NSBRI Bone Loss Team Strategic Plan

3.0 BONE LOSS

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3.1 INTRODUCTION

The musculoskeletal system is uniquely dependent on Earth's gravity. Although human adaptation to the microgravity environment has allowed astronauts to maintain overall function, the musculoskeletal system rapidly degrades once the force of gravity is removed. Muscle atrophy has been documented by biopsy after 11 days in flight. The loss of cortical and trabecular bone follows the loss of mechanical strain normally transmitted from muscle. Several studies, American and Russian, have demonstrated that bone loss during flights lasting 4 to 6 months approximates 1-2% per month. However, some researchers have reported the range of bone loss in Mir cosmonauts varies from 0% to 24% per month when measured in cancellous and cortical bone in the tibia. Bone loss of this magnitude has been observed in human bed rest studies and in individuals following spinal cord injury. The loss of bone mass compromises bone strength, and diminished bone strength increases the risk of fracture, presenting a hazard to astronaut health and function and a threat to mission success.

The NSBRI Bone Loss Team aims to develop an effective countermeasure to bone loss. Countermeasures applied to date, including current in flight exercise regimens, dietary and vitamin supplements, and pre-flight conditioning, have not prevented bone loss during long duration flights such as those on Mir. Exercise regimens are currently being re-evaluated. Pharmacological interventions are also under investigation. However, as discussed below, progress in these areas will require a better understanding of the basic mechanisms that alter bone cell function in a microgravity environment. The current Bone Team research program includes basic and applied research targeted at countermeasure development and testing; each project focuses on issues relevant to mechanisms of bone loss, as well as, means that may be employed in the near future to mitigate the negative effects of microgravity on bone cells.

3.2 RISKS

The following risks in the Bone Loss Discipline Area have been identified in the Critical Path Roadmap (CPR) (risk number in parentheses):

- Development of Osteoporosis (9)
- Fracture and Impaired Fracture Healing (10)
- Injury to Soft Connective Tissue, Joint Cartilage, and Intervertebral Disc Rupture w/ or w/o Neurological Complications (11)
- Renal Stone Formation (12)

The majority of astronauts/cosmonauts have delayed return of bone density to normal following prolonged space fight causing two postflight health hazards: 1) prolonged fracture risk during active post-flight re-conditioning and 2) a life long increase in fracture risk and the risk of related soft tissue injury if bone density fails to attain pre-flight levels. As a result, the Bone Team has added an additional risk to the list currently found in the CPR:

- Delayed Return of Bone Mass to Normal Mass and Strength Following Extended Exposure to Weightlessness.

3.3 GOALS

The Bone Loss Team has the following goals for its program:

Risk-Based Goals

Goal 1: *Reduce the risk of accelerated bone loss leading to osteoporosis.*

Goal 2: *Reduce the risk of fracture and evaluate the potential for impaired fracture healing*

Goal 3: *Reduce the risk of injury to soft connective tissue, joint cartilage, and intervertebral disc rupture*

Goal 4: *Reduce the risk of renal stone formation*

Goal 5: *Promote the return of bone mass and strength to normal following an extended exposure to weightlessness*

Non Risk-Based Goals

Goal 6: *Collaborate with the NSBRI Muscle, Radiation and Technology Development Teams on the development of methods for inflight assessment of bone health and the appropriate monitoring, diagnosis and treatment for bone loss, fractures and soft tissue injury. Develop methods for the inflight assessment of renal stone risk and the prevention and treatment of renal calculi developed during flight.*

Goal 7: *Develop Earth-based applications of countermeasures to reduce increased bone loss and fracture risk found in health hazards, such as in children with non-weight bearing disorders and in adults following CNS and spinal cord trauma*

Goal 8: *Develop Earth-based applications of low weight, sensitive bone density machine*

Goal 9: *Integrate research and analysis*

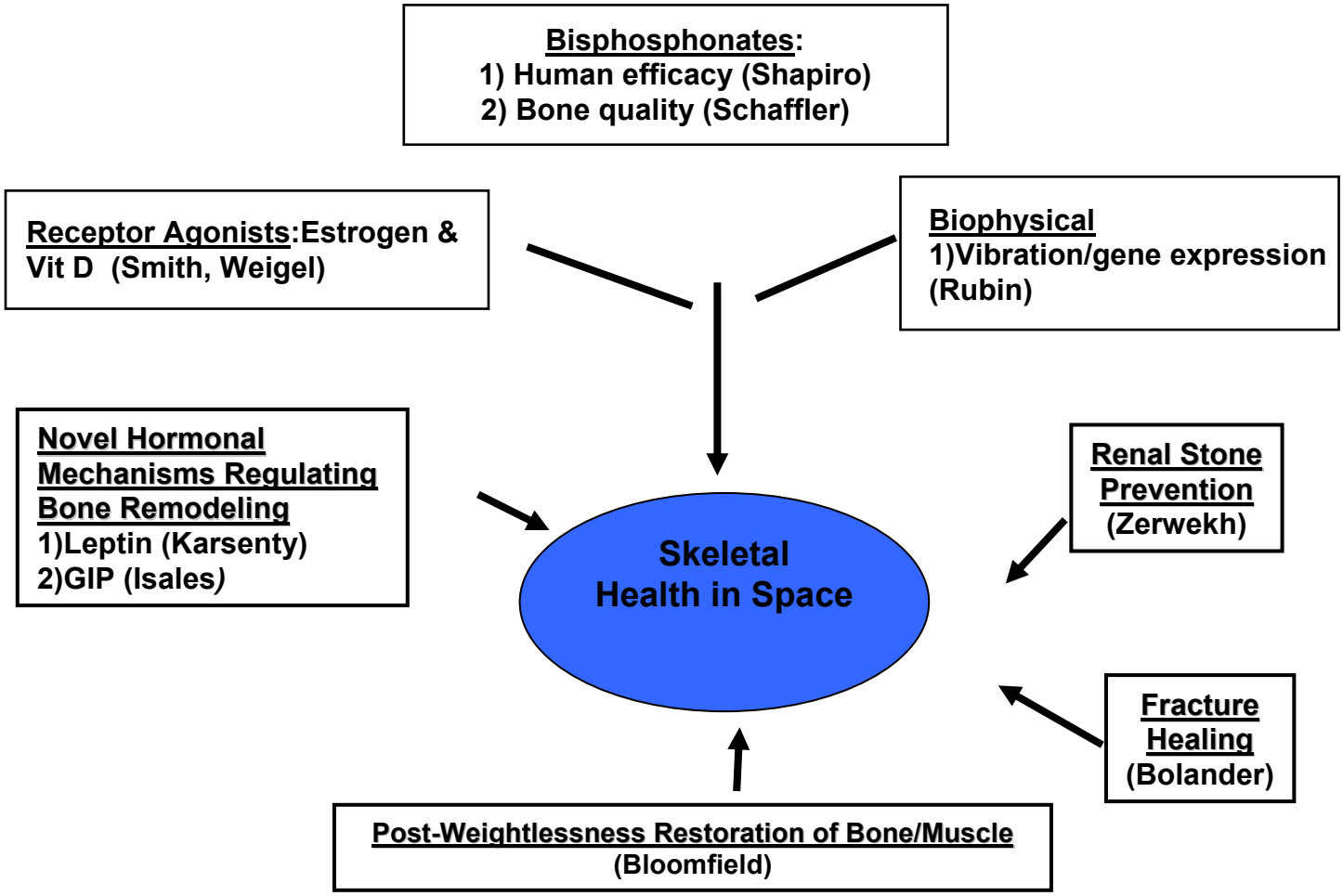
3.4 DESCRIPTION AND EVALUATION OF CURRENT PROGRAM

Current Research Projects

The current NSBRI program in bone includes both basic research and clinical research aimed at the development of countermeasures that may be tested during flight of animals or crew within the next 3-5 years. Project Countermeasure Readiness Levels (CRL) range from Level 2 for a

basic research investigation of leptin and GIP function as potential hormonal targets for countermeasure development to Level 6 for testing of new intravenous bisphosphonates in spinal cord injury patients as a model of microgravity in parallel to testing of use of intravenous bisphosphonate prior to spaceflight and testing of KMgCit for renal calculus prevention. Current and anticipated future countermeasures include exercise, pharmaceuticals, mechanical methods, and nutritional methods. Figure 3.1 summarizes the different approaches taken by the projects in the Bone Loss Program.

Figure 3.1.



Operational Bone Team Activities

Members of the Bone Team are active participants in operational programs and planning at JSC. For instance, Dr. Shapiro serves on the Integrated Products Team for Bone, Muscle and Exercise. Recently, he has advised JSC flight surgeons on the evaluation and potential treatment of astronauts whose bone mass may have been negatively impacted by multiple flight exposures. This effort is joined with physicians at the MD Anderson Hospital in Houston. Additional efforts in this area have involved participation in a NASA/NSBRI committee planning the evaluation of clinical biochemical testing in astronaut crew before, during and after flight. As another example, Dr. Sue Bloomfield, Texas A & M University, is an active member of the Critical Path

Roadmap development team which has revised and expanded issues of relevance to the Critical Path Roadmap program.

Each project is briefly summarized here and Table 3.1, presenting the basic research focus and experimental design, countermeasure focus, and contribution to achievement of team goals:

Basic Research Projects (Countermeasure Readiness Levels 2-4)

1. *Leptin as a Regulator of Bone Formation in Microgravity Bone Loss*: Karsenty Elefteriou, Dacquin, (Baylor): Leptin is a recently defined polypeptide hormone produced by adipocytes which binds to hypothalamic receptors and which decreases bone formation rate. Increased bone mass in obesity, a condition in which leptin levels are diminished, has led to studies showing that the absence of leptin can lead to high bone mass even in hypogonadal and hypercortisolemic states. In addition, it appears that leptin must bind to a hypothalamic receptor to control bone formation. Leptin indirectly appears to control bone formation by acting on the osteoblast via an yet undefined factor(s). The study seeks to :1) determine whether leptin controls bone mass by releasing a humoral substance following its binding to hypothalamic receptors, 2) to determine if the sympathetic nervous system is involved in mediating leptin control of bone formation, and 3) to determine whether a naturally occurring soluble form of the leptin receptor can prevent leptin inhibitory action on bone.

Significance: This research is focused on the relationship of the CNS to peripheral multifunctional hormones, which are now recognized to modulate osteoblast function and thus to influence bone mass. It addresses Goal 1, Reduce risk of accelerated bone loss leading to osteoporosis. Understanding the mechanism of leptin action promises to open a new route for the therapeutic control of bone mass, suggesting potential new countermeasures. Furthermore, the role of the CNS in the regulation of bone mass may have important implications for the problem of bone loss during spaceflight. The countermeasure development focus of this project, therefore, addresses central regulation of hormonal modulation of bone remodeling.

2. *Therapeutic Modulation of Systemic Glucose-Dependent Insulinotropic Peptide Levels to Counteract Microgravity-Induced Bone Loss*: Isales, Bollag, Mulloy (Medical College of Georgia): mRNA for the GIP receptor (GIPR) has been found in osteoblast cell lines and in isolated rat osteoclasts. Also, GIP appears to promote osteoblast differentiation and maturation, to stimulate type I collagen mRNA expression and to increase alkaline phosphatase activity in SaOS2 cells. GIP inhibits PTH-induced bone resorption. Transgenic mice over expressing GIP show increased bone mass. Intermittent GIP injection prevents bone loss in ovarectomized mice. The study will determine: 1) whether elevations in endogenous GIP leads to an increase in bone formation in GIP over expressing mice, including observing GIP effects in bone loss associated with estrogen and androgen deficiency and the effects of nutritional alterations on GIP effects on bone and 2) whether endogenous elevation in GIP prevents bone loss in GIP over expressing mice subjected to hindlimb suspension. These studies may establish GIP as the potential link between food intake and bone metabolism.

Significance: The problem of nutritional modification of bone loss during spaceflight has long been a subject of discussion and intervention but without success. Altering salt, protein or

carbohydrate intake or caloric intake has not provided answers to control of bone loss. This project, directed at Goal 1, addresses a novel relationship between diet and bone cell function and may result in novel countermeasures that expand nutritional impact on bone mass during extended spaceflight.

3. *Receptor Countermeasures to Bone Loss in Microgravity*: Smith, Weigel, Bloomfield, Narayanan, Suva (Baylor, U. Arkansas): Space flight is associated with decreased gonadal steroid levels and 25(OH) D and 1,25(OH)D2 levels. This study examines specific pharmacological alternatives, estrogen and vitamin D receptor agonists, as countermeasures to bone loss. It is hypothesized that the appropriate administration and or combination of receptor active agent(s) will improve calcium absorption, promote bone formation and decrease bone resorption. These studies focus on novel vitamin D receptor agonists (VDR) such as EB189 and selective estrogen receptor modulator agents (SERMs) such as raloxifene. This study targets the ability of novel receptor agonists of the vitamin D receptor and estrogen receptor alone or in combination to modulate osteoblastogenesis, mature osteoblast function and osteoclastogenesis in vitro and in vivo. The study currently addresses these effects on preserving bone mass in the hindlimb suspended rat model of microgravity. Attenuation of disuse bone loss by estrogen and raloxifene in the hind limb suspended rat has been demonstrated. Altered osteoblast differentiation and preservation of bone mass during hindlimb suspension has been demonstrated using VDR agonists.

Significance: Hormonal alterations during spaceflight impact bone remodeling and potentiate bone loss. These changes assume greater significance as extended microgravity exposure is anticipated. Understanding the cellular mechanisms responsible for these changes are basic to defining and suggesting effective countermeasures to hormonal imbalance and thus bone loss. Flight testing with animal models is critical to correcting hormonal perturbations secondary to microgravity. This project addresses Goal 1. Countermeasure development involves receptor agonists used during spaceflight to correct gonadal/vit D imbalance.

<u>Applied Research Projects (Readiness Levels 4-6)</u>

4. *Muscle Bone Inbalance After Non-Weightbearing*: Bloomfield, Hogan Smith, Warren, Schultheis (Texas A & M): This study utilizes the hind limb suspended rat model to examine: 1) the time course of recovery of functional properties in a muscle bone pair of the hindlimb during reambulation after 28 days of skeletal unloading, 2) the mechanisms affecting the rate of recovery during periods of maximal mismatch between muscle and bone functional properties, 3) the effectiveness of two exercise regimens and a biomechanical intervention to promote return of bone strength during recovery, 4) the effectiveness of PTH treatment and growth hormone treatment as anabolic countermeasures during recovery.

Significance: Fracture risk, soft tissue injury and renal calculus formation continue into the post-flight period. This project addresses post-flight muscle/bone imbalance, and anticipates countermeasures to minimize continuing injury risk. It contributes to the achievement of Goal 5, Promote return of bone mass and strength to normal following an extended exposure to weightlessness. This project will develop countermeasures that may combine effective exercise regimens and medication to improve osteoblastic bone formation following flight.

5. *A Biomedical Countermeasure for Disuse Osteopenia*: Rubin, Hadjiargyrou, Zhi, Judex, Dowd, Donahue (State University of New York at Stony Brook and Brookhaven National Labs). Whole body vibrational impact may provide an effective countermeasure to bone loss during exposure to microgravity. Using the tail-suspended rat and an oscillating plate to deliver mechanical strain, this study addresses 4 specific aims: 1) to correlate bone remodeling activity with spatial and temporal gene transcriptional activity in hind limb bone, 2) to correlate bone remodeling activity in the hind limb in the presence of 10 min. daily osteogenic mechanical stimulus (0.3 g @ 30 Hz) with the spatial and temporal transcriptional activity in the bone, 3) to correlate bone remodeling activity in the hind limb which arises from 23 h, 50 min of disuse interrupted daily by 10 minute osteogenic mechanical stimulus (0.3 g @ 30 Hz), 4) to correlate recovery of bone mass and transcriptional activity in the hindlimb following 28 days of disuse followed by 7 or 28 days of normal weightbearing vs. 28 days of disuse followed by 7 or 28 days of normal weightbearing plus 10 minutes/day of mechanical stimulus (0.3 g@ 30 Hz), vs. control. Evaluation will include histomorphometry, microtomographic imaging to present 3D models of the femur, gene expression patterns (actin, integrin β-3, osteopontin, collagen 1, connexin 43, BMP-2, MMP-1, MCSF, and ODF). IGF-1 levels in serum will be measured as indicative of coupled bone resorption and formation.

Significance: Several studies, animal and human have documented the beneficial effect of impact loading via vibrational stimuli on bone gene expression and bone mass. Impact loading in this manner may prove an effective countermeasure to bone loss in space. This project addresses Goal 1. Since this study requires early flight testing to facilitate the design of effective instrumentation, Dr. Rubin has submitted one plan for flight testing for review. Countermeasure development involves the design and construction of an effective device for vibrational mechanical loading.

6. *Resorption Suppression and Bone Health in Disuse Bone Loss*: Schaffler (Mt. Sinai School of Medicine): This protocol tests the hypothesis that long-term suppression of bone remodeling with bisphosphonate in a disuse situation will result in preserved bone mass and architecture but reduced resistance to fracture because of decreased osteocyte viability and integrity.

Significance: This study assesses potential risks in the utilization of chronic bisphosphonate as a countermeasure during extended duration spaceflight or following return to Earth after shorter microgravity exposure, and addresses Goals 1 and 5. Countermeasure plans include the eventual design of bisphosphonate with specific bone growth activities.

7. *The Effect of Microgravity on Fracture Healing/Ultrasound as a Possible Countermeasure*: Bolander, Turner, Greenleaf (Mayo Clinic): This program will determine the effect of hindlimb unloading on fracture healing in the rat model of microgravity. The study seeks to identify major cellular and molecular targets for the adverse effects of hindlimb unloading on fracture healing. Fracture healing will be evaluated by comparing histology and histomorphometry as well as by mechanical testing at different time points during fracture healing. The study explores the use of low intensity ultrasound to affect the rate of fracture healing

during hind limb suspension. It is expected that ultrasound will promote cartilage callus formation and thus enhance the rate of fracture healing.

Significance: There exists no information about fracture healing in microgravity. This study will apply biomechanical testing and histomorphometry to fractured regions using the hindlimb suspended rat. Addressing Goal 2, Reduce risk of fracture and evaluate potential for impaired fracture healing, the results of this study will orient plans for medical care during flight and extraterrestrial exploration where fracture risk will be substantially increased and the issue of healing fractures will be a major concern. Countermeasure development includes the utility of ultrasound as a means to facilitate fracture healing.

8. *SCI as Model for Microgravity: Effect of Zoledronate*: Shapiro, Toerge, Ballard, Baldwin, Beck, Ruff, Burman, Mustapha (Unformed Services University, Johns Hopkins University and the National Rehabilitation Hospital). This protocol will utilize subjects with spinal cord injury (SCI, tetraplagia and paraplegia) as models of the muscle and bone loss experienced by astronauts during extended spaceflight. The essential elements of this program include 1) measurements of rates of bone loss during non-weightbearing, 2) measurements of rates of loss of muscle mass, 3) determination of biochemical alterations in muscle tissue during prolonged non-weightbearing, 4) geometric and structural analysis of femur bone loss, including 3 D finite element analysis of femur bone before and after treatments for estimation of fracture risk, and 5) an evaluation of the effectiveness of the tertiary potent intravenous bisphosphonate, zoledronate, as a countermeasure to prevent bone loss in these subjects and in astronauts during space flight.

Significance: This study, which addresses Goals 1 and 5, has two achievable outcomes. The first is establishment of the spinal cord injured patient as an Earth-bound surrogate for space-induced bone loss. This task has been accomplished. The second is obtaining data about the effectiveness of a third level bisphosphonate on Earth and during spaceflight. Bisphosphonate testing during flight has not yet been initiated; however, such analysis is on the horizon. Countermeasure development involves the administration of long active bisphosphonate prior to, during and after flight.

9. *Prevention of Microgravity-Induced Stone Risk by KMgCitrate*: Zerwekh, Wuermeser, Pak, Antich, (UT Southwestern Medical Center at Dallas): Both clinical observations and evaluation of the composition of urine related to stone-forming factors indicate an increase risk of stone formation during and after extended spaceflight. The objective of this research study is: 1) to determine the effectiveness of potassium magnesium citrate (KMgCitrate) as a countermeasure to the propensity for stone formation and skeletal mineral loss sustained during spaceflight, 2) to evaluate the effect of KMgCit in averting the diminished muscle Mg and K concentrations that may occur during microgravity-related muscle atrophy, and 3) to assess the efficacy of KMgCit supplementation in reducing microgravity-induced increase in bone resorption and urinary calcium. These specific aims will be studied in healthy volunteers on chronic bed rest for 5 weeks. Study phases will include 1 week of ambulatory evaluation (A), 2 weeks of bed rest (weeks 2-6) (B) and 2 weeks of reambulation (weeks 7-8) (C). Subjects will receive Relyte tablets, 3 tabs with breakfast, and 3 with dinner to equal 42 mEq K, 21 mEq Mg, and 63 mEq citrate. Placebo controls are included in the study design.

Significance: Renal calculus formation has occurred in cosmonauts, and this study addresses Goal 4, Reduce risk of renal stone formation. Stone formation is a major health hazard, and previous clinical studies point to the utility of KMgCit as a useful countermeasure. KMgCit is currently under consideration or in use for flight testing. KMgCit may offer additional benefits now under study in the bed rest model. Flight-testing is appropriate for this agent.

Achieving Non Risk-Based Goals

The team's activities towards achieving Goal 6, which includes the development of monitoring methods for bone loss inflight, involve the development and utilization of the AMPDEXA machine for measurement of bone mineral density during extended flight. The instrument will be of use on the ISS to measure sequentially and in real time changes in bone mass, estimated at 1-2%/month during flight. These types of measurements are of value because they permit: 1) recording differences in rates of bone loss of individual astronauts and the correlation of changes in bone biomarkers and hormones related to rates of bone loss, and 2) development of specific countermeasures applied at the time bone loss is evident. This complements preventive measures applied prior to flight. This machine is ready to be used in healthy volunteers. Plans will be made for its utilization at the Johns Hopkins Applied Physics Laboratory and for chronic bed rest studies at the National Rehabilitation Hospital using Dr. Shapiro's current protocol.

It should be emphasized that several components of the bone program impact the delivery of medical care on Earth (Goals 7 and 8). Investigating factors responsible for a potential muscle/bone functional gap during recovery from weightlessness (Bloomfield) addresses an issue of great significance to the rehabilitation community. In addition, it raises the question of factors responsible for the delay in recovery of osteoblast function following microgravity exposure. Definition of mechanisms involved in bone loss during weightlessness and treatment with a novel intravenous bisphosphonate (Shapiro) is relevant to pediatric and adult non-weightbearing populations at risk of fracture but currently untreated. Schaffler addresses the question of potential long term risk secondary to bisphosphonate treatment, a matter of importance to a large number of elderly currently under treatment for osteoporosis. These analyses and others on the team address Goal 7, Develop Earth-based applications of countermeasures for bone loss and fracture risk found in health hazards. Initial work towards achievement of Goal 8, Earth-based applications of low weight, sensitive bone density machine, involves completion of experiments in Spinal Cord Injury (Shapiro) project to provide a monitoring arm (AMPDEXA machine) to correlate bone loss rates with biomarker changes.

Achieving Goal 9, Program Integration, is summarized in Table 3.2: The members of the Bone Team maintain a constant level of contact through teleconferences, individual conferences and team meetings at national symposia and meetings. A current program at the Applied Physics Laboratory involves 3 D finite element analysis, which will be expanded for modeling purposes as fracture risk data is obtained.

Needs for Bone Loss Program

Bone loss during extended space flight and habitation on extra terrestrial bodies at reduced gravity poses a significant health hazard for Astronaut crews. This program is developing 3D finite element analysis for estimates of fracture risk. In view of a rate of bone loss during flight that is 10 fold that seen in postmenopausal women this risk of fracture that could approach a 10-15% level during extended spaceflight. This fact is emphasized in NASA Critical Path statement.

The development of novel countermeasures directed at minimizing bone loss and thus fracture risk, requires a greater understanding of the responsible mechanisms at the cellular level. Our current understanding of bone loss however is limited to the level of the "effector" mechanisms, i.e., bone forming and bone resorbing cells. We do not understand what the mechanosensitive cells in bone actually experience as a result of microgravity, which in turn leads to responses from the effector cells. Accordingly, our ability to specifically target mechanical and exercise countermeasures and more effectively develop and utilize pharmacological intervention is limited. Critical issues for skeletal research, therefore, include issues as fundamental as the detailed characterization of the Earth-based loading environment of the skeleton. Central to this effort is developing a detailed understanding of how muscle loss alters function of bone cells, and whether countermeasures for prevention of muscle atrophy in space will also prevent bone loss. Unweighting effects and signal transduction mechanisms in bone warrant significant attention because of their importance in defining the optimal targets, both mechanical and biological, for countermeasure development. The current NSBRI Bone Loss Program strives to obtain some of this needed information, but more mechanistic studies are required.

The development of new and effective means for measuring bone mass during space flight, the application of effective resistive exercise for the maintenance of muscle and bone mass, and evaluation of biomechanical methods (vibrational impact loading) and new pharmacological agents all require evaluation under microgravity conditions. This analysis applies to both animal and human subjects. Unfortunately, the microgravity cannot be exactly approximated on Earth that may require revisiting the process under which certain projects are considered for flight testing. Targeted countermeasure research in this area in general is hampered by the problem of reproducing the microgravity environment for animal or human studies conducted on Earth. It is necessary that a coordinated effort between NSBRI Bone Team members and JSC staff facilitate flight testing for both animal models and human evaluation.

More research is needed in certain areas to achieve all the risk-based goals of the program. The currently (2000-2003) funded program of the Bone Team addresses Goals 1, 4, and 5 and to a insufficient extent, Goal 2. However, in spite of a broadly distributed request for proposals initiated in February 2000, only one proposal was received for Goal 2, fracture healing, and 2 proposals, neither judged fundable, were received for Goal 3, connective tissue injuries. These issues still need to be addressed. Accordingly, the current NSBRI solicitation again focuses on a call for research to address gaps in the bone program, specifically, fracture healing and soft connective tissue injury.

Evaluation of Current Countermeasure Technologies and Ideas to Further Accelerate Development

One objective of this strategic plan should be to conduct broad-based critical evaluations of current countermeasures nearing the stage of flight-testing. This process could serve to stimulate the search for new and novel methods of protecting Astronauts from the risk of fracture under hazardous conditions.

1) In-Flight Exercise Programs: Resistive/endurance exercise programs are currently being tested under protocols considered by the Integrated Product Team for Bone**,** Muscle and Exercise. Resistive exercise may be an important adjunct to maintaining muscle mass and bone mass; however, its efficacy in microgravity remains to be determined. At this time 2 methods are considered for flight testing, the Interim Resistive Exercise Device and the cycle ergometer (Tesch).

Recommendations: Critical evaluation of the potential usefulness of each method, in flight, is

needed in a timely manner, so that modifications can be considered if the basic protocols are determined to not be effective during flight. A combined and focused NASA/NSBRI review to stimulate novel methods for effective-exercise regimens, under the aegis of the NSBRI Exercise team, is also recommended. Also, determination of the effectiveness of current resistive exercise regimens by tracking changes in bone mineral density (BMD) with varying intensity, duration or frequency of training is suggested. Certain biomarker measurements such as serum CTX (a resorption biomarker) could be obtained concurrently to establish their usefulness in predicting changes in BMD.

2) Pharmacological agents: New bisphosphonates and receptor agonists (SERMs and Vit D receptor agonists) are among agents currently being developed by or in clinical study by NSBRI Bone Team members. It is necessary that NASA regulations regarding testing agents of potential usefulness be modified to allow flight experience to be gained within the limits of astronaut safety.

Recommendations: Bisphosphonate testing on short-term shuttle flights for determination of acute metabolic effects is recommended. The initial study should pre-treat astronauts with intravenous pamidronate, an extensively used agent about which extensive clinical and toxicological information exists. Completion of Earth-bound studies of chronic effects and drug safety is also required. Administration of potent intravenous bisphosphonates for immediate metabolic effects on shuttle fights and subsequently on the ISS is suggested. Animal studies, on mice or rats, to permit testing novel pharmacological agents, e.g., SERMs and VDR agonists, leptin and GIP in-flight is also encouraged. Note that commercial (Amgen) studies of osteoprotegerin have been completed.

3) Mechanical Methods: These techniques include human centrifuge methods to simulate gravity that are based on cycling or rotation of a sled or table and vibrational mechanical stimuli to provide osteogenic signals to bone in a weightless environment. These methods have been demonstrated in animals and humans to increase bone formation.

Recommendations: Funding for commercial development of a flight-ready device for providing vibrational strain to the lower extremities is suggested. The development for Earth testing of a suitable vibrational impact instrument that could be flight tested within 5 years is encouraged. In contrast to current commercially designed units, this instrument would be specific for use in flight.

4) Nutritional Methods: KMgCit is being tested to decrease renal propensity to calculus formation.

Recommendations: Once safety data have been acquired from bed rest studies, this compound should be tested on Shuttle flights and later on the ISS. Application of nutritional regimens for testing in bed rest protocols to improve spontaneous eating patterns during flight and to provide adequate nutritional supplements in preparation for testing during spaceflight is suggested.

Additional studies and monitoring to consider to initiate

- Biomechanical and radiological testing of fracture healing models during flight and postflight.

- Assessment of soft tissue injury during and after extended flight. This assessment would include radiological procedures (MRI) to define the anatomy of the intervertebral space, vertebral body, and joint space.
- Testing of the AMP DEXA machine now in preparation at the Johns Hopkins University Applied Physics Laboratory (JHU APL) on the ground in control and bedrest or spinal cord injured subjects. Testing on the ISS within 5 years.
- Testing of the mass spectrometer for biochemical analysis (R. Potember, JHU APL) in flight on the Shuttle. This testing would permit real-time assessment of blood, urine and saliva biochemistries.
- Animal studies to determine if reduced blood flow to the lower limbs (as observed during hind limb suspension in rats) impacts on interstitial fluid flow and hence on mechanotransduction in bone during (simulated or real) weightlessness.
- Identification of key molecular responses in bone which initiate bone loss in space flight. For example, definition of the signal transduction system that couples gravity-related muscle pull with bone formation/resorption could be made. This examination will facilitate development of optimally targeted pharmacological and mechanical countermeasures.
- Testing transgenic animal (specificity to be defined) models in flight. Considerations would include site-specific over-expression of locally acting osteogenic factors. Suitable agents could be those affecting osteoblast/osteoclast function in bone, application of the "coupling factor" when this is defined, identification through knock out experiments of critical transcription and growth factors to maintain bone mass in the face of microgravity.
- Advanced testing of novel pharmacological agents during ISS flight. These may be growth factors to maintain bone formation, novel antiresorptive agents, hormone receptor agonists, and agents such as leptin and GIP, whose role is yet to be defined.
- Genetic analysis of flight candidates for fracture risk. Family bone density survey, initial screening for specific osteoporosis related polymorphisms would initiate development of a data base to serve as a platform for expanded studies as new findings are available.
- Flight-based determination of bone mineral density. Includes application of analytic programs, including considerations of bone geometry, and modeling for fracture risk
- Correlation of new nutritional and optimized resistive exercise programs with bone mineral density changes during 6-month flight experience. In addition to bone density, this analysis would require the development of novel imaging methods for estimation of bone strength.
- Treatment for fracture healing tested under flight conditions in animals. This test could include current proposals (ultrasound), as well as, the dermal application or fracture-site use of instilled agents (e.g., bone morphogenetic protein-like agents)

Post-flight Rehabilitation Suggestions

Various conditioning training programs are currently in use to assist in the return of bone to normal levels post-flight. These programs extend for 6 weeks following return and evaluate conditioning programs with respect to post-flight alterations in bone mass and bone strength and the return of these parameters to pre-flight values.
Recommendations: Review of the effectiveness of current conditioning programs in American Astronauts in a joint NSBRI/NASA forum and from that data, design of a standardized rehabilitation program that will serve to highlight alterations in individual response to conditioning. This review, in turn, will permit analysis to determine mechanisms responsible for failure to regain pre-flight mineral mass (e.g., muscle bone mismatch).

NSBRI/JSC Collaboration

A short-term aim should be to increase the collaborative efforts between the NSBRI Bone Team and scientists at JSC and Ames. As currently structured, JSC has a limited capability to deal with countermeasure development for bone loss. Bone loss, as a Critical Path risk, is a matter of second priority at the Bone, Muscle, and Exercise Integrated Products Team because of the current emphasis on flight-testing exercise protocols. This may reflect: 1) failure on the part of investigators to bring bone related protocols forward, 2) the probability that certain countermeasures for bone loss are not ready to be flight-tested, and, 3) the slow process in bringing pharmacological agents to the stage of flight testing. Funding for bone loss research (HEDS Program) has been significantly hampered by budget problems within NASA as well as administrative issues. It is strongly recommended that the JSC/NSBRI program be critically reviewed so that program strategies provide the maximum collaboration between JSC scientists, engineers, flight surgeons and funded NSBRI investigators and their respective institutions. An initial step in this effort was taken at the NSBRI meeting at Del Lago, January 2002, where a combined NSBRI Bone Team/JSC staff meeting was held to open discussions about NASA targets and current NSBRI research program.

The recommendations presented in this plan are predicated on appropriate funding and the imposition of a countermeasure targeted research program. Members of this team recommend, that in contrast to "bottom up" NIH/NSF type funding programs, NASA/NSBRI research should be a "top down" system in which research funding follows mission requirements. In turn, mission requirements should reflect the best consensus about basic and applied priorities.

3.5 OBJECTIVES AND STRATEGIC ACTIVITIES

Goal 1: *Reduce risk of accelerated bone loss leading to osteoporosis*

Objective 1A: Assess risk and target level of acceptable risk.

- Assess initial and sequential bone mass, bone biomarkers and genetic background, preflight in astronauts.

Objective 1B: Determine Mechanisms

- Determine mechanisms of bone loss using geometric/structural DEXA analysis pre flight and on interval flights if applicable.
- Determine mechanisms of bone loss using bone cell populations obtained from the hindquarter suspended rat or mouse model of microgravity. Mechanisms of bone cell response to microgravity such as altered signal transduction mechanisms are of critical importance in the eventual development of new countermeasures.

Objective 1C: Develop countermeasures.

- Study combined effect of related stressors such as radiation and weightlessness and countermeasures to lessen muscle loss
- Bring bisphosphonate administration and vibrational mechanical loading to point of flight testing. Achieving this feat may require short-circuiting established pre-flight testing currently required by NASA. Access exercise protocols.
- Study leptin, GIP, SERMs and Vit D analogues in flight experiments. Build on the database aimed at the development of new and novel countermeasures. Use a five-year window for development of new agents. Join with industry to facilitate new drug development.

Goal 2: *Reduce Risk of Fracture and Impaired Fracture Healing*

Objective 2A: Assess risk and target level of acceptable risk

- Using the initial data on fracture healing and callus strength now available through the Bolander study, recruit additional protocols to focus on these mechanisms and countermeasure development re: the integrity of the fracture callus.
- Collaborate with Hopkins Applied Technology Group to test the new AMP DEXA in humans with the aim of a flight model in 2004 for estimation of fracture risk
- Use 3-D finite element analysis derived from CT scans to model fracture risk during flight, in extraterrestrial environment and post-flight.

Objective 2B: Determine Mechanisms

- Determine mechanisms using cell models derived from fracture site, callus

Objective 2C: Develop Countermeasures

- Develop countermeasures including ultrasound, mechanical/electrical stimulation

Goal 3: *Reduce Risk of Injury to Soft Connective Tissue, Joint Cartilage, and Intervertebral Disc Rupture w/ or w/o Neurological Complications*

Objective 3A: Assess risk and target level of acceptable risk

- Initiate more funded studies. It is recognized that back pain presumably due to intervertebral disc disease is a significant problem with astronauts. The extent of cartilage impairment is undetermined. The risk must be determined using specific pre- and post flight studies of both animals and astronaut vertebrae and joints: biochemical and histopathological data is required.

Objective 3B: Determine mechanisms

- Study soft connective tissues in the hindlimb suspended model: sequential cartilage changes, expand the data base on intervertebral disc alterations. The mechanisms involved in these processes and their prevention/repair are significant issues.

Objective 3C: Develop Countermeasures

- Begin to assess potential countermeasures to injury to include: physical therapy, exercise programs.

Goal 4: *Reduce Risk of Renal Stone Formation*

Objective 4A: Assess risk and target level of acceptable risk.

- Review recent and current astronaut/cosmonaut experience re: stone formation and use of K Citrate.
- Zerwekh program addresses these issues.
- Re-evaluate urine stone propensity data collected by Drs Pak/Whitson

Objective 2A: Determine mechanisms

Objective 2A: Develop Countermeasures

- Test KMgCit in flight setting

Goal 5: *Reduce Risk of Delayed Return of Bone Mass to Normal Mass and Strength Following Extended Exposure to Weightlessness*

Objective 5A: Assess risk and target level of acceptable risk

- Review current data on stress fractures during re-conditioning.
- Evaluate data obtained in the Bloomfield study (bone/muscle mismatch)

Objective 5B: Determine Mechanisms

Objective 5C: Develop Countermeasures

- Work with the NASA IPT Committee for Bone, Muscle, and Exercise and JSC flight surgeons to formulate an effective and long duration rehabilitation program post-flight.

Goal 6: *Collaborate with the NSBRI Muscle, Radiation and Technology Development Teams on the development of methods for inflight assessment of bone health and the appropriate monitoring, diagnosis and treatment for bone loss, fractures and soft tissue injury. Develop methods for the inflight assessment of renal stone risk and the prevention and treatment of renal calculi developed during flight.*

- Collaborate with the Technology Team on developing and testing methods for monitoring of bone mass (e.g., AMPDEXA machine)
- Collaborate with the Muscle Team to correlate biochemical and genetic patterns of bone loss with changes in bone mass during weightlessness.
- Collaborate with the Radiation Team to evaluate the separate and interactive effects of radiation and weightlessness on bone cell functions.

Goal 7: *Develop Earth-based applications of countermeasures to reduce increased bone loss and fracture risk found in health hazards, such as in children with non-weight bearing disorders and in adults following CNS and spinal cord trauma*

- Complete experiments in Spinal Cord Injury project to provide treatments (such as bisphosphonates) that are useful to general public. (Shapiro et al)

Goal 8: *Develop Earth-based application of low weight, sensitive bone density machine*

- Complete experiments in Spinal Cord Injury project to provide a monitoring arm (AMPDEXA machine) to correlate bone loss rates with biomarker changes that are useful to general public. (Shapiro et al)

Goal 9: *Integrate Research and Analysis*

Objective 9A: Integrate Research Within the Muscle Alterations and Atrophy Team

- Teleconferences and team meetings at national symposia.

Objective 9B: Integrate Research With Other Teams, using modeling as well as other approaches

- Use Modeling (3-D finite element analysis, DEXA scan analysis)
- Collaborate with the Radiation Team to study independent and interactive effects of radiation on bone mass.

Objective 9C: Integrate Research with Scientists Outside of NSBRI

- This is in progress with scientists at the Armed Radiation and Radiobiology Institute, AFRRI.

3.6 SUMMARY

Two major hazards to human extended duration spaceflight are radiation exposure and bone loss. It is necessary to coordinate NSBRI research efforts with those of the NASA extramural HEDS program so as to create a focused, countermeasure development and testing program aimed at addressing problems of bone loss. In the current program, basic and applied protocols examine regulation of bone mass under weightlessness conditions, as well as, potential pharmacological, nutritional, exercise and biomechanical methods of countering bone loss. Questions related to accelerated bone loss, post-flight fracture, and renal calculus formation are addressed; however, some risks to astronaut health, such as inflight fracture risk and healing and soft tissue injury risk, are not being adequately addressed at this time. Investigation of cellular mechanisms modulating bone cell function should be a more prominent part of the program. Coordination of

countermeasure development with in-flight and post-flight monitoring should be an integral part as well.

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Table 3.1. Project Research Activities

PI/Project	Risk(s) Addressed	Countermeasure Target	Experimental System	Phase 1 Activities: Focused Mechanistic Research	Phase 2 Activities: Preliminary Countermeasure Development Research	Phase 3 Activities: Mature Countermeasure Development Research
BLOOMFIELD/ Bone and Muscle Recovery from Simulated Microgravity	Post-flight fracture risk	• Pharmacological • Exercise	Hind limb suspended rat	Muscle/bone imbalance	Exercise regulation, PTH, growth hormone	
BOLANDER/The Effect of Microgravity on Fracture Healing: Ultrasound as a Possible Countermeasure	Microgravity impact on fracture healing	Ultrasound	Hind limb suspended rat	Integrity of fracture healing callus formation	Ultrasound action on fracture healing	
ISALES/Therapeutic Modulation of Systemic Glucose-Dependent Insulinotropic Peptide Levels to Counteract Microgravity-Induced Bone Loss	Accelerated bone loss leading to osteoporosis	Diet	Mice, transgenic mice	GIP effect on bone remodeling	Nutritional intervention to increase GIP levels and lesson bone loss	
KARSENTY/Leptin as a Regulator of Bone Formation in Microgravity	Accelerated bone loss leading to osteoporosis	Pharmacological	Mice	Leptin (humoral factors)/CNS inhibition role in bone remodeling; cntrl mechanisms	Test hindlimb suspended animals for this system and potential cms	
RUBIN/A Biomechanical Countermeasure for Disuse Osteopenia	Accelerated bone loss leading to osteoporosis	Mechanical stimulation	Mice	Gene expression related to vibratory stimuli	Mechanically stimulate bone via impact loading; operational features for testing in human	Flight protocol under review at JSC

Table 3.1. Project Research Activities (continued)

PI/Project	Risk(s) Addressed	Countermeasure Target	Experimental System	Phase 1 Activities: Focused Mechanistic Research	Phase 2 Activities: Preliminary Countermeasure Development Research	Phase 3 Activities: Mature Countermeasure Development Research
SCHAFFLER/Resorption Suppression and Bone Health in Disuse	Fracture risk after bisphosphonate treatment	Pharmacological	Dog with immobilized limb	Characterization of osteocyte integrity	Propose alterations in bisphosphonate drug dose, duration of administration	
SHAPIRO/Defining and Preventing Bone Loss: A Microgravity Model	• Accelerated bone loss leading to osteoporosis • Fracture risk after bisphospho-nate treatment	Pharmacological	Patients with spinal cord injury	Characterize rates and patterns of bone loss, muscle loss in non-weight bearing subjects	Administration of intravenous potent bisphosphonate (zoledronate) to protect bone loss	Flight testing of bisphosphonates under consideration
SMITH/Receptor Countermeasures to Bone Loss in Microgravity	Accelerated bone loss leading to osteoporosis	Pharmacological	Hind limb suspended rat	Characterize receptor agonist effects on bone cell and prevention of bone loss	Testing with SERMs and vitamin D agonist to decrease bone loss; in progress in hindlimb suspension model, flight testing anticipated.	
ZERWEKH/Prevention of Microgravity-Induced Stone Risk by KMgCitrate	Renal calculus prevention	Nutritional	Normal human volunteers at bed rest.		Effects of treatment with KMgCit on bone and muscle loss, renal stone propensity	Flight testing to be proposed.

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Table 3.2. Integration Activities

	BLOOMFIELD	BOLANDER	ISALES	KARSENTY	RUBIN	SCHAFFLER	SHAPIRO	SMITH	ZERWEKH
Internal Communication	Monthly teleconferences, team meetings 2-3 times per year	same	same	Same	same	same	same	same	same
Integrated Experiment Development	Collaboration with Smith project								
Sample Sharing			Collaboration with Zerwekh bedrest project				Muscle team collaborates on this project		With Isales GIP project
Synergistic Studies of Opportunity							Collaborate with Technology Team for AMPDEXA evaluation		
Development of Computer Model of Integrated Human Function									

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Table 3.3. Achieving Goal 1: Reduce Risk of Accelerated Bone Loss Leading to Osteoporosis

Countermeasure Development Phases	Pre 2001	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Phase 0: Observational & Phenomenological Research													
Phase 1: Focused Mechanistic Research													
• Understand gravity effects on leptin • Understand gravity effects on GIP • Complete evaluation of chronic bisphosphonate effect on bone cells • Determine how spinal cord injured (SCI) affects biochemistry of atrophy of human muscle													
• Model fracture risk in SCI with 3D finite element analysis • Evaluate alterations in fracture healing in weightlessness • Assess bone/muscle gap during reconditioning • Define geometric/structural parameters on bone loss in spinal cord injured patients													
Phase 2: Preliminary Countermeasure Development Research													
• Assess chronic bisphosphonates use on osteocyte integrity • Test SERM/Vitamin D agonist effects during flight in animals • Determine role of ultrasound in fracture healing in weightlessness													
• Assess role of exercise, PTH and growth hormone during reconditioning of hind limb suspended rats.													
Phase 3: Mature Countermeasure Development Research													
• Develop integrated exercise, nutritional, and pharmacological countermeasure and test in humans: test exercise activity on bone mass													
• Determine whether vibrational mechanical loading can be adapted to flight conditions: correlate engineering requirements for flight testing • Evaluate AMP DEXA apparatus with Technology Team													
Phase 4: Countermeasure Evaluation & Validation													
• Test intravenous bisphosphonate during flight • Test KMgCit during flight													
Phase 5: Operational Implementation of Countermeasure Strategy													