsteoAnalyzer (SXA). Tibia and fibula midshaft structural parameters (area, $A$; principal moments, $I_{\text{max}}$ and $I_{\text{min}}$; and polar moment, $J$) were computed from three registered lower leg scans.

Results
Runners in both age groups were significantly ($P<0.05$) lighter than non-runners. Calcaneal bone density normalized by body weight was significantly higher in runners. Tibia $A$, normalized by body weight, and $I_{\text{max}}$ and $J$, normalized by body weight and tibia length, were significantly
higher in runners in both age groups with no dependence on age. No differences were found in the fibula. The stress exponent was determined from this study to be 6.07, falling within previously predicted values (i.e. 3-8), although the correlation between bone density and a measure of the “daily stimulus” was low (r= 0.439).

Conclusion
The daily history of the vertical component of the ground reaction force, which includes a constant force component (body weight) and a dynamic force component, provides a quantitative measure of lower limb musculoskeletal loading. When accounting for body size, activities (running) generating high dynamic loads increased bone density and parameters of long bone stiffness and strength. Our finding of a correlation between our model prediction and measured bone density is encouraging and we believe it will improve with volumetric (CT) bone imaging combined with an exercise intervention study providing a step increase in the loading history.

References

FUTURE PLANS
Our objectives are to determine the accuracy and precision of our new quantitative computed tomography (QCT) technology and to make the technology practical to use. The technology corrects beam hardening errors in the CT image and registers serial scans using a surface-based registration algorithm. This work will be done in collaboration with Dr. Chye Yan at DSO National Laboratories in Singapore. Dr. Yan was the principal developer of the registration and beam hardening correction algorithms while at Stanford University obtaining his PhD.

Research Co-Investigators: Chye Yan, PhD, DSO National Laboratories, Singapore; Gary Beaupré, PhD, RR&D Center, Veterans Administration Medical Center, Palo Alto, CA; Norbert Pelc, ScD, Department of Radiology, Stanford University Medical School, Stanford University, Stanford, CA; Cliff Les, PhD, Bone and Joint Center, Henry Ford Hospital, Detroit, MI; John Vogel, MD, UC Davis, School of Medicine, retired.

INDEX TERMS: bone density, bone geometry, quantitative computed tomography, QCT, exercise, countermeasures, calcaneus

ACKNOWLEDGMENTS
Dr. Sara Arnaud served as Medical Monitor for Dr. Bowley’s study and Dr. John Vogel collaborated on the calcaneal scans using his OsteoAnalyzer.
MECHANOTRANSDUCTION IN BONE: THE ROLE OF CALCIUM CHANNELS AND INTRACELLULAR CALCIUM RELEASE IN THE RESPONSE OF OSTEOBLASTS TO MECHANICAL STIMULATION

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INTRODUCTION
A major biomedical concern in the exploration and exploitation of space is the rapid and continual loss of bone during extended weightlessness. Similar responses occur during immobilization and skeletal unloading on earth. Conversely, bone tissue responds to mechanical loading with changes in mass and architecture to reduce strains incurred by the load. These responses to the mechanical environment appear to be mediated by changes in the function of cells of the osteoblastic lineage since increases in resorption in response to skeletal unloading is transient. However, the mechanisms through which osteogenic cells convert a biophysical stimulus into a cellular response are poorly understood.

One of the earliest responses of osteoblasts and osteocytes to mechanical stimulation is a rapid increase in intracellular calcium ([Ca^{2+}]_i) that is dependent on both Ca^{2+} entry into the cell via Ca^{2+} channels and Ca^{2+} release from intracellular stores (1). We have previously characterized a mechanosensitive, cation-selective channel (MSCC) that we have postulated interacts with the L-type voltage-sensitive Ca^{2+} channel (VSCC) to play an important role in this Ca^{2+} signal. This hypothesis appears to be strengthened by evidence that inhibition of the MSCC significantly reduced PGE2 secretion in osteoblasts and osteocytes (2). However, we have recently shown that mechanically induced gene expression is dependent on intracellular Ca^{2+} release rather than Ca^{2+} entry. A possible explanation for these seemingly paradoxical observations is that Intracellular Ca^{2+} release would increase the [Ca^{2+}] levels throughout the interior of the cell whereas rapid Ca^{2+} entry via channels would elevate the Ca^{2+} concentration near the membrane (3) where many membrane bound enzymes and signaling molecules reside. This mechanism would provide a unique mechanism to raise [Ca^{2+}], in discrete domains within the cell. Release of intracellular Ca^{2+} from internal stores would be optimally placed for activating mechanisms associated with gene expression while channel-mediated Ca^{2+} entry would activate a variety of membrane bound kinases and enzymes, for example, phospholipase A2, which cleaves arachidonic acid from phospholipids in the membrane for synthesis of prostaglandins. To test this hypothesis, we examined the role of Ca^{2+} entry and intracellular Ca^{2+} release on NFκB translocation and tyrosine, focal adhesion and MAP kinase activation in MC3T3-E1 osteoblast-like cells in response to fluid shear. We also examined the effects of attachment of these cells to different extracellular matrix proteins on Ca^{2+} signaling.

CURRENT STATUS OF RESEARCH

Methods: Application of fluid shear: MC3T3-E1 cells, an osteoblast-like, non-transformed cell line, were grown on fibronectin-coated or type I collagen-coated glass slides. Once the cells had reached confluency, the slide was mounted on a parallel plate chamber that subjects the cells to uniform laminar flow across the plate. During the flow experiments, cells were maintained at 37°C with the flow media pH held constant by aeration with 95% air/5% CO2. Flow was kept constant at 12 dynes/cm² for the duration of the experiment. Determination of c-fos, COX-2 production, NFκB translocation, kinase activity and actin cytoskeletal changes following shear: Following application of shear, cells were fixed in 4% paraformaldehyde and processed for immunofluorescence using 1° and 2° antibodies against c-fos, COX-2, NFκB, FAK, tyrosine kinase or MAP kinase using rhodamine-phalloidin or FITC-phalloidin. Images were recorded on Tmax 400 film using a Nikon Optiphot II microscope. To determine the expression of these factors, mRNA or total protein was isolated immediately following application of shear and Northern or Western analyses conducted respectively. To determine the effects of Ca^{2+} channels and intracellular Ca^{2+} release, various channel and release inhibitors were introduced 30 min prior to application of shear and kept in the shear solution for the duration of loading.

Results: Effects of fluid shear on NFκB translocation: Immunostaining of the p65 subunit of NFκB demonstrated that fluid shear induced NFκB translocation in a time dependent manner. In non-flowed cells NFκB was mainly distributed in the cytoplasm. Nuclear staining for NFκB in cell began to appear at 30 min of fluid shear, indicating
the translocation of NFkB from the cytoplasm to the nucleus. Nuclear NFkB staining peaked at 1h, with NFkB reappearing in the cytoplasm after 2h. To explore the role of [Ca\(^{2+}\)]\(_i\), in this translocation, cells were pretreated with BAPTA (30 μM), a membrane permeable Ca\(^{2+}\) chelator, for 30 min prior to shear. BAPTA completely blocked the fluid shear-induced NFkB translocation. Block of Ca\(^{2+}\) entry through the MSCC or VSCC with gadolinium or nifedipine, respectively, had no effect on this translocation. However, inhibition of intracellular Ca\(^{2+}\) release with thapsigargin or block of phospholipase C with U73122 completely inhibited this translocation. Unlike our previous findings where disruption of the actin cytoskeleton with cytochalasin D completely inhibited production of fos and COX-2, application of cytochalasin D had no effect on this translocation.

The role of Ca\(^{2+}\) in activation of kinase activity: Increases in tyrosine phosphorylation (pTYR) immunostaining in response to fluid shear in MC3T3-E1 cells occurred primarily at the cell periphery with focal adhesion kinase (pp125\(^{\text{FAK}}\)), paxillin and MAPK 1/2 being the dominant tyrosine phosphorylated proteins. Chelation of intracellular Ca\(^{2+}\) by BAPTA reduced pTYR staining at focal adhesion sites and abolished flow-induced increases in FAK pTYR immunoreactivity. Inhibition of the MSCC decreased kinase activity whereas disruption of the cytoskeleton by cytochalasin D nullified fluid flow-induced increases in pTYR staining at focal adhesion sites. Fluid flow-induced increases in actin stress fiber formation and density in MC3T3-E1 cells were also abrogated by pretreatment with tyrosine kinase inhibitors, genistein and herbimycin A. However, inhibition of protein kinase C or phosphatidylinositol 3-kinase did not affect flow-induced cytoskeletal rearrangements.

The role of the extracellular matrix in Ca\(^{2+}\) signaling: Since all previous studies had been conducted on fibronectin-coated plates, a series of experiments were made on type I collagen coated plates to determine if cellular attachment changed the pathway for Ca\(^{2+}\) signaling. When MC3T3-E1 cells were sheared for 1hr and immunostained for fos and COX-2 at 2d and 5d, post-seeding, it was found that the increase in fos and COX-2 was dependent on intracellular Ca\(^{2+}\) release at 2d, but dependent on MSCC and VSCC activity at 5d. This corresponded to translocation of the VSCC from the cytoplasm to the membrane in MC3T3-E1 cells.

Conclusions: These results demonstrate that fluid shear induces NFkB translocation in a transient manner and increases tyrosine kinase activity. However, while both processes are dependent on an increase in [Ca\(^{2+}\)]\(_i\), the pathway for this Ca\(^{2+}\) increase is mediated differently. Tyrosine kinase activation appears to be dependent on Ca\(^{2+}\) entry via Ca\(^{2+}\) channels while NFkB translocation is mediated by phospholipase C-dependent intracellular Ca\(^{2+}\) release. These data support our hypothesis that [Ca\(^{2+}\)]\(_i\) can be changed within discrete domains to activate different cellular processes. Furthermore, we have demonstrated that Ca\(^{2+}\) signaling can be altered by cell attachment, indicating that the extracellular matrix can dictate how the cell functions.

FUTURE PLANS
We will continue to examine the activation of different Ca\(^{2+}\) signaling pathways on the response of osteoblasts to fluid shear. One factor that is important to the response of bone to mechanical loading is prostaglandins. Several studies have indicated that Ca\(^{2+}\) can greatly influence the release of PGE\(_2\) and PGI\(_2\) in a variety of cells. Whether this is in response to an increase in COX-2 expression or an increase in release is still unclear. We will also continue to study the role of the extracellular matrix protein on osteoblastic function. We hypothesize that type I collagen induces a more differentiated phenotype, while fibronectin produces a proliferative response. Changes in the Ca\(^{2+}\) signaling pathways may mediate this response.

INDEX TERMS: Ca\(^{2+}\) channels, intracellular Ca\(^{2+}\), osteoblasts, NFκB, tyrosine kinase, extracellular matrix, mechanotransduction.

REFERENCES:


INTRODUCTION

The biological actions mediated by the estrogen receptor (ER) and vitamin D receptor (VDR) play key roles in the normal control of bone growth and skeletal turnover that is necessary for skeletal health. These receptors act by controlling the differentiation and/or function of osteoblasts and osteoclasts, and other cell types within the bone and bone marrow microenvironment. The appropriate use of selective ER modulators (SERMs) which target bone and vitamin D analogs that favor bone formation over resorption should make it possible to prevent the reduction in bone formation and increase in bone resorption that normally contribute to the bone loss induced by weightlessness. Indeed, there may be synergistic interactions among these receptors that enhance the actions of any one used alone.

CURRENT STATUS OF RESEARCH

Methods

We have: 1) assessed the in vitro ability of novel ER and VDR agonists, alone or in combination, to modulate osteoblastogenesis and mature osteoblast function under conditions of 1g and simulated microgravity; 2) assessed the in vitro ability of novel ER and VDR agonists, alone or in combination, to modulate osteoclastogenesis; and 3) carried out baseline studies on the in vivo actions of our novel ER- and VDR-based therapeutics in the rat in preparation for their use in hindlimb suspension model of unloading induced bone loss.

Results

In our VDR studies, we have examined the ability of the VDR agonist, EB1089, which is less calcemic than calcitriol, to regulate osteoblastic gene expression and find that it is more potent than calcitriol. In addition, gene expression in the MG-63 osteoblastic cell line was characterized in the Slow Turning Lateral Vessel (STLV) culture system, which approximates many aspects of microgravity. Many genes were down-regulated in comparison to monolayer cultures grown at unit gravity, and responses to VDR agonists were less robust. In ongoing hindlimb suspension studies in male rats, EB1089 was able to prevent unloading-induced bone loss measured at the proximal tibia, while calcitriol was able to increase bone mineral density. However, increases in serum calcium in calcitriol-treated animals, not observed in EB1089-treated rats, indicate that the latter is a superior countermeasure. EB1089 is also a less potent stimulator of osteoclast formation in comparison to calcitriol. Finally, our ER studies have revealed that osteoblastic gene expression patterns induced by estradiol and the SERMs idoxifene and raloxifene, are distinct even though all agents are capable of inhibiting bone loss due to sex steroid depletion. Raloxifene does not reduce bone mineral density in normal female rats and has only modest effects on biochemical markers of bone turnover suggesting its use in gonad-intact populations should not increase the risk of bone loss via inhibiting endogenous estrogens. In ongoing hindlimb suspension studies in ovariectomized female rats, raloxifene and estradiol, individually, appear able to prevent loss of bone mineral density. Since raloxifene...
does not exert undesirable, estrogen-like effects in reproductive tissues, it has the potential to be an acceptable countermeasure to disuse-induced bone loss. Ongoing studies will continue to examine the use of EB1089 and raloxifene, alone and in combination, to prevent bone loss in male and female rats induced by hindlimb suspension.

Conclusion
Collectively these studies suggest that manipulation of VDR and ER activity has the potential to reduce the risk of bone loss resulting from the microgravity environment encountered during Space travel. Importantly, our data also suggest that novel ligands for these two receptors that significantly attenuate the negative side effects of the natural ligands can be effectively employed to reduce unloading-induced bone loss, and the ensuing risks of bone fracture.

FUTURE PLANS
The VDR studies indicate that osteoblastic gene expression is compromised under conditions that mimic several aspects of microgravity. The responsiveness of these genes to the natural VDR ligand, calcitriol, is reduced, and cell signaling pathways are altered suggesting that more research is required to better understand the cellular and molecular changes that accompany microgravity. The ER studies reveal that estradiol and SERMs, although both able to prevent bone loss associated with sex steroid depletion, do not appear to regulate osteoblastic gene expression in a similar manner. This suggests that estradiol and SERMs either regulate bone mineral density through different pathways or that the molecular events through which the ER regulates bone density are distinct from our current understanding of ER action. Either possibility should be further explored because it has the potential to reveal new mechanisms for enhancing bone structure and function that may facilitate the identification of a high potency, high specificity agents for treating or preventing bone loss.

INDEX TERMS: Estrogen Receptor, Vitamin D Receptor, Osteoblast, Osteoclast, SERM, Calcitriol, Hormone, Hindlimb Suspension
INTRODUCTION
The current tenet of mechanically related bone adaptation suggests that mechanical signals must be large in magnitude to stimulate bone formation. In contrast to this “bigger is better” perspective, recent studies have demonstrated the strong anabolic potential of extremely low magnitude - but high frequency - mechanical signals. Considering the osteogenic character of these high frequency mechanical stimuli, we hypothesize that introducing these signals will serve as an effective countermeasure for the bone loss which parallels disuse. Importantly, these low level signals may play a critical role in defining and maintaining normal bone mass and morphology, as they persist over long durations, including passive actions such as standing, and therefore represent a dominant component of bone's functional strain history. Not surprisingly, therefore, conditions such as microgravity (or a model of this pathology such as rat-tail suspension) may abolish this key regulatory stimulus, and thus permit resorptive activity. In an effort to “reintroduce” these low-level mechanical stimuli, we have devised a prototype, categorized as "non-significant risk" by the FDA, which can increase bone formation by inducing extremely low level mechanical stimuli into the lower appendicular and axial skeleton. Importantly, this unique biomechanical intervention affords the ability to examine the molecular basis of an osteogenic signal, thus identifying novel targets for drug development. Osteoclast differentiation factor (ODF) is a cytokine involved the recruitment and activity of osteoclasts and in vitro studies have linked its upregulation to the absence of mechanical strain. Here, we first examined the osteogenic efficacy of low-level high frequency mechanical stimuli and their ability to reverse the bone loss which arises under microgravity. We then hypothesized that the expression of ODF would be inversely related to altered tissue level bone formation rates.

METHODS
Female 6 months old Sprague-Dawley rats were assigned to controls (n=30), mechanically stimulated (n=21), tail suspension related disuse (n=11), disuse interrupted by 10min/d of normal weight bearing (n=7), and disuse interrupted by 10min/d of 90Hz stimulation at 0.25g (n=19). All experimental procedures were applied for 28d. Mechanical stimulation consisted of whole body vibration at 90Hz (0.25g). All rats were given injections with demeclocycline prior to the beginning of the study and calcein on day 18 of the protocol to determine histomorphometric indices of bone formation. ODF mRNA levels were quantitated in three animals of each group (except disuse + weight bearing group) via Northerns. RNA was extracted from whole left tibiae, including bone marrow and cartilage.

RESULTS
Body mass of the rats did not change significantly in any of the groups during the course of the 28d study. Mechanical stimulation at 90Hz for 10 min/d proved to be a strong osteogenic stimulus as indicated by increased trabecular bone formation rates (+97%, Fig. 1a). Hindlimb suspension significantly decreased trabecular bone formation rates by 92% as compared to controls. This suppression was not significantly different from the animals subject to disuse for most of the day (23h, 50min) and then allowed to freely bear weight for 10 min/d (D+WB). In contrast, when low-level mechanical stimulation was applied for 10min/d to combat disuse, the countermeasure served to normalize bone formation rates back to control values.
Mechanical stimulation for 10 min/d decreased ODF mRNA levels by 78%. Disuse increased the expression of ODF by 72% with respect to control values while disuse interrupted by 10 min of daily mechanical stimulation decreased ODF levels by 49% (Fig. 1b). When linear correlation was used to relate bone formation rates to ODF expression levels across groups, the $r^2$ value was 0.79 (inverse correlation).

![Graph](image)

**Figure 1a.** Tibial trabecular bone formation rates (BFR/BV) of age matched controls (LTC) and after 28 days of mechanical stimulation for 10 min/d at 90Hz (90Hz), tail suspension (Dis), disuse interrupted by 10min of weightbearing (Dis + WB), and disuse interrupted by 10 min of mechanical stimulation (Dis + 90Hz). b. Relative expression of ODF in control, 90Hz stimulated, disuse, and disuse interrupted by 90Hz vibration rats (mean ± SD).

**CONCLUSION**

The anabolic potential of extremely low level, high frequency mechanical stimuli is evident even when applied for a very short daily duration. Importantly, from a bioastronautics perspective, these signals effectively prevented disuse osteopenia from occurring, even when the bone was subjected to 23 hours and 50 minutes per day of this strong stimulus for resorption. This mechanical intervention has evolved from efforts to define the osteogenic components within functional activity, and then establishing a non-invasive way of “re-introducing” these anabolic signals to a skeleton deprived of them due to disuse or aging. Long-term animal studies have shown that both trabecular bone density and strength are actually augmented by such a prophylaxis. Further, preliminary clinical studies on a post-menopausal population are also encouraging; in a randomized double-blind study, short exposure (20 minutes per day) to such mechanical loads inhibits site-specific bone loss (femur and spine) in postmenopausal women. The strong inverse relation that we found between mechanically altered bone formation rates and the expression of a gene involved in osteoclastogenesis expression suggests that mechanical stimulation directly or indirectly influences the expression of this gene.

**FUTURE PLANS**

Preliminary data indicate that the anabolic capability of this low-level biomechanical countermeasure is also effective in the mouse. The mouse is the animal model of choice (Osborn et al., NRC report 1998) for space research due to space constraints in space stations. Further, the mouse genome has been extensively deciphered and we will be able to probe gene function by using specific mouse strains with different bone phenotypes (e.g., high and low BMD) and selective knockouts.

**INDEX**

trabecular bone, mechanical stimulation, strain, frequency, formation, disuse, osteopenia, gene expression, osteoclast differentiation factor
PARATHYROID HORMONE MODULATES THE RESPONSE OF
OSTEOBLAST-LIKE CELLS TO MECHANICAL STIMULATION

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INTRODUCTION
Fluid shear is one form of mechanical stimulation to which bone cells respond. It is hypothesized that fluid shear may be induced in vivo by movement of interstitial fluid through the microporosity of bone. For an anabolic response to occur in bone, however, the level of mechanical stimulation must meet or exceed a mechanical threshold. Parathyroid hormone (PTH) is one factor that may reduce the mechanical threshold, increasing bone formation at lower magnitudes of mechanical stimulation. One of the earliest detected responses in osteoblasts to fluid shear and PTH is an increase in intracellular calcium ([Ca²⁺]i). The increase in [Ca²⁺]i due to these stimuli may be mediated through two different Ca²⁺ channels shown to be present in osteoblasts, mechanosensitive calcium channels (MSCC’s) and voltage-sensitive calcium channels (VSCC’s). These channels have been shown to be modulated by protein kinase A (PKA) and protein kinase C (PKC) signaling, which both PTH and mechanical stimulation activate in osteoblasts. Therefore, the purpose of this study was to determine if addition of PTH prior to subjecting MC3T3-E1 cells, an osteoblast-like cell line, to fluid shear enhanced shear-induced [Ca²⁺]i signaling. In addition, the Ca²⁺ channels involved in the response, as well as modulation of these channels by PKA or PKC activation were explored.

CURRENT STATUS OF RESEARCH
Methods
MC3T3 cells (P6-P19) were grown 4 d on collagen-coated quartz slides. For flow experiments, cells were loaded with 3 µM fura-2/AM (Molecular Probes), a fluorescent Ca²⁺ probe. A parallel-plate flow chamber was used to subject the cells to fluid shear. Flow was introduced to the chamber through a syringe mounted on a Harvard Syringe Pump (PhD Programmable, Harvard Apparatus) that controlled the flow rate. To establish a fluid flow [Ca²⁺]i baseline, MC3T3-E1 cells were exposed to fluid shear of 1 dyne/cm² for 3 minutes. Fluid shear magnitude then remained at 1 dyne/cm² or was increased to 12 or 25 dynes/cm² for 3 minutes.

The effects of PTH on the [Ca²⁺]i response were determined by treating the cells with bPTH(1-34) 50 nM 10 min prior and during shear. The MSCC inhibitor gadolinium (Gd³⁺, 10 µM) or the VSCC inhibitor nifedipine (Nif, 5 µM) were used to assess Ca²⁺ channel activity during shear or shear+PTH. PTH fragments selective for PKA or PKC activation were utilized to determine the role of kinase activation in the [Ca²⁺]i response. hPTH(1-31) 50 nM selectively activates PKA and not PKC, whereas bPTH(3-34) 50 nM activates PKC and not PKA. The PKA activator, forskolin (FSK, 10 µM) and the PKC activator, PMA (10 nM) were also used.
Results
There was a shear magnitude-dependent increase in the mean peak \([\text{Ca}^{2+}]_i\) response. Shear stress of 25 dynes/cm\(^2\) significantly increased the mean peak \([\text{Ca}^{2+}]_i\) response, and the percentage of cells responding to shear compared to shear stresses of 1 and 12 dynes/cm\(^2\). PTH addition significantly enhanced the response at each level of shear stress studied. Gd\(^{3+}\) significantly inhibited the response to shear and shear+PTH. The VSCC inhibitor, Nif, did not significantly inhibit the response to shear alone, but did significantly reduce the mean peak \([\text{Ca}^{2+}]_i\) response to shear plus PTH. Activation of PKC did not alter the \([\text{Ca}^{2+}]_i\) response to shear. However, PKA activation significantly increased the \([\text{Ca}^{2+}]_i\) response to lower magnitudes of shear. An interaction between PKA and PKC signaling may occur, as bPTH(1-34) which activates both PKA and PKC induced a greater response to shear than either PKA activation or PKC activation alone.

Conclusion
PTH is able to significantly enhance a mechanically-induced initial signaling event in osteoblast-like cells. PTH appears to reduce the mechanical threshold required to elicit an increase in \([\text{Ca}^{2+}]_i\) in these cells. PTH appears to primarily mediate this effect through activation of VSCC’s and MSCC’s. Activation of PKA and PKC also plays a role in the enhancement of the \([\text{Ca}^{2+}]_i\) response by PTH in mechanically stimulated osteoblasts.

FUTURE PLANS
Patch clamping studies will be conducted to more specifically determine channel involvement in the response to PTH and shear in osteoblast-like cells.

INDEX TERMS
osteoblast, parathyroid hormone (PTH), fluid shear, mechanical, calcium channels, protein kinase A (PKA), protein kinase C (PKC)
THE EFFECTS OF PARTIAL WEIGHTBEARING, MECHANICAL LOADING
AND IBANDRONATE ON SKELETAL TISSUES IN THE ADULT RAT
HINDQUARTER SUSPENSION MODEL OF MICROGRAVITY.

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INTRODUCTION
The future of manned spaceflight depends on the discovery of effective countermeasures for bone loss. Our central hypothesis is that pharmacological modification of bone remodeling and/or precisely designed mechanical loadbearing may provide sufficient skeletal protection. The four specific aims were: (1) To determine whether partial weightbearing is a countermeasure that will preserve bone quality. (2) To determine whether young, growing animals and older mature animals respond to partial weightbearing conditions in the same way. (3) To determine the efficacy of a potent bisphosphonate. (4) To determine the efficacy of dynamic loading similar to exercise.

This study focuses on partial weightbearing set to 50% of the normal weightbearing to provide a benchmark for the effects of Martian (0.38G) and artificial gravity on bone. Ibandronate is a powerful third generation bisphosphonate that can be administered intravenously every 3 months in humans to prevent bone loss due to osteoporosis. Mechanical loading is known to modulate bone mass, structure and anisotropy.

CURRENT STATUS OF RESEARCH

Methods
To simulate Martian gravity, we modified the hindquarter rat suspension model such that the front limbs of hindquarter suspended rats were supported by a platform set to 50% front limb weightbearing and controlled by a digital computer in negative feedback. This platform could be programmed to resonate with specified frequencies of impact. Based upon evidence that osteogenesis is particularly sensitive to specific components of dynamic bone strain, we monitored a single axis-strain gauge aligned with the shaft of humerus simultaneously with the ground reaction force on the forepaw. The strain frequency spectrum was centered at 2-3 Hertz with less than 1% of the energy coming from components over 10 Hertz. The gain ratio of bone strain/platform acceleration as a log relation of platform vibration frequency (Bode plot) indicated low-pass filtering properties of the rat foreleg for oscillations greater than 1 Hertz. Thus, 3 Hertz is a principal component of normal impacts of ambulation. Voluntary activity of skeletal muscles can force the skeleton to synchronously oppose motion of the platform at 3 Hertz as form of controlled exercise.

A comprehensive assessment of bone quality was carried out using both non invasive (longitudinal studies) and invasive techniques (end point studies) from the cell to organ level. In vivo, longitudinal changes in bone quality were assessed by peripheral quantitative computed tomography (pQCT) on days 0 and 35. The pQCT measured parameters were cortical and trabecular bone density and structural properties of cortical bone. These include cortical bone area which is an indicator of axial strength and polar moment of inertia and section modulus which are indicators of torsional and bending rigidity and strength respectively. On day 35, the animals were sacrificed, the bones harvested and used for histomorphometry, mechanical testing, cell culture studies and biochemical analyses. Six experimental conditions were studied in female, 3 or 5 months old (at the beginning of the 35 day trial period) Sprague Dawley rats. (1) Free roaming rats with full weightbearing on their forelimbs and unrestrained activity, (2) free roaming rats pretreated with ibandronate, (3) hindquarter suspended rats with half of the normal load supported by the forelimbs i.e., partial (50%) weightbearing rats, (4) hindquarter suspended rats with 50% forelimb weightbearing, pretreated with ibandronate and (5) hindquarter suspended rats with 50% forelimb weightbearing treated with daily episodes of dynamic loading at 3 Hertz on their forelimbs as a simulation of ambulatory exercise.

Results
Longitudinal studies were carried out by assessing in vivo changes in pQCT measured bone properties between days 0 and 35. Over a period of 35 days, full weightbearing rats (3 and 5 month old) significantly increased their cortical bone structural properties but showed no significant changes in cortical or trabecular bone densities. In contrast, partial weightbearing rats showed no significant changes in cortical bone structural properties but significantly decreased trabecular bone density. Younger partial weightbearing rats, also showed significant increases in cortical bone density. Temporal changes in bone quality observed in young partial weightbearing rats
that received ibandronate or additional mechanical loading at 3 Hertz were similar to those observed in free roaming animals.

A longitudinal comparison of changes in bone properties between groups suggests that structural properties of cortical bone are significantly compromised in partial weightbearing rats as compared to free roaming rats or partial weightbearing rats treated with ibandronate or dynamic loading at 3 Hertz. Changes in structural properties of cortical bone are similar in free roaming rats, free roaming rats treated with ibandronate and rats treated with ibandronate or dynamic loading at 3 Hertz.

Properties of trabecular bone (density) are also significantly compromised in partial weightbearing rats and partial weightbearing rats that received additional dynamic loading at 3 Hertz as compared to free roaming rats. Ibandronate attenuated trabecular bone loss in partial weightbearing animals and significantly increased trabecular bone density in full weightbearing rats. Preliminary data suggests that high frequency dynamic loading at 500Hertz can improve trabecular bone properties but not cortical bone properties.

End point study results suggested that pre-treatment with ibandronate was a sufficient countermeasure to prevent bone loss (higher bone formation rates, higher mechanical testing indices, higher collagen and proteoglycan concentrations as compared to partial weightbearing rats). Histomorphometric assessment of bone formation rate and mineral apposition rate suggested that partial weightbearing alone was a sufficient countermeasure against bone loss when compared to free roaming rats. Mechanical properties were generally comparable for all groups. Collagen and proteoglycan concentrations were depressed in partial weightbearing rats but osteocalcin concentration increased in comparison to full weightbearing rats. There were no significant differences in vitamin D levels from retro-orbital blood between partial weightbearing and full weightbearing rats.

End point results from pQCT indicated higher cortical and trabecular bone densities in free roaming rats as compared to partial weightbearing rats but lower cortical bone structural properties. There is an interesting contrast between the pQCT results from end point and longitudinal studies. End point studies assume that bone properties are comparable in the different groups at the beginning of the study. Our pQCT data from day 0 indicated that there were significant differences in bone properties between groups.

Finally, indicators of systemic stress suggested that there were no significant differences in urine catecholamines levels between the free roaming full weightbearing and suspended partial weightbearing rats. In summary, a comprehensive assessment of bone quality based on end point study data suggests that partial weightbearing is sufficient to maintain bone properties from the molecular to tissue level but at the organ level properties are compromised when compared to treated partial weightbearing rats. Ibandronate helps maintain bone quality at all levels of structural hierarchy.

Conclusion

The results of this study provide evidence that different countermeasure modalities provide skeletal protection to different aspects of bone quality as assessed at different levels of structural hierarchy. Our results suggest that 50% partial weightbearing, our simulation of Martian gravity is insufficient to prevent significant trabecular bone loss and deterioration in structural properties of cortical bone as compared to free roaming rats. There is no significant difference in bone properties when 3 month old and 5 month old rats are compared but differences are best evaluated when partial weightbearing animals are compared to full weightbearing animals. Pharmacological modification of bone remodeling using a powerful third generation bisphosphonate drug, ibandronate coupled with partial weightbearing can attenuate trabecular bone loss and significantly improve structural properties of cortical bone. Mechanical loading at 3 Hertz as a simulation of muscular exercise superimposed on partial weightbearing significantly improves structural properties of cortical bone. Both pharmacological and additional mechanical loading change bone structural properties in a manner similar to changes observed unrestrained rats at full weightbearing. Our results underline the importance of in vivo longitudinal measurements of different bone properties in the same animal at both cortical and trabecular bone envelopes. Finally, our results suggest that a combination of mechanical and pharmacological countermeasures along with partial weightbearing may yet be effective in the prevention of deleterious changes in bone properties due to unloading as experienced in spaceflight. The exact combination of mechanical loading and pharmacological intervention to be used as a countermeasure needs to be determined.

FUTURE PLANS

To elucidate the exact combination of mechanical loading (force, frequency and duration of dynamic loading) and dosage of pharmacological intervention that would preserve bone quality for quantitatively defined levels of weightbearing. 

INDEX TERMS

Bone quality, artificial gravity, partial weightbearing, mechanical loading, pharmacological interventions, countermeasures, exercise, ibandronate, low frequency dynamic loading.
ADULT CORTICAL BONE RECOVERS FROM LONG TERM DISUSE OSTEOPOROSIS BY CHANGING ITS ARCHITECTURE

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INTRODUCTION:
The ability of bone to recover from disuse osteoporosis is unclear. Studies in rodents suggest that complete recovery of bone mass can occur after disuse. In the skeletally mature canine skeleton, Jaworski and Uhthoff (2) and Lane et al (3) found that cortical bone mass did not recover from long-term disuse. However, the previous studies have focussed on restoration as bone mass as the *sine qua non* for recovery; the potential contributions of architecture in the bone recovery process have not been studied. In the current studies, we sought to determine whether bone mass and bone architecture might contribute differentially to the recovery process from long-term disuse osteoporosis.

CURRENT STATUS OF RESEARCH

METHODS:
Right forelimbs of adult female Beagle dogs (4-7 years old, N=32) were immobilized using a splint device (4). To facilitate remobilization all animals had their splint removed twice weekly for passive range of motion to minimize joint stiffening; during ROM therapy, animals were suspended in a hammock to prevent weight bearing. After either 3 or 6 months, bones were recovered from half the animals (groups: 3 mo IM; 6 mo IM) to sample long-term and very long-term disuse osteoporosis. The remaining dogs at each time period were removed from their splints and allowed resume weight-bearing in normal daily activities; long bones were recovered from these animals at 12 month after remobilization (groups: 3 mo IM-12 Rem; 6 mo IM-12mo Rem). Age-matched non-immobilized animals (N=6) were used as controls. Animals received bone fluorochrome double labels prior to sacrifice. All experimental procedures were carried out with approval from the governing institutional animal care committees. Cortical bone recovery from disuse osteoporosis was evaluated at the metacarpal mid-diaphysis using histomorphometry. Statistical comparisons were made among groups using the Kruskal-Wallis ANOVA, with post-hoc comparison of treatment groups versus control performed using the Mann-Whitney U-test. Data are shown as mean ± s.d.

RESULTS

*Immobilation:* Consistent with previous studies, immobilization resulted in a profound loss of bone, with bone area reduced nearly 15 percent from control levels by 3 months of disuse and by approximately 35 percent from control levels after 6 months (Fig 1). Bone loss during immobilization occurred principally from endocortical surfaces, resulting in an almost 250 percent increase in marrow cavity size by 6 months of immobilization (Fig 2). Periosteal surfaces did not contribute to bone loss; hence total cross-sectional area remained unchanged (Fig 3).

*Recovery of bone mass:* Animals immobilized for 3 month recovered approximately 95 percent of their bone mass with remobilization (p=n.s. vs. control; Fig 1). In contrast, with remobilization after 6 months of immobilization bone mass, remained approximately 15 percent below control levels. In both remobilization groups, histomorphometric indices of periosteal and endocortical bone formation and resorption were similar to control levels, indicating that the diaphyses had achieved a steady state in their adaptation.

*Mechanism of bone recovery:* The architectural bases for recovery of diaphyseal bone mass were different from those associated with bone loss. Recovery of some bone occurred by apposition along the endocortical surface; however, in both remobilization groups, marrow cavity size remained at least 60 percent larger than that in controls (Fig 2). However, with recovery, there was marked periosteal expansion of the bones, such that remobilized bones were 10-18% larger than control bones.
DISCUSSION

The current studies show that after long-duration immobilization, cortical bone mass does not recover to control levels with restoration of loading. This is consistent with previous studies in which a bone mass deficit remained with up to 32 weeks of remobilization after long-term disuse in the canine skeleton (2,3). The current studies show that some disuse osteoporosis is maintained even after 1 year of remobilization.

However, the current studies indicate that recovery of bone mass does not adequately reflect the recovery potential for long bone diaphyses. During recovery from disuse, long bones appear to adapt their architecture so as to provide the best mechanical advantage for the tissue that is deposited. Remobilized diaphyses undergo a compensatory expansion at the periosteal envelope during the recovery process, resulting in increased cross-sectional dimensions for the remobilized diaphyses. It is the cross-sectional dimensions (total cross-sectional area, moment of inertia) of the diaphysis, rather than bone mass, that are the key determinants of structural strength in long bones. In aging human diaphyses, small amounts of periosteal expansion readily offset the deleterious effects of significantly greater amount of endocortical bone loss on the mechanical strength of long bones (5). Previous studies of showing inadequate recovery of bone masses after long-term disuse suggest that protracted immobilization results an osteoblastic deficit, which underlies the inability of adult bone to recover bone mass after disuse (2). However, the current studies suggest that may not be correct; the focus on bone mass provides an erroneous picture of the recovery potential of long bone from long-term disuse. The restoration of bone the original bone mass (i.e., that bone loss from the endocortical surface), is mechanically unnecessary if new bone is deposited where it will provide the best mechanical advantage.

FUTURE PLANS
Efforts are underway to 1) characterize the structural-mechanical properties of long bone diaphyses, and 2) to evaluate the architectural bases and functional-mechanical aspects of cancellous bone recovery from disuse osteoporosis.


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INDEX TERMS: bone, disuse, immobilization, osteoporosis, recovery, bone structure, mechanical properties
ALTERATION IN SKELETAL PERFUSION WITH HINDLIMB UNLOADING:
A POSSIBLE MECHANISM FOR BONE REMODELING

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INTRODUCTION
Abnormalities in bone and mineral metabolism have been identified in both humans and rodents after spaceflight (Turner JAP 89, 2000). Although these skeletal changes are associated with altered calcium homeostasis and abnormal bone turnover, the precise relationships between endocrine changes, reduced calcium absorption, increased calcium excretion, altered bone formation and resorption, and reduced impact loading have yet to be resolved. Furthermore, differences in net bone loss between young growing rats and adult animals confound the literature.

In order to study spaceflight-induced bone loss, the hindlimb unloaded (HU) rat has been used as a model of weightlessness because of the mechanical unloading of hindlimb bones. In addition, like that in astronauts, HU elicits a headward fluid shift which increases perfusion pressure in the thorax and head and decreases perfusion pressure to the hindlimbs.

CURRENT STATUS OF RESEARCH
Methods: Six-month-old male Sprague-Dawley rats remained in a standing position \( (n=11) \) or were placed in a head-down position by elevating the hindlimbs to an approximate spinal angle of 40-45° from horizontal; this was maintained for 10 min \( (n=11) \), 7 days \( (n=11) \) or 28 days \( (n=7) \). Using radioactive microspheres, blood flow was measured to the marrow and various compartments of the femur and tibia, as well as other forelimb and head bones. Bone mass was also determined in these animals. In a separate group of animals, histomorphometric analysis of fluorochrome-labeling was used to determine mineral apposition rate and bone formation rate at the tibio-fibular junction in control (CON) and 7, 14, 21 and 28 day HU rats.

Results: The data demonstrate that HU rapidly (10 min) diminishes blood flow to the femoral (Figure 1) and tibial metaphysis (cancellous bone), diaphysis (cortical bone) and marrow, and that prolonged unloading (28 day) further decreases perfusion of the femoral shaft and marrow. Chronic unloading was also necessary to decrease fibular perfusion rate. These declines in blood flow appear to coincide with decreases in bone mass in 28 day HU rats (femur, \( P<0.05 \); tibia and fibula, \( P<0.1 \)). Histomorphometric analyses of fluorochrome-labeled bone also reveal large decrements in mineral apposition rate (60% decline) and bone formation rate (90% decline) at the tibio-fibular of mature adult rats with 21 and 28 days of unloading.

In contrast to the hindlimb bones, HU acutely (10 min) increases blood flow to the humerus, clavicle, skull and mandible. These acute elevations in blood flow to forelimb, shoulder and head bones appears to coincide with increased bone mass in HU rats (present study, Roer & Dillaman, J Appl Physiol 68, 1990).
Conclusion: The data demonstrates that mechanical unloading of bone rapidly diminishes blood flow to hindlimb bones and marrow. These decreases in flow appear to be the result of both increases in vascular resistance and decreases in perfusion pressure. In contrast, hindlimb unloading acutely elevates blood flow to the humerus, clavicle, mandible and skull. The increased flow to the skull was the result of a decrease in vascular resistance, while the increased flow to the other forelimb and facial bones was the result of an increased perfusion pressure. The decline in blood flow to the hindlimb bones appears to coincide with a diminished mineral apposition rate, density and mass of both cortical and cancellous bone observed in hindlimb unloaded rats. Correspondingly, the acute increase in blood flow to forelimb, shoulder and head bones appears to coincide with reports of increased mass. Therefore, we speculate that alterations in bone perfusion and associated changes in interstitial or intravascular shear stress may provide a stimulus for altering the balance between bone resorption and bone formation with simulated microgravity. The impact of these findings on human space flight warrants continued investigation.

FUTURE PLANS: Investigate mechanisms that may link blood flow and tissue fluid pressure to bone remodeling.

INDEX TERMS: hindlimb unloading; unweighting; simulated microgravity
EFFECTS OF HIGH-VOLUME PHYSICAL ACTIVITY ON BONE STIFFNESS, AND BONE TURNOVER IN COLLEGE-AGE FEMALES

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INTRODUCTION
Investigators have demonstrated positive effects of certain chronic exercise interventions on skeletal integrity. However, there appears to be a transient vulnerability of bone during an exercise induced remodeling process, where continued physical activity results in degradation of skeletal micro-architecture. Decreased indicators of bone formation have been reported at 4 weeks post-initiation of a running program, after which a subsequent increase in bone formation markers followed at 8 weeks. Mechanisms by which exercise leads to these changes are not fully understood. Traditionally, researchers have employed measurements of bone mineral density (DXA) and/or the biochemical markers of bone metabolic activity to describe bone adaptations to exercise. Although these tools are useful, they provide a surrogate measure for bone strength. The mechanical response tissue analyzer (MRTA) is a newly developed tool to measure the bending stiffness (EI) of long bones in vivo. The MRTA variable, EI, is the product of Young’s modulus of elasticity (E) and the cross-sectional moment of inertia (I). EI quantifies a physical property in bone associated with its macro- and micro-architecture. Serial MRTA measurements potentially increase the opportunity to identify compromised skeletal integrity attributable to exercise induced transient vulnerability. Inclusion of MRTA measurements along with DXA and biochemical markers of bone turnover may elucidate potential mechanisms by which bone is degraded, and subsequently enhanced.

CURRENT STATUS OF RESEARCH

Methods
The subjects were 30 volunteer females from the Virginia Polytechnic Institute and State University community. Of this total, 15 were randomly assigned to an exercise-training group (EX), and 15 were assigned to a non-training group to serve as controls (NT). The exercise group participated in high-intensity isokinetic resistance training, ~25 min/d, 3 d/wk, using the Biodex® isokinetic dynamometer that allows concentric/eccentric exercise with the legs, and 30 min/d, 3 d/wk running, for a total of 6 d/wk activity. The control group (NTX) did not participate in any structured exercise program during the 6 wk study. Bone mineral density (BMD), as well as isokinetic leg strength was assessed at baseline, and following the 6wk exercise training program. Tibial bending stiffness as assessed with mechanical response tissue analysis (MRTA), and serum biochemical markers of bone turnover (serum osteocalcin and serum NTx) were assessed at baseline, 2wk, 4wk, and post training.

Results
DATA COLLECTION FOR THIS STUDY IS COMPLETE. HOWEVER, MEASUREMENT ANALYSIS, AS WELL AS DATA ANALYSIS IS CURRENTLY ONGOING. IT IS ANTICIPATED THAT ALL MEASUREMENT AND DATA ANALYSIS WILL BE FINALIZED FOR PRESENTATION AT THE BIOASTONAUTICS INVESTIGATORS WORKSHOP

Conclusions
FUTURE PLANS
Future plans for this research include application of an improved 9-parameter and 12-parameter algorithm for estimation of tibial bending stiffness. The current 7-parameter algorithm for estimation of tibial bending stiffness under estimates the bending stiffness.

INDEX TERMS
Mechanical response tissue analysis (MRTA), exercise, osteocalcin, NTx, isokinetic strength, bone mineral density, DXA
In this project, musculoskeletal connective tissue metabolism was assessed in non-human primate rhesus monkeys (*Macaca mulatta*) before and after 14 days of spaceflight from daily urine samples and portions of small muscle biopsies obtained from lower leg extensor and flexor muscles. In an extension of the BION 11 spaceflight experiments, musculoskeletal connective tissue metabolism was measured before, during and after chronic 2G hypergravity in urine samples collected daily from rhesus monkeys. This project abstract is divided into three sections (BION 11 spaceflight urines, BION 11 muscle connective tissue biopsies, and Chronic 2G urines) highlighting the major findings.

**BION 11 Connective Tissue Metabolism Urine Biomarkers:** (Urine connective tissue metabolism biomarker analyses has been previously published in abstract form: Journal of Gravitational Physiology 7(1)S169-S170, 2000). Changes in urinary connective tissue and bone metabolites are thought to precede alterations at the tissue macromolecular level. Astronauts and Cosmonauts have also shown suggestive increases in urinary by-products of mineralized and non-mineralized tissue degradation. Thus, the idea of assessing connective tissue and bone response in spaceflight monkeys by measurement of biomarkers in urine has merit. The purpose of this investigation was to evaluate mineralized and non-mineralized connective tissue responses of non-human primates to microgravity by the non-invasive analysis of urinary biomarkers. **Methods:** 24 hr. samples were obtained intermittently before flight and after flight, before and after 1 G simulations of flight, and from vivarium monkeys. Urine volumes were measured and aliquots were frozen for subsequent analyses. Collagen biomarker concentrations of urinary hydroxyproline (Hyp), hydroxylysylpyridinoline (HP cross-links), and lysylpyridinoline (LP cross-links) were assayed by reverse-phase high performance liquid chromatography (rp-HPLC). Urinary creatinine (Cr) was quantitated using a colorimetric assay to measure potential muscle rhabdomyolysis and to normalize urine concentrations of biomarkers. Bone mineral metabolism was analyzed by assaying pre- and post-flight urinary calcium (Ca²⁺) by atomic absorption. Urinary osteocalcin (Oc), a biomarker for bone formation was analyzed by concentrations of biomarkers. Bone mineral metabolism was analyzed by assaying pre- and post-flight urinary calcium (Ca²⁺) by atomic absorption. Urinary osteocalcin (Oc), a biomarker for bone formation was analyzed by

**BION 11 Muscle Connective Tissue Analyses:** Short-duration space flight and hindlimb unloading cause pronounced skeletal muscle atrophy and weakening of the contractile elements, thereby subjecting the muscle and supporting connective tissue to increased ground reaction forces during reloading. Collagen, the major component of the muscle ECM, aids in the transmission of contractile forces to tendons and bones and prevents tissue deformation.
Very little is known regarding the plasticity of skeletal muscle collagen following acute spaceflight. Therefore, the objectives of this study are twofold: 1) To measure changes in muscle collagen gene expression from muscle biopsies following spaceflight [soleus (SOL) medial gastrocnemius (MG) and tibialis anterior (TA)] and 2) To assess the adaptive changes in muscle collagen concentration and maturation following micro-gravity. Methods: Lower limb pre-flight and post-flight muscle biopsy specimens were obtained from two rhesus monkeys flown on the Russian/US joint BION 11 Space Project for 14 days. The expression of the major muscle collagen genes, type I (COL1A2) and type III (COL3A1) were measured by quantitative-competitive RT-PCR. Collagen concentration (Hyp) and maturation (HP and LP cross-link content) were assayed by reverse phase-HPLC. Results: Muscle biopsy RT-PCR results indicate a significant decrease in COL1A2 (-25%) and COL3A1 (-16%) in the TA, but no significant changes in the SOL or MG muscles. Likewise, post-flight hydroxyproline concentration decreased significantly from pre-flight levels in the TA, but not in the MG or SOL. Post-flight HP and LP cross-link contents in the TA were significantly elevated compared to pre-flight contents with no changes in MG and SOL. Conclusions: These results indicate that muscle collagen adaptation may be dependent on muscle function and/or muscle duty cycle during spaceflight.

Connective Tissue Metabolism Urine Biomarkers During 2G Hypergravity: Loading of the skeleton is known to alter the metabolism of bone during growth. It is also known that many physical, electrochemical and hormonal stresses can collectively change bone and other nonmineralized connective tissues. Metabolic by-products of tissue metabolism measured in urine of astronauts, cosmonauts, and rhesus monkeys, suggests that an increased degradation of connective tissue occurs during short duration exposure to microgravity. Increases in the urinary excretion of hydroxyproline, collagen cross-links and mineral salts in astronauts is evidence that degradation of connective tissue can be enhanced by altered load environments, such as weightlessness. Little consensus data has been collected on the non-invasive measurement of collagen degradation products associated with an enhanced weight-bearing stress (hypergravity) on the skeleton. The purpose of this study is to assess the urinary collagen metabolic profiles of 6 rhesus monkeys during Pre-2G, Chronic 2G and Post-2G recovery periods. Methods: 24 hour collections were obtained from 6 individual rhesus monkeys. Urine volumes were recorded and stored at -85°C. Urine Hyp and collagen cross-links (HP and LP) were measured by rp-HPLC. Creatinine was measured using a kit assay. Results: During the 2G exposure period, our results showed a significant elevation of HP and LP cross-links and Hyp per volume of urine midway through the chronic 2G period followed by a decline in urinary content of cross-links and Hyp towards the end of the chronic 2G period. However, the 2nd week was still significantly elevated above resting pre-2G levels. Recovery to Pre-2G collagen biomarker levels was not achieved until the later half of the Post-2G period. Conclusions: Non-invasive measurement of collagen biomarkers are excellent physiological indicators of collagen metabolism during 2G hypergravity in rhesus monkeys. Work in progress: urine analyses to be added to this study will include urine Ca\(^{2+}\) and osteocalcin data. Future daily, in-flight, non-invasive collections of urine would be a useful tool to assess musculoskeletal plasticity in astronauts and cosmonauts during spaceflight.

Conclusions/Future Plans: Non-invasive assessment of connective tissue metabolism through the analysis of urine biomarkers has been measured in two BION experiments (BION 10,11) and a chronic 2G hypergravity study in rhesus monkeys by our laboratory. The levels of Hyp, HP & LP collagen cross-links and creatinine show an increase immediately post-flight compared to preflight levels indicating changes in the remodeling of connective tissue and muscle. Chronic 2G exposure also elevates Hyp, HP & LP collagen cross-links and creatinine concentrations initially during the first phase of a chronic 2G insult. We conclude that connective tissue metabolism during microgravity and hypergravity involve an initial resorption phase and can be used to measure total body connective tissue adaptation. Future in-flight 24 hr. urine collections in animals, including volume measurements, would improve our understanding of the temporal changes in microgravity induced connective tissue metabolism as it has for the 2G rhesus monkey experiments. Future long duration spaceflights may involve intermittent exposure to hypergravity as a possible countermeasure to spaceflight induced bone loss in humans. Non-invasive urine biomarker analysis during extended stays on the International Space Station may be a useful clinical tool to determine changes in connective tissue metabolism from pre-flight levels.

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Index Terms: collagen remodeling, musculoskeletal metabolism, non-reducible cross-links, biomarkers, resorption,
INTRODUCTION

Limb proprioception is one component of the neuromuscular system important to the execution of coordinated movements. Numerous reports from astronauts and cosmonauts indicate that the ability to accurately sense and control the position of the limbs is altered during and/or following spaceflight. While multiple sensorimotor systems (e.g. visual, vestibular) contribute to weight bearing postural control and the execution of coordinated movements, the present study investigated the neuromotor control of isolated joints in the absence of weight bearing. It was hypothesized that the ability to estimate requested positions of the elbow and ankle joints would be altered by microgravity, but would be improved during low level agonist muscle contractions as compared to passive joint movements.

METHODS

Four male astronauts, age 43.8 ± 3.8, aboard the 17-day STS-78 mission performed a series of tests estimating requested positions of the elbow and ankle joints. Preflight testing occurred 29/30 (L-30) and 11/12 (L-12) days before launch. During flight, the arm was tested on flight days (FD) 1, FD4/5, FD9/10, and FD15/16 and the leg was tested on FD2/3, FD7/8, and FD13/14. Postflight, the arm was tested on recovery (R+) days R+1, R+5, R+9, and R+16 and the leg was tested on R+0, R+2, R+4, R+8, and R+15 days. Subjects were asked to estimate joint angles during passive (relaxed) and active (10% maximal agonist effort) isokinetic extension (EXT) and flexion (FLX) of the elbow (90° or 135°) and plantarflexion (PF) and dorsiflexion (DF) of the ankle (100° or 120°). The velocity of isokinetic movement was 10° • sec^{-1} in each direction and the range of movement was within ±5° of full voluntary range of motion. Subjects also estimated these joint positions during low resistance isotonic elbow EXT and ankle PF. For each angle, six (isokinetic modes) and three (isotonic mode) trials were requested in random order.

RESULTS

Ankle (Fig. 1)

The joint angle estimates for 120° were reduced (P<0.05) during isotonic PF on the 2nd and 7th days of flight compared to specific pre- and/or post-flight days. Estimates of angles during isotonic PF (mean error -6° for 100° and -18° for 120°) were less than passive isokinetic PF, but greater than passive isokinetic DF (P<0.05). No effects of spaceflight occurred for any isokinetic condition. For passive isokinetic movements, angle estimates were most accurate during PF (mean error +3° for 100° and -6° for 120°), whereas mean estimates were 15° lower (P<0.05) for both angles during DF. No differences occurred between passive vs. active isokinetic PF or DF.

Figure 1. Ankle joint estimation for 100° (a.) and 120° (b.).
* significant decrease during flight
Elbow (Fig. 2)
During spaceflight, joint angle estimates were lower during isokinetic FLX and EXT for 90° (P<0.05) and 135° (P< 0.10) compared to preflight. Spaceflight also decreased angle estimates during isotonic FLX and EXT (P<0.05). Elbow angle estimates had returned to preflight values one day after return to 1G. Across all test conditions, the mean angle estimate ranges were 93°-118° and 119°-152° for requested angles of 90° and 135°, respectively. Subjects overestimated the 90° position across all conditions, whereas the accuracy of the 135° estimate varied. Angle estimates were most accurate during isokinetic FLX for 90° (mean error +9.8°) and isokinetic EXT for 135° (mean error +0.7°). No differences occurred between passive vs. active isokinetic EXT or FLX. Estimates of each joint angle during isokinetic EXT were less than isotonic EXT, but greater than isokinetic FLX (P<0.05).

CONCLUSION
These data demonstrate that ankle and elbow joint angle estimation was: 1) decreased during spaceflight, particularly for agonist muscle exertion during isokinetic movements. In conclusion, the proprioception of isolated joint position is altered by microgravity, but rapidly readapts to preflight control values after return to 1G. These spaceflight effects are manifested in isolated, non-weight bearing joints and apparently occur independent of visual or vestibular sensorimotor systems.

INDEX TERMS
proprioception; joint position; motor control
With the advent of cDNA microarrays, it has become possible to profile the changes in expression of large numbers of genes under different conditions. In an effort to establish a comprehensive picture of the many transcriptional adaptations which lead to the loss of muscle mass, we have undertaken to define the changes in mRNA expression in muscle atrophying due to different causes. In response to food deprivation, protein synthesis falls in muscle, and protein degradation increases primarily to supply amino acids for gluconeogenesis. This increase in muscle proteolysis seems to involve similar mechanisms to that seen in cancer, and disuse (e.g., in microgravity) as well as many systemic diseases (e.g., cancer, sepsis, diabetes). In different animal models, we previously found that such muscles all exhibit a common set of adaptations that indicate an activation of the ubiquitin-proteasome pathway, e.g., mRNAs for components of the Ub-proteasome pathway increase 2-3 fold. The ubiquitin-proteasome pathway was previously believed to degrade primarily short-lived proteins, but more recently, we showed that long-lived proteins, e.g., myofibrillar components, are also degraded by this pathway.

There must exist a number of other atrophy-related genes critical for the development of muscle wasting. To identify such components and to analyze the transcriptional adaptations in muscle atrophy, we have used cDNA microarrays to compare muscle from normal and food-deprived mice. Fasted animals were chosen for initial analysis because the various changes in energy metabolism and protein breakdown have been well characterized. Surprisingly, the levels of the vast majority (98%) of gene transcripts do not change in the atrophying muscles after food deprivation. As expected, we found a coordinate up-regulation of ubiquitin and many 20S and 26S proteasome subunit genes, as well as a generalized decrease in mRNAs for many myofibrillar genes, and enzymes of glycolytic and oxidative metabolism. In addition, we find a group of previously unidentified genes (we termed atrophins) whose transcripts increase markedly in the atrophying muscles. We have cloned one of these genes, atrophin-1, whose expression increases 9-fold specifically in skeletal muscle, but not in other tissues of the food-deprived mice. It also increased sharply in other forms of muscle atrophy. It is expressed specifically in muscle and rises in other forms of atrophy. This gene contains an F-box, a motif found in the SCF class of Ub-protein ligases (E3s), and a nuclear localization sequence. It therefore may be involved in the degradation of critical regulatory proteins in the atrophying muscles.
THE EFFECTS OF ALTERED LOADING STATES ON MUSCLE MASS AND REGULATORY MARKERS OF PROTEIN TRANSLATIONAL PROCESSES

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INTRODUCTION

Skeletal muscles used extensively during locomotor activity undergo both a rapid and marked degree of atrophy that can result in as much as a ~40% loss in mass and protein content in response to chronic unloading of rodents (spaceflight and hindlimb suspension). The atrophy process involves an imbalance in protein synthetic activity relative to protein degradation processes such that there is a net loss in muscle protein, the chief of which involves the contractile machinery. Strategies that are aimed at attenuating muscle wasting must therefore focus on understanding those factors that control protein translational and/or degradative processes. In order to address this issue our laboratory has initiated studies examining the effects of different loading states (chronic functional overload, intermittent resistance loading and hindlimb unloading models) on 1) regulatory proteins that impact the putative rate limiting steps involving the initiation process of protein translation (Fig. 1), and 2) associated signaling pathways involving muscle insulin like growth factor 1 (IGF-1), a factor that is thought to impact the compensatory growth of muscle via an autocrine/paracrine mode of action. The central hypothesis being tested is that both continuous and intermittent increases in muscle loading state up regulate expression of muscle derived IGF-1 and subsequent signaling molecules (phosphorylation state of ERK-MAPK kinase and P70 S6 kinase) and functional activity of protein factors that are strategic in regulating a) the ternary binding process [via phosphorylation state of eukaryotic Initiation factor 2α (eIF2α-P)], and b) the mRNA 5’ cap-binding process [via the phosphorylation state of eukaryotic Initiation Factor 4E Binding Protein (4E-BP), e.g., two processes that affect global protein synthesis (Fig. 1 and 2). Augmentation of these processes favors hypertrophy of muscles in load bearing animals; whereas, unloading states either reduce or fail to augment translational activity to a level sufficient to compensate for the increase in protein degradation that occurs.

CURRENT STATUS OF RESEARCH

Methods.

Groups of adult female rodents were either maintained as normal controls (C) or subjected to the following: 1) Functional Overload (OL) in which the synergists of the plantaris/soleus muscles were surgically removed and the animals were allowed to weight bear from 1 to 6 days; 2) Resistance Training (T) in which the animals performed 2-3 consecutive training sessions consisting ~60 4-sec isometric contractions per session separated by 24 hrs of rest; and 3) continuous Hindlimb Suspension (HS) for varying durations up to 14 days. At the appropriate time points, animals were euthanized and the muscles were removed and analyzed for the above mentioned markers using either Western blot or RT-PCR based mRNA analyses.

Results.

IGF-1 Expression. OL induced an ~4-fold increase in the level of IGF-1 peptide in the first 24 hrs of overload and this response was maintained throughout an 8-day period (Fig 3). Additional analyses revealed that the mRNA for both IGF-1 and a muscle-specific variant (mechano growth Factor (MGF)) was increased in both OL and T treated...
Collectively, these results suggest that when increased loading is either continuously or intermittently imposed on skeletal muscle, regulatory processes favoring general protein translation of mRNAs, as well as specific mRNAs involved in regulating translational events (such as protein elongation factors), are augmented. In contrast, when muscles such as the soleus, which are normally used extensively in weight bearing activities, are unloaded, there are transient reductions in phosphorylation of P-70S6 kinase and 4E-BP (Fig. 7), thereby establishing a functional state consistent with atrophy and net protein loss in the muscle. Consistent with this interpretation is the observation that a marker enzyme of protein degradation, E2 14k ubiquitin carrier enzyme, undergoes increased mRNA expression in response to unloading state and decreased expression in response to OL (Fig. 8).

**Conclusion and Future Directions.**

Based on the findings reported herein, it appears that T induces augmented potential for muscle growth; whereas, unloading induces the opposite based on their respective effects on protein translational/degradation processes. Therefore, future research needs to focus on how the interaction of T and HS control these regulatory processes in the context of their overall effect on protein balance and the maintenance of muscle mass. Supported by NSBRI NCC9-58-A.
Wound Healing Response of the Medial Collateral Ligament During Hindlimb Unweighting in Young Rats

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Introduction: The likelihood of an astronaut receiving an injury or needing an invasive medical treatment that would require repair and the prevention of infection is highly probable during an extended duration spaceflight mission (SpaceStation or Manned Mars Mission crews). Some astronauts participate in extra-vehicular activity (EVA), by moving, manipulating and constructing high mass hardware and/or participate in other tasks that may increase the probability of being injured. Unfortunately, there is very little spaceflight data from human, non-human primate or rodent experiments on injury repair or trauma associated with the musculoskeletal system. The few pieces of data obtained from rat studies have indicated that weightlessness retards the repair of bone and skeletal muscle (4). Previous studies from our laboratory have demonstrated that exposure to hindlimb suspension (HS) causes a compromise of the musculoskeletal system. In particular, rodents subjected to 14 and 21 days of HS had significantly weaker ligament-bone-ligament junction (9). Furthermore, spaceflight and/or HS induces muscular atrophy and decreases in strength (8). In essence, HS and/or microgravity have a deleterious effect upon the strength and energy storage capacity of connective tissue.

Wound healing and tissue repair is highly dependent upon the optimal regulatory control of local and systemic hormones, sequential release of essential cytokines, optimal load histories, and nutrient delivery to the injured area. Following spaceflight and/or HS, a diminished immune system response has been reported (7). Decreased IL-1, IL-2 and TNF-α production was recorded from rat spleen cells flown on the SLS-2 mission (5). A decreased lymphocyte function was recorded in Russian cosmonauts following spaceflight (1), while rhesus monkeys flown 14 days have exhibited a decrease in IL-1 production and a decrease in IL-2 receptor expression (6). Other endocrine functions are compromised during spaceflight and hindlimb suspension, specifically the secretion of growth hormone (GH). Hindlimb unloaded rats showed a diminished secretion of bioassayable GH, a decreased responsiveness to growth hormone releasing factor (GRF) (2), and a decreased immunoassayable plasma GH in intact rats (10) and decreased plasma GH concentration following spaceflight (2). Non-human primates showed a marked suppression of GH secretion after 14 d of flight on the BION 10 (Cosmos 2229) (3). Thus, it is apparent that changes in immune and endocrine functions occur in humans (astronauts and cosmonauts), non-human primates and rodents following spaceflight and could compromise the wound healing processes. Therefore, the primary objective of our project is to use the rodent knee ligament injury repair model to study the impact HS has on the wound healing processes.

Current Status of Research: Protocol Design: A timeline of the experimental design is illustrated on Figure 1.

The study is divided into two parts: Year 1 (Y01) is investigating the effects of HS on wound healing of rodent knee ligaments; Year 2 and Year 3 (Y02&Y03) will assess the efficacy of recombinant human growth hormone (rhGH) supplementation as a treatment for ligament wound healing during HS. In each study, a time-course of ligament healing will be studied: 3 week and 7 week injury repair. An n=24/group has been created to increase the power of the statistical analyses for biochemical & molecular biology measurements (n=12/group), and biomechanical & scanning electron microscopy measurements (n=12/group).

Figure 1. Time-line of Y01-Y03 Wound Healing Experiments

Animal model and surgical lesions: All surgeries are being performed at the Animal Care Facility at NASA-Ames. Young rodents (~200g) are induced with isoflurane (inhalation) anesthesia and treated aseptically. The bilateral MCLs are transected at mid ligament with a scalpel. The fascia and skin incision are closed with one to two sutures. Animals are allowed to recover for 24hrs. and given analgesics, then assigned to one of three experimental groups. Hindlimb Unloading: The animals are being suspended for periods of 3 weeks or 7 weeks, using the HS protocol of Wronski and Morey-Holton (11). Rats are fed and watered ad libitum and checked daily for overall health, skin incision healing, food/water consumption, and the condition of their tails. Growth hormone: In Y02&Y03 of the study subcutaneous injections (s.c.) of recombinant human growth hormone (rhGH) and vehicle will be given to the
appropriate groups twice daily at 0730 and 1630 hrs. The rhGH is best administered in two equal doses (0.5 mg/kg BW) s.c., to simulate the pulsatile release that occurs in vivo.

**Plasma Growth Hormone and IGF-I Detection:** Radioimmunoassays (RIAs) specific for rat GH and rat insulin-like growth factor-1 (IGF-1) are being used to document the circulating plasma concentrations in Y01, Y02, and Y03.

**Biochemical and Gene Expression Experiments on Rodent MCL:** In one MCL, biochemical markers of collagen concentration (hydroxyproline), amino-sugars concentration and collagen maturation (hydroxylysylpyridinoline) will be measured by rhPRLC. Ligament hexuronate and DNA content will be assayed colorimetrically using a platereader. In the contralateral ligament, total RNA will be isolated and reversed transcribed into cDNA and stored at -85°C. Quantitative-competitive RT-PCR will be performed on aliquots of total RNA isolated from each ligament and analyzed in triplicate. Collagen type I (Col1A1), type III (Col3A1) type V (Col5a1) plus other genes coding for fibrillar and nonfibrillar proteins involved in the wound healing process will be compared.

**Biomechanical and Scanning Electron Microscopy (SEM) Analyses:** Testing of the MCL is being done with femoral and tibial insertion sites intact and immersed in Hank's balanced salt solution. Strain is being measured using video image analysis. The bone-ligament-bone complex is pulled to failure at a grip speed of 1.0 mm/s. Strain at each location in the specimen is determined from the four optical markers by video dimensional analysis and structural stiffness will be computed for each region. **SEM:** The morphology of the ligament scar tissue and collagen fiber matrix microstructure will be compared to normal MCLs by SEM analyses.

**Statistical Analyses:** The quantitative data obtained from biomechanical, biochemical, and molecular biology methodology will be analyzed with a series of two factorial analysis of variance: each dependent variable will be analyzed individually as they provide separate information.

**Results:** As of this abstract submission, the surgical procedures to induce the wound and hindlimb suspension of over 48+ animals plus controls (n=144+), during two different suspension/healing time courses (3 weeks and 7 weeks) has not been completed. Body weight analyses from the 3 week group show 10% less weight gain in the HS+lesion animals compared to Amb+lesion controls as shown in Figure 2.

**Preliminary biomechanical data demonstrates a large decrease in the load to failure (Fig.3) and stress (Fig.4) in the MCL of the HS+lesion group versus Amb+lesion controls in the 3 week group. Both lesion groups were significantly less than non-surgical Shams. Further analyses will be performed on the remaining tissues.**

**Conclusions/Future Plans:** The surgeries and hindlimb unweighting experimental protocol has been a success. We plan to perform further biochemical and biomechanical testing on tissues from the 3 week group. The 7 week suspension group will be taken down from their suspension harnesses on 11/1/00, weighed, euthanized and dissected.

**Index Terms:** wound, ligament, collagen, remodeling, extracellular matrix, maturation, hindlimb suspension, atrophy.

INFLAMMATORY CELL FUNCTION IN MUSCLE INJURY AND REPAIR

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INTRODUCTION

Muscle weakness, injury and inflammation are important obstacles to normal locomotory function in individuals subjected to periods of muscle unloading followed by normal loading. These defects are expected to greatly impair the activity of personnel upon return to normal gravitational loading following spaceflight. The design of optimal preventative or therapeutic treatments to minimize the muscle defects that arise upon reloading will rely upon identifying the factors that contribute to muscle damage during this reloading period.

Our findings support the view that myeloid cells contribute to promoting injury and repair during muscle reloading. We hypothesize that neutrophils promote injury to reloaded muscle via free radical mediated events. Furthermore, we propose that the reduction in the expression of nitric oxide synthase (NOS) during muscle unloading renders muscle susceptible to neutrophil-mediated damage, because NO can normally protect muscle from damage by free radicals. Finally, we hypothesize that macrophages participate in muscle repair, so that interventions that affect their presence or activity in muscle can influence the repair process.

CURRENT STATUS OF RESEARCH

Methods
We have used in vivo and in vitro models to assess that contribution of inflammatory cells to muscle injury that occurs during reloading following periods of unloading. Our in vivo model consists of rat or mouse hindlimb suspension followed by reloading, in which we measure muscle inflammation by quantitative histopathology assays and measure muscle cell membrane injury by measuring the presence of extracellular tracer dyes in the muscle cell cytoplasm. Our in vitro model consists of measuring muscle cell lysis in co-cultures of muscle cells and selected myeloid cell populations, using chromium release cytotoxicity assays. We also incorporate in vitro muscle cell loading protocols into cytotoxicity assays to assess the relationship between muscle cell lysis by myeloid cells in the presence and absence of myeloid cells.

Results
1. Time course studies of soleus muscle cell membrane injury during reloading following hindlimb suspension shows a close relationship between the invasion of reloaded muscle by neutrophils and the occurrence of membrane damage.
2. In vitro cytotoxicity assays show that neutrophils in co-cultures with muscle cells lyse muscle cells by superoxide-dependent processes.
3. Inhibition of nitric oxide synthase (NOS) activity increases muscle cell killing in neutrophil-muscle co-cultures.
4. Muscle unloading by hindlimb suspension decreases muscle NOS expression and activity.
5. The return of muscle nNOS expression during reloading to the levels that normally occur in ambulatory muscle corresponds with end of increased muscle cell membrane injury during reloading.
Conclusions
Our findings support the hypothesis that interactions between myeloid cells and muscles experiencing modifications in loading are important in determining the course of muscle injury and repair. Our findings indicate that muscle unloading may compromise the free radical defense mechanisms of muscle by reducing nNOS expression. This impairment renders the unloaded muscle more susceptible to superoxide-mediated damage by invading neutrophils upon muscle reloading.

FUTURE PLANS
We are testing whether normalizing muscle-derived NO production in unloaded soleus muscles to ambulatory control levels will diminish muscle injury during reloading. We have generated a transgenic mouse in which there is a muscle specific overexpression of nNOS so that we can modulate NO production in unloaded muscles to the levels that occur in ambulatory mouse muscles. Muscle inflammation and fiber injury that occur during reloading following hindlimb suspension are being compared in transgenic mice and wild type mice. In addition, we are testing whether experimental manipulation of neutrophil concentrations or superoxide-generating capability in reloaded muscle can reduce muscle injury.

INDEX TERMS
muscle; muscle injury; muscle inflammation; neutrophil; macrophage; nitric oxide; nitric oxide synthase; superoxide
LONG TERM ACTIVITY LEVELS OF FLEXOR AND EXTENSOR MOTOR POOLS OF THE ANKLE AND ELBOW OF HUMANS BEFORE, DURING AND AFTER SPACEFLIGHT

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INTRODUCTION

Both supraspinal and proprioceptive inputs to spinal motor pools are altered by microgravity but little detail is available as to what these changes are or what impact these changes have on the output of the different motor pools. To begin to address this problem, we recorded the chronic levels of EMG activity in ankle and elbow flexor and extensor muscles during spaceflight and during routine activities on Earth.

To gain a further understanding of how the human neuromotor system is shaped by gravity we designed experiments that document also how the sudden onset and a more prolonged exposure to microgravity affects the activity levels of the flexor and extensor motor pools. That is, what are the patterns and levels of activity of motor pools that control muscles of nonweightbearing vs. weightbearing limbs and muscles that work directly against gravity to maintain an upright position.

MATERIALS AND METHODS

Four healthy male astronauts, with no previous spaceflight experience, participated voluntarily in the present study, one component of the National Aeronautics and Space Administration (NASA) Life and Microgravity Spacelab STS -78 mission. Mean age for these subjects was $43.8 \pm 3.8$ years. EMG electrodes were placed directly over the belly of the soleus (Sol), medial gastrocnemius (MG), tibialis anterior (TA), biceps brachii (BB), and triceps brachii (TB) muscles. EMG signals were amplified using custom made amplifiers with a gain of 1000 and the signals were sampled with 12 bit resolution at a 1 kHz sampling rate and stored on an IBM ThinkPad hard drive. The analysis program rectified the EMG data, calculated averages for successive 40 msec segments throughout the day and constructed amplitude histograms from the averages. The averaging process approximated a low pass filter with a cutoff of 12.5 Hz. Integrated EMG levels were calculated by summing the product of the amplitude represented by each bin and the bin count. Histograms of baseline noise were used to determine which bins represented zero activity. This was typically the first two or three bins of the histograms. Counts in the remaining bins were used to calculate the duration of the activity. Two to three 24-hour recording sessions before, during and after flight on four male astronauts from the NASA STS-78 mission were completed.

RESULTS

These data show that activation of the motor pools of the TA, an ankle dorsiflexor, increased substantially during spaceflight while the ankle extensors (Sol, MG) were
maintained at approximately normal activation levels during spaceflight. The total activity of both an elbow flexor (BB) and extensor (TB) was significantly greater during than after or before flight. Together, these data indicate that spaceflight is not necessarily a model of reduced or even low levels of activation for many muscles in humans.

We suggest that the elevated activity in the TA is due in part to a lack of reciprocal inhibition that is routinely derived from the activation of extensors during weight bearing. It is also likely that some of the elevated activity can be attributed to routine motor tasks performed routinely during flight; that is, securing the body in a stable position using foot loops. Activation of the dorsiflexors occurs routinely when the foot is placed in these loops. However, based on both the total integrated activity and the duration of periods when activity is present, it would appear that the use of the loops cannot be the total explanation for the elevated integrated activity in the TA.

It is also of interest that there was not a reduced level of activity in the MG or Sol during space flight. Some of the activity in these muscles must also be attributed to the busy work schedule of each crew member during flight. It is also important to understand that numerous experiments, on this particular flight particularly, involved extensive neuromuscular testing. From this perspective the activity levels more accurately reflect how the neuromuscular activity patterns responds to a series of programmed motor tasks in a microgravity environment rather than to a microgravity environment per se.

CONCLUSIONS

The elevated activity of both flexor and extensor motor pools controlling the elbow reflects a marked increase in the use of the upper limbs for generating whole body mobility, as well as for performing detailed motor tasks with the hands while in flight. The elevated activity of the TA may reflect an absence of reciprocal inhibition as occurs during weight bearing at 1G.

INDEX TERMS

Electromyographic activity, flexor and extensor muscle function
RESISTANCE TRAINING USING FLYWHEEL TECHNOLOGY PROMOTES HYPERTROPHY OF UNLOADED MUSCLE

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INTRODUCTION
Spaceflight compromises skeletal muscle function and size. Because resistance exercise promotes muscle hypertrophy and increases in muscle strength, it also appears attractive as an in-flight countermeasure. Resistance exercise simulating weight-training on Earth is however not readily executed in microgravity. We have developed a mechanical non-gravity dependent exercise system, which uses the inertia of one or more flywheels to produce resistance during both concentric and eccentric actions. This study examined the efficacy of this exercise system to prevent muscle atrophy during lowerlimb unloading - a spaceflight analog.

CURRENT STATUS OF RESEARCH
Methods
Male and female volunteers performed unilateral lowerlimb unloading (n=10; UL) by walking on crutches for five weeks. Another group (n=10; ULRE) in addition, performed resistance exercise using their unloaded limb. A third ambulatory group (n=10) performed resistance exercise (RE) only. Twelve exercise sessions consisted of four sets of seven coupled concentric and eccentric knee extensions on an inertial ergometer 2-3 times weekly. Before and after UL, ULRE and RE, muscle cross-sectional area (CSA) of individual knee and ankle extensor muscles was measured by means of magnetic resonance imaging (MRI). Also, maximal isometric strength was measured during knee extension and leg press, respectively and maximal concentric and eccentric strength assessed during knee extension.

Results
UL produced decreases (p<0.05) in muscle CSA and strength, whereas RE produced marked increases (p<0.05) in knee extensor muscle CSA and strength. The ULRE group showed an increase (p<0.05) in muscle CSA but no increase (p>0.05) in strength. The hypertrophic response was similar in RE and ULRE. Performance across the twelve exercise sessions increased (p<0.05) in both RE and ULRE. UL and ULRE showed a similar decrease (p<0.05) in ankle extensor muscle CSA.

Conclusion
These results suggest muscle atrophy induced by five weeks of unilateral lowerlimb unloading is prevented by resistive exercise using flywheel technology performed two or three times weekly. In fact, it appears the potential for exercise-induced muscle hypertrophy is not reduced in unloaded muscle. We believe this novel resistance exercise technique, should be employed as an exercise countermeasure in space.
FUTURE PLANS
The flywheel technology will be employed in an exercise configuration that will comply with NASA’s requirements of a resistive exercise device designed for the International Space Station.

INDEX TERMS
Countermeasure, Muscle atrophy, Space flight, Strength training
Dr. Susan Bloomfield

Dramatic losses of bone mineral density (BMD) and muscle strength are two of the best-documented changes observed in humans after prolonged exposure to microgravity. Recovery of muscle upon return to a 1-G environment is well studied, however, far less is known about the rate and completeness of BMD recovery to pre-flight values. Using the mature tail-suspended adult rat model, this proposal will focus on the temporal course of recovery in tibial bone following a 28-d period of skeletal unloading. Through the study of bone density and muscle strength in the same animal, time-points during recovery from simulated microgravity will be identified when bone is at an elevated risk for fracture. These will occur due to the rapid recovery of muscle strength coupled with a slower recovery of bone, producing a significant mismatch in functional strength of these two tissues. Once the time-point of maximal mismatch is defined, various mechanical and pharmacological interventions will be tested at and around this time-point in an attempt to minimize the functional difference of bone and muscle. The outcomes of this research will have high relevance for optimizing the rehabilitation of astronauts upon return to Earth, as well as upon landing on the Martian surface before assuming arduous physical tasks. Further, it will impact significantly on rehabilitation issues common to patients experiencing long periods of limb immobilization or bed rest.
THE BIOMECHANICS OF EXERCISE COUNTERMEASURE

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In the last 5 years, considerable NASA resources have been devoted towards the design and construction of a vibration isolated treadmill (TVIS) that is soon to be installed in the International Space Station. This device is attractive from a countermeasure standpoint since it may, with properly designed programs, allow simultaneous exercise countermeasures to be applied against bone, muscle, and cardiovascular adaptations. Despite the need for on-orbit exercise using TVIS, almost no research has been conducted to determine the acceptability of the subject load device and harness that must be used to tether the crew member to the treadmill during exercise. Data regarding the bone strains produced by exercise countermeasures, such as running in reduced gravity, are also unavailable, yet there is little doubt that bone strain plays an important intermediary role in the signal transduction pathway responsible for bone maintenance and functional adaptation. We hypothesize that treadmill running on-orbit, under tolerable gravity replacement loads, alters limb kinematics, ground reaction forces, and muscle action, resulting in significantly different mechanical stimuli for bone maintenance than treadmill running in a terrestrial 1G environment. To test this hypothesis we propose to use an integrated series of studies that will take advantage of two unique ground based simulators. The first is the Zero Gravity Locomotion Simulator (ZLS) in which human subjects can walk and run in simulated zero gravity. An important feature of the ZLS is that it uses the same subject load devices (SLDs) that are used on-orbit to accelerate the subject back to the treadmill surface. We will use the ZLS to define estimates of user comfort, kinematics, ground reaction forces, and muscle activity during a series of exercise protocols that might be used on-orbit. These data will then be input to the second simulator, the Dynamic Gait Simulator (DGS), to directly measure bone strains in cadaver limbs moving through accurate kinematic and kinetic reconstructions of ground-based and orbit-based locomotion. These two sequential experimental approaches will provide unique psychophysical and biomechanical information having direct operational relevance (i.e. the design of better exercise countermeasures to bone loss on the ISS). The experiments will also provide fundamental new data on the state of strain in human bone and its relationship to external loading.
PRECISION BONE STRUCTURAL MEASUREMENTS BY ADVANCED MULTIPLE PROJECTION DUAL ENERGY X-RAY ABSORPTIOMETRY (AMPDXA) TECHNIQUES FOR SPACEFLIGHT APPLICATIONS*

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INTRODUCTION
An Advanced Multiple Projection Dual Energy X-ray Absorptiometry (AMPDXA) Scanning System is being developed to monitor the deleterious effects of weightlessness on the human musculoskeletal system during prolonged spaceflight. The AMPDXA is designed to measure bone mineral density (BMD), decompose soft tissue into fat and muscle, and derive structural properties (cross-sections, moments of inertia). Such data permits assessment of microgravity effects on bone and muscle and the associated fracture risk upon returning to planetary gravity levels. The protoflight scanner will be designed to minimize volume and mass (46 kg goal), while maintaining the required mechanical stability for high-precision measurement. The AMPDXA can detect 1% changes in bone mass and geometry and 5% changes in muscle mass.

RESEARCH STATUS
It is known that the elimination of gravitational effects on the human body produces an adaptation response resulting in the wasting of body skeletal muscle and bone mass. The stimuli for maintaining homeostasis of muscle and bone appear to be different; moreover, there also appear to be differences between losses in weight bearing and non-weight bearing body regions. Bone mass lost from the vertebrae, pelvis, and proximal femurs of astronauts average between 1 and 1.6% per month. This compares to the loss of about 0.8 to 1.3% per year in postmenopausal women. The ultimate concern is that the loss of mass will lead to degradation in mechanical competence and possible failure. During prolonged space travel, bone fracture may prove to be catastrophic, especially since healing in the absence of mechanical stimulus (load) is believed to be degraded. Clearly, effective countermeasures to stem the loss as well as some method for dynamically monitoring countermeasure effectiveness are required. On earth, skeletal load causes mechanical strains within the bone, which tend to be greatest on the subperiosteal surface. Normal bone turnover accompanying the aging process causes a net loss of bone from endocortical and internal surfaces. This loss causes skeletal strains to increase, but in long bones the increase is greater on the subperiosteal surface, not at the internal surfaces where the mass loss occurred. New bone forms on the subperiosteal surface sufficiently fast to maintain the section modulus. Since it takes less new bone on the subperiosteal surface to compensate for bone loss from internal surfaces, strength can be maintained in the presence of net bone loss. This mechanism requires skeletal loading, which is absent on the lower skeleton during spaceflight. Bone loss accelerates under diminishing loading and evidence from Cosmonaut data on Mir suggest that no compensatory changes take place. This means that astronauts may be at a greater risk of fracture for the same loss of bone mass. Hence, it is important to determine the geometrical configuration of the bone structure.

Methods
The AMPDXA development is being carried out in three stages: (1) Laboratory Test Bed for instrument development, (2) Clinical Test System for ground-based human testing and (3) a protoflight design for space applications.

Results
The full-sized Laboratory Test Bed (1 meter source to detector distance) was constructed to verify principles and theoretical predictions. Scanning is provided by high-precision rotating and translating stages. The test bed, in conjunction with a high-resolution detector and our analysis software, has produced some exciting preliminary results. Figure 1(a) is a BMD image of a human femur immersed in a cylinder of water (simulates fatty tissue). The same bone was imaged on a new commercial DXA scanner located at the Johns Hopkins Hospital as shown in Figure 1(b). The improvement in spatial and contrast resolution with our scanner is quite evident by comparing the two figures. This improvement is further elucidated by the graph in Figure 1(c). The curves are measured bone projected thicknesses on a slice through the femoral shaft. The fine variations on the AMPDXA profile are not
noise, but reflect small changes in the actual bone thickness. Using multiple projections, as shown in Figure 2, about the bone axis allows structural properties (e.g., bending strength) to be obtained independent of patient position. To do this at least three arbitrary projections over 90 degrees (two of which are orthogonal) must be obtained. Such analysis can provide maximum and minimum moments of inertia for bending or torsion in any plane. Our experiments to date with different sets of three projections show that the principal moments of inertia can be determined within 3 to 4%. Additional projections (above 3) reduce this number further. Our experimental systems also have some known non-linearities which when removed will drop the error in the three projection estimation of moments to less than 1%.

CONCLUSION
An Advanced Multiple Projection Dual Energy X-ray absorptiometry (AMPDXA) System has been designed and two ground-based test systems developed. Results from these systems indicate that an AMPDXA system would provide the accuracy and repeatability necessary to monitor bone (muscle) loss in space as well as to develop and monitor the efficacy of countermeasures. The availability of an AMPDXA offers significant potential for fostering future musculoskeletal research in a number of disciplines.

FUTURE PLANS
The AMPDXA is capable of real-time monitoring of bone and muscle loss at extremely high precision. Since the results are patient-specific and not tied to volumetric averages and statistical norms, the AMPDXA is a very useful tool for monitoring the effectiveness of countermeasures as well as determining risk of fracture under various loading conditions and activity scenarios. To bring the AMPDXA to its full potential, the following specific research problems must be addressed: (1) Human testing to develop the final instrument parameters, (2) refinement of soft tissue extraction algorithms (3) solution to the 2-D vs. 3-D reconstruction problem, (4) refinement of bone strength vs. risk of fracture algorithms, (5) develop lightweight power supply, and (6) software refinements to allow radiograph collection for the diagnosis of injury and disease.

INDEX TERMS
dual energy x-ray absorptiometry (DXA), bone mineral density (BMD), bone loss, muscle loss, osteoporosis, microgravity effects, advanced multiple projection DXA (AMPDXA)

Figure 1. Comparison AMPDXA vs. conventional DXA. (a) AMPDXA BMD image. (b) Commercial DXA BMD image (same bone as (a)). (c) Bone mass profiles with distance across a given bone section.

Figure 2. Multiple DXA projections using rotational stage.
MICRO-GRAVITY INDUCED CHANGES IN THE CONTROL OF MUSCLES

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INTRODUCTION

The detrimental effects micro-gravity on muscle function are well-known. These effects may be mediated through two factors: 1) changes in muscle mass and mechanical properties, and 2) the control of muscles by the central nervous system. While there have been a number of studies addressing atrophy and fiber type changes in astronauts, the effects of micro-gravity on the control aspects of muscles have received very little attention. This work is geared toward filling this gap in the current understanding by focusing specifically on the micro-gravity induced changes in the neural control of muscles, both in terms of the regulation of the firing of motor units that comprise a muscle and in terms of the coordination of a group of muscles which control a given body joint.

The specific aims of the project can be outlined as:

1. Understand the effects of micro-gravity on the control of motor units,
2. Compare the effects of micro-gravity on two muscles that are used to varying extents in space, in order to assess the potential of muscle use to curtail micro-gravity induced disturbances in control properties of motor units,
3. Understand the effects of micro-gravity on the force contributions of muscles controlling a given joint,
4. Determine the course of recovery after re-exposure to Earth’s gravity.

CURRENT STATUS OF RESEARCH

Methods

In order to determine the effects of micro-gravity on muscle control, we will test at least 4 astronauts (depending on availability) before and after spaceflight. We will repeat baseline measurements on three consecutive days two weeks before launch in order to establish the inter- and intra-subject variability. The astronauts will be tested immediately upon return to Earth (R+0) and the tests will be repeated 5 more times on days R+1, R+2, R+4, R+7, R+13 to study the recovery and adaptation back to gravity.

Intramuscular EMG will be used to assess the control properties of motor units within a given muscle. Two muscles have been identified for investigation: the First Dorsal Interosseous (FDI) of the hand and a knee extensor, the Vastus Medialis (VM). The investigation of these muscles will enable us to observe any rehabilitative effect of continued use of the muscle under micro-gravity conditions. Specifically, we will determine if exercising the muscle in space slows down any detrimental effects of micro-gravity on the neural control of muscles, in addition to attenuating atrophy. In evaluating the control of motor units, we will employ approaches that are similar to those depicted in Figure 1. This figure summarizes the assessment of the effects of aging on the control of motor units through the investigation of mean firing rates and recruitment thresholds of motor units, as well as the correlation among the firing patterns of concurrently active motor units.

Surface EMG will enable us to characterize the activation levels of individual muscles controlling a joint. By comparing the relative contributions of Vastus Lateralis, Vastus Medialis, Rectus Femoris and Biceps Femoris muscles which control the knee joint, before and after space flight, we will assess any micro-gravity induced changes on the make-up of joint torque by contributions from different muscles.

The project will also include a system development component. In this stage, we will design and implement a portable version of the laboratory equipment we currently use in data collection. This new system will enable us to perform the necessary data acquisition from the astronauts. It will also include online data quality evaluation procedures, which will ensure that high quality data are acquired from the astronauts. This is especially important in the return experiments which cannot be repeated if data quality is found to be low in subsequent analyses.

IMPACT

In addition to answering the basic scientific question regarding the effects of micro-gravity on the control of muscles, this study will accomplish some major milestones in the design of countermeasures: 1) It will determine the neural component of the micro-gravity induced deteriorations in muscle function and strength, enabling the design of well-informed rehabilitative interventions (such as exercises to be performed in space or on Earth) that target the specific alterations in muscle control; 2) It will provide the knowledge base necessary to identify a ground-based model for micro-gravity. The availability of a validated model for micro-gravity will facilitate the
development of countermeasures by enabling the testing and validation of proposed countermeasures on Earth; 3) Knowing whether low-level activity is useful in counteracting some of the effects of micro-gravity will be important in the design of countermeasures; 4) The results of this study will determine if in addition to exercise regimens, biofeedback should be considered as a countermeasure to encourage ‘corrective’ activation of muscles; 5) The knowledge regarding the time course of recovery will guide the rehabilitation regimens to be offered to the astronauts after return to gravity. In addition to these contributions to countermeasures development, this study will yield a portable intramuscular EMG acquisition system, which will be immediately useful in the clinical environment as well as research labs. Last but not least, this study may lead to the validation of aging as a ground-based model for micro-gravity, in which case future studies on astronauts will also aid research on aging.

INDEX TERMS
Micro-gravity, muscle, motor unit, control, EMG, disuse, aging, exercise, rehabilitation, countermeasure, model

Figure 1. Aging and motor unit firing behavior. A) (Top row) Time course of mean firing rates (light lines) and force (dark solid line) in young and elderly subjects. Note the orderly arrangement of firing rates in young subjects and the disturbed form of firing rates in the elderly. (Bottom row) - Cross-correlation among motor unit firing rates in the same subjects. B) (Left) Average firing rate as a function of recruitment threshold. Note the decreased values in the elderly. (Right) Percentage of motor units recruited at various thresholds for young and elderly subjects. Note that in elderly subjects more are recruited at thresholds below 20% MVC. Similar analyses will be employed in studying the effects of micro-gravity.
We propose a novel combination of $^{31}\text{P}$ NMR spectroscopies, ultrasound functional images, biomechanical analyses and multi-level modeling for analysis leading to an integration of human limb muscle function. We will integrate macroscopic properties in terms of molecular mechanisms. Human limb muscle will provide an exemplar for the integrated human function team: the analysis and modeling of different cell types and tissues in the limb as a functional organ will provide enabling concepts and technology for larger scale modeling of the “digital human” and guide strategies for database and global computer system development. We believe the current best strategy to develop milestones and to make progress on the ambitious goal of the "digital human" is to commence work on one body part that includes important pieces of NASA's critical path analysis. An understanding of limb muscle function is crucial to the planning of training exercises and to selecting personnel for the most strenuous activities with optimal efficiency and minimal risk. The science of this proposal evaluates the mechanisms responsible for transient and steady state performance of limb muscle. This analysis requires the specification of: 1) the mechanical power output by specific muscles during limb functions, 2) the analysis of the properties of different muscles in the same individual and of the same muscles in different individuals, 3) the quantification of energy demand by mechanical output, 4) the division of energy supply between glycolytic and oxidative processes and analysis of their inter-related controls, and 5) analysis of the response of these components with integrated models of the system. The information obtained through these experimental approaches is crucial to develop a model-based approach to the study of in vivo muscle energy balance in humans for two reasons: 1) the relevant data is not available, and 2) more importantly, the conceptual basis for integrating the component mechanisms can only be evolved from these new observations. We will show that the tissues in the limb have ideal properties and components that render hierarchical modeling feasible. Many properties of these processes are known and characterized in vitro but in vivo they form an integrated system, the characteristics and regulation of which is largely unknown. We will expand a mathematical model for intracellular energetics to include mechanics and blood flow. The goal is a hierarchical and mechanistic model of these crucial components of limb muscle function which can be extended to include additional metabolic and cellular features developed by other investigators, bone mechanical properties and, eventually, cardiovascular and respiratory analyses currently under development in other teams. We expect the intermediate milestone of the limb functional model will be a powerful tool for analyzing altered physiological responses to space environment and for testing efficacy of countermeasures.
SWEAT LOSS OF CALCIUM DURING BED REST WITH AND WITHOUT EXERCISE
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INTRODUCTION

Routine Calcium (Ca) balance measurements usually do not include dermal loss of Ca. We have previously shown that the skin patch can be used as a non-invasive method to determine the amount of calcium in human sweat. Our NASA bed rest study, simulating the skeletal unloading that occurs during space flight, includes a group of subjects that perform resistive exercise. Exercise can cause a significant Ca loss in the sweat, when performed at a normal room temperature. For this reason, the bed rest subjects in the current study exercise in a cool temperature (60°F). It is important to determine if ignoring sweat loss of Ca is causing significant error in the routine total body Ca balance measurements in the bed rest subjects. It is assumed that dermal or sweat loss of Ca is relatively constant with and without 1 hour of exercise performed in a cool environment as in the current study. This study tested the validity of this assumption by using a skin patch to collect sweat Ca.

CURRENT STATUS OF RESEARCH

Methods

The participants in the ongoing NASA funded bed rest study, conducted at the Baylor College of Medicine, Biomedical Research Bed Rest Facility located in The Methodist Hospital, Houston, Texas, were recruited as subjects for the sweat Ca study. The participants were between 19-50 years of age. There were two groups of subjects: group 1 (Ex, n=4) performed a resistive exercise program with a workload that progressively increased throughout the bed rest period; group 2 (NonEx, n=10) did not exercise for comparison with the other group. The ten NonEx subjects included 4 subjects taking alendronate. Subjects wore the patches (Osteopatch, Pacific Biometrics Inc.) at 5 different time periods: once during the baseline ambulatory period, at 6, 12 and 17 weeks of bed rest and during the second week of re-ambulation. These time periods were selected to coincide with other tests done on the subjects during the study. Sweat samples were collected for two consecutive days during each collection period. The participants in the Ex group performed resistive exercise 6 days a week and rested one day on Sunday. Sweat collection over two successive Sundays were used as the non-exercise day for the Ex group during their bed rest period. To minimize sweating, the room in which the subjects exercised was maintained at a temperature of 64°F or less while the rest of the unit was between 73 and 79°F.

Results

The average difference in total body sweat Ca between exercise and non-exercise (n=4) periods of bed rest in the Ex group was 1±7 mg/day. The average difference between sweat Ca during the ambulatory period (before or after the bed rest) and during bed rest...
in the NonEx group was 3±12 mg/day (n=10). Previously published data indicate that the average calcium balance during bed rest is about –180mg/day. Since the difference in sweat Ca loss due to either bed rest or exercise was small, the error in net total body Ca balance from ignoring sweat Ca during exercise conducted in a cool environment would be small.

**Conclusion**
Changes in sweat calcium from a brief period (1hr/day) of exercise performed in a cool (60° F) environment during bed rest do not significantly affect total body Ca balance.

**FUTURE PLANS**
Measure the sweat resorption bone markers using atomic absorption and correlate with corresponding urine bone markers.

**INDEX TERMS**
Sweat Ca, Total body calcium, Bed rest, Exercise, Calcium balance study.
Muscle and bone loss are major obstacles to extended spaceflight and habitation at extraterrestrial sites. Chronic immobilization or weightlessness lead to muscle atrophy and progressive bone loss which increase the risks of fracture and loss of function. This sequence is common in patients with spinal cord injury, in the elderly and in astronauts exposed to microgravity. This research program addresses the problem of bone loss following spinal cord injury (SCI) in tetraplegic/paraplegic subjects and it utilizes these subjects as a model for the bone loss experienced during extended spaceflight. The spinal cord injured patient shows impairment or loss of motor and sensory function resulting from injury to cervical or upper thoracic segments of the spinal cord. Loss of muscle mass occurs immediately after spinal cord injury. Bone loss in the lower trunk and lower extremities simulates that experienced by astronauts. During three years at the National Rehabilitation Hospital, we will determine sequential changes in bone mineral density (BMD), as well as changes in femur bone geometry and structural parameters measured from DEXA scans by established 2-D curved beam analysis methods. Using femur CT we will apply 3-D finite element analysis to estimate fracture risk. Serum and urine bone biochemical markers and bone-related hormones will be assayed. We will determine the relationships between sequential changes in thigh muscle mass measured by CT, muscle fiber histology and multiple cellular factors regulating muscle protein synthesis and proteolysis during the period of active bone loss. Patterns of bone and muscle loss and biochemical parameters in SCI patients will be compared to those reported during spaceflight. Using BMD and femur scan structural analysis as the prime indicators of bone integrity this protocol will evaluate the effectiveness of the third-generation bisphosphonate, zoledronate, as a countermeasure to acute bone loss and the effect of zoledronate on changes bone biomarkers and hormone patterns. Dose and effect studies of bisphosphonate action on bone BMD are limited to large clinical trials or oral agents. In an effort to further understand dose/effect relationships of intravenous bisphosphonates, we will measure skeletal retention of zoledronate in these subjects in relation to its effect on radiologic measurements and biomarkers using a new time-of-flight mass spectrometer. The objectives of this research are: 1) to develop a regimen for minimizing bone loss in immobilized subjects which is applicable to extended exposure to weightlessness, and 2) to assess the relative contribution of muscle atrophy to bone loss under conditions of lower extremity immobilization.