

FINAL REPORT

ARCHITECTURE AND MECHANICAL FUNCTION IN BONE WITH RECOVERY FROM DISUSE OSTEOPOROSIS

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PRINCIPAL INVESTIGATOR: MITCHELL B. SCHAFFLER, PH.D.

**CO-INVESTIGATORS: DAVID P. FYHRIE, PH.D.
KARL J. JEPSEN, PH.D.
ROBERT D. BOYD, ED.D.**

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INSTITUTIONS:

HENRY FORD HEALTH SCIENCES CENTER, DETROIT, MI

MOUNT SINAI SCHOOL OF MEDICINE, NEW YORK, NY

CRITICAL PATH ROADMAP QUESTIONS ADDRESSED

IS BONE LOSS REVERSIBLE AND WITHIN WHAT TIME FRAME: CAN GEOMETRY AND ARCHITECTURE RETURN TO BASELINE AS WELL AS BMD? (CPR 2.19)

PRIORITY: 1

RISK FACTOR 1

AIMS

Our studies show that disuse-induced bone loss in the canine skeleton evolves through a loss of trabecular bone structural elements, involving both thinning and subsequent perforation and loss events. Moreover, during the bone loss process with disuse, there is a discrete, temporal separation of thinning of trabeculae from the later perforation and complete loss of trabecular elements. In these experiments, we took advantage of this sequence of bone changes in the development of disuse-induced bone loss to establish different architectural baseline points from which recovery of structure and mechanical function with reloading were be examined.

The objectives of the proposed project are to characterize the microarchitectural and structural-mechanical correlates of recovery from osteoporosis secondary to decreased mechanical usage. Specifically these experiments will examine recovery from disuse osteoporosis in a canine model so that the bone processes can be studied in all bone envelopes, in a skeletal system that has a physiology similar to that in adult humans. While recovery in trabecular bone is the principal focus of these studies, recovery and function in diaphyseal compact bone also will be studied. In support of these objectives, the specific aims of this proposal are:

1. To determine experimentally whether bone architecture can recover from the changes resulting from long-term disuse osteoporosis.

These experiments will test the hypothesis that with recovery after long-term disuse, bone will be apposed only to existing surfaces, resulting in restoration of bone mass but not microarchitecture.

2. To determine experimentally whether bone mechanical properties can recover from long-term disuse osteoporosis.

We will determine the contributions of bone microarchitecture and mass to mechanical properties with recovery from disuse osteoporosis. These experiments will test the hypothesis that there are negative functional-mechanical consequences to having fewer thick trabeculae versus smaller more interconnected trabecular elements after recovery from extant osteoporosis.

BACKGROUND:

The rate of bone loss resulting from hypodynamic states, such as space flight and immobilization is estimated to be an order of magnitude greater than that in any other metabolic disorder of bone. Reversal of an extant osteoporosis is thought to result in recovery of bone mass but not the restoration of microarchitecture or the replacement of lost trabecular elements. Specifically, it is thought that restoration of bone mass after osteoporosis occurs through

compensatory thickening of remaining trabecular elements; restoration of trabecular bone microarchitecture (lost trabeculae, interconnectedness of trabecular elements) is thought not to occur. However, there is very little direct information available to support or refute these assertions. The functional-mechanical consequences of having fewer thick trabeculae versus smaller, more numerous interconnected trabecular elements have been the subject of extensive discussion, but again there is very little direct data on these structure-function relationships. Understanding the recovery potentials of the osteoporotic skeleton, both architecturally, mechanically and biologically, has considerable clinical and functional significance.

Studies show that during disuse-induced bone loss in the canine skeleton, there is a discrete, temporal separation of thinning of trabeculae from the later perforation and complete loss of trabecular elements. In the proposed experiments, we took advantage of this sequence of bone changes in the development of disuse-induced bone loss to establish different architectural baseline points from which recovery of structure and mechanical function with reloading were examined. These experiments used cast immobilization of the canine forelimb for up to 6 months to establish baseline points for remobilization. After remobilization, bone microarchitecture was evaluated using by histomorphometry, and then tested mechanically to determine the mechanical integrity of bone after recovery from disuse.

MATERIALS AND METHODS:

The purpose of this experiment was to characterize the tissue microarchitectural and mechanical consequences of bone recovery from short- and long-term disuse osteoporosis. Accordingly, we took advantage of the temporal separation of thinning of trabeculae versus later perforation and complete loss of trabecular elements evident in the canine disuse model, in order to establish two distinct architectural baseline points in trabecular bone (thinning versus loss of trabecular elements) for initiating studies of the recovery from disuse osteoporosis. Specifically these experiments examined recovery from disuse osteoporosis in a canine model so that the bone physiology and resulting mechanical properties could be studied in all bone envelopes, in a skeletal system which has a physiology similar to that of adult humans.

1. Animal Model: In these experiments, we built on our existing data set for evolution of disuse-induced bone loss (osteoporosis) in Beagle dogs originally performed in collaboration with the Department of Energy Radiobiology Laboratory at the University of Utah.

Use of an adult canine model allowed us to study this physiology on all bone envelopes (both cortical and trabecular bone), in a well characterized skeletal system, which has been demonstrated to possess a remodeling physiology similar to adult humans and other primates in each skeletal envelope. In addition, canine metaphyses are sufficiently large to allow us to make direct mechanical assessments of the functional mechanical integrity of the recovered bone architecture. Rodent models for disuse effects (tail-suspension and hindlimb immobilization) would not suffice for the proposed experiments, for the following reasons: 1) cortical bone loss in rodents is not caused by the intracortical processes which effect bone loss in larger mammals, 2) because trabeculae in rodents are much thinner than those in larger mammals, the trabecular bone loss pattern in rodents does not allow effective temporal separation of thinning versus loss

of elements and 3) the size of trabecular bone regions in rodent long bones is inadequate for the mechanical testing needed to assess functional integrity. We used 5-7 year old bred for purpose retired breeder Beagle dogs (12-15 kg body weight). Use of a consistent breed minimized phenotypic variation among experimental animals. Only female animals were examined to control for possible hormonal differences. Dogs were housed singly, in 8.5 ft x 4.75 ft kennel runs in the Animal Resources Facility at Henry Ford Hospital. Dogs were fed a standard diet with water available *ad libitum*. Body weights were determined weekly.

2. Immobilization and Remobilization Procedures

The purpose of this experiment was to characterize the tissue microarchitecture, mechanical properties and biological mechanisms of bone recovery from both acute and chronic disuse osteoporosis. Accordingly, nontraumatic immobilization was used to establish two distinct architectural baseline points for initiating studies of the recovery from disuse osteoporosis.

Immobilization Procedure: Right forelimbs of adult Beagle dogs (females, N=28, 5-7 years old) were non-traumatically immobilized using a modified body jacket-splint. The splint was custom-formed for each animal using thermoplastic resin (Aquaplast, *Smith and Nephew, IN*) This device is superior to the body cast method used in previous experiments, as it can be removed easily to allow frequent inspection of the skin of the immobilized limb and allow passive mobilization of the limb to minimize joint contractures in the long-term animals, which could complicate the remobilization portions of these investigations. Right forelimbs were splinted with the elbow flexed at 90 degrees and the carpal joint volar-flexed slightly. The splint devices was removed 3 times per week and the animals placed in a canvas hammock with the forelimbs placed freely hanging through opening in the hammock so as to be non-weight-bearing. Fifteen minutes of passive flexion-extension motion applied manually at that time to reduce the effects of immobilization on the development of joint contractures. Dogs were conditioned to handling so that no sedation was needed during splint removal and treatment.

Remobilization: Immobilization lasted either 3 or 6 months. These time periods to start remobilization were chosen (based on our preliminary data) to correspond to microarchitectural events. At 3 months (acute disuse), there is loss of bone volume corresponding to thinning of trabeculae, but there is no loss of trabecular elements. After 6 months of immobilization (chronic disuse), there is a significant reduction in number of trabecular elements. Groups of animals were sacrificed after 3 and 6 months of immobilization to provide baseline values for the disuse osteoporosis. For the remaining acute and chronic disuse groups, splints were removed and the animals allowed to resume quadrupedal gait as tolerated. Normal weight-bearing function was achieved by 4-6 weeks after splint removal, assessed from ground reaction force determined using a strain gage force-plate. Remobilized animals were euthanized in after 12 months of remobilization

This long time period form remobilization was chosen based on several criteria. First, it encompasses the completion of at least 2 remodeling periods in dogs* to provide a steady state for long-term recovery period (*the remodeling period, sigma, in dogs is reported to be from 3 - 5 months; mean and standard deviation for the remodeling period in our control Beagles from

our previous studies is 111±47 days). Second, it assures that newly formed bone during the recovery process is completely mineralized, so that mechanical property measurements are not confounded by the latency of normal secondary mineralization processes. Comparisons of final architecture and mechanical function were based principally on the basal, immobilized and long-term remobilized groups.

Figure 1. Summary of Experiments

Bone Recovery from Acute Disuse

IM	0 - - - - - 3 (months)
REMOB 1	Start - - - - - 12 (months)
Sacrifice	XX XX

Bone Recovery from Chronic Disuse

IM	0 - - - - - 6 (months)
REMOB 2	Start - - - - - 12 (months)
Sacrifice	XX XX

Basal Controls XX

Methods for specific studies are presented in context to each study.

RESULTS AND DISCUSSION

1. Cortical bone loss and recovery in long-term disuse osteoporosis

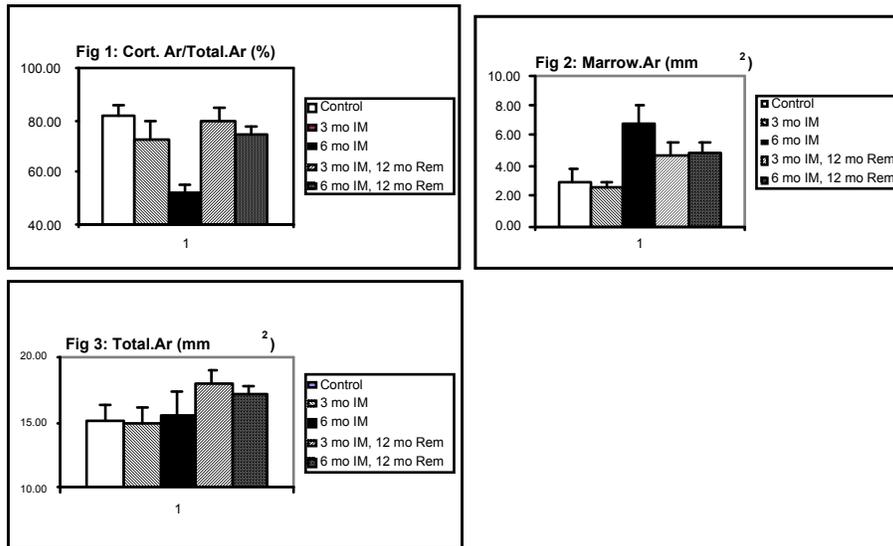
The ability of bone to recover from disuse osteoporosis is unclear. Studies in rodents suggest that complete recovery of bone mass can occur after disuse (1). In the skeletally mature canine skeleton, Jaworski and Uthoff (2) and Lane et al (3) found that cortical bone mass did not recover from long-term 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

Methods: The animal model and experimental design and Immobilization and remobilization methods are described above. Histomorphometry was used to assess the architectural bases for recovery from disuse osteoporosis. Cortical bone recovery from disuse osteoporosis was evaluated at the metacarpal mid-diaphysis. Metacarpal-2 was embedded in PMMA and 100 µm thick undecalcified cross-sections prepared from the mid-diaphysis. Histomorphometric studies were performed using combination of point count stereological methods and direct digitizing methods (OsteoMeasure). 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: Immobilization: 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 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 significantly 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. These 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. 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. 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.

2. DOES MECHANICAL FUNCTION IN LONG BONES RECOVER AFTER LONG-TERM DISUSE OSTEOPOROSIS?

Methods: Mechanical function of long bones was evaluated from metacarpal-3 from the immobilization studies described above. Four-point bending tests were performed to assess structural-mechanical properties. Prior to mechanical testing, pQCT scans were performed to determine the axial moments of inertia at mid-diaphysis. Testing was performed using a servohydraulic materials test system. Intact long bones were positioned in the test apparatus with the dorsal side in compression and volar side in tension. The mid-diaphysis was centered over two supports positioned 21mm apart with upper loads spaced 7 mm apart. Bones were loaded to failure at a displacement rate of 0.1mm/sec. Structural properties (stiffness, ultimate load, work to failure) were determined from the load-displacement curves. Tissue level properties of cortical bone (bending modulus) were derived using pQCT-determined moments of inertia. Differences among groups were tested using the ANOVA with post-hoc testing using the Mann-Whitney U test; significance values reported $p < 0.05$.

Results: *Immobilization:* Bones at 3 months of IM showed significant reductions in stiffness (-23%; Fig 1), strength (-34%) and work to fracture (-29%). A 17% loss of bone tissue modulus was also observed (Fig 2). After 6 months of IM, whole bone stiffness (Fig 1), strength and work to fracture were reduced to about half that of control bones. Tissue modulus was more than 60% lower than control (Fig 2), suggesting an impairment of bone matrix properties beyond that expected from the osteoporosis alone.

Remobilization: Structural properties of bones remobilized after either 3 or 6 months of IM were not significantly different from the control group ($p > 0.22$; Fig 1). In contrast, remobilized bone tissue modulus remained approximately 13% less than normal tissue (Fig 2). Moments of inertia of remobilized bone were approximately 10-15% larger than those in the control group (Fig 3).

Figure 1: Stiffness (N/m)

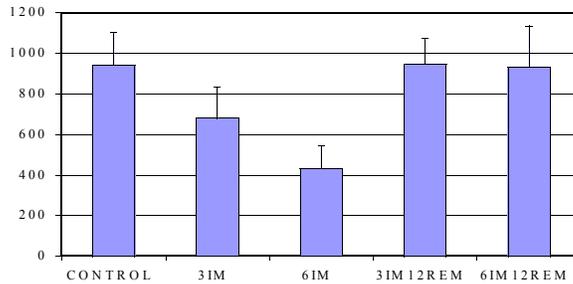


Figure 2: Tissue Bending Modulus (GPa)

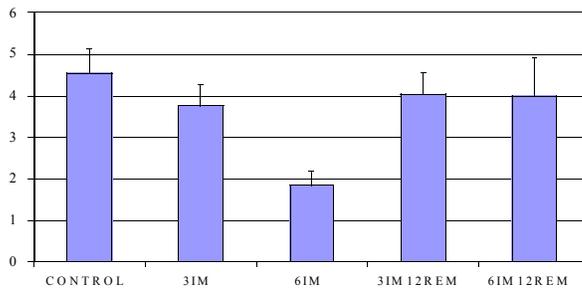
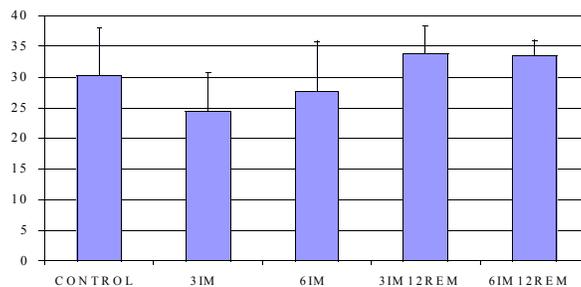


Figure 3: Moment of Inertia (mm⁴)



Discussion: It has long been believed that long-term disuse osteoporosis (> 3 months duration) results in non-reversible architectural and mechanical impairments in bone. Previous studies by our group and others show that after long-duration immobilization, cortical bone mass does not recover to baseline levels, even one year after restoration of loading. However, our group recently demonstrated that in recovery from long-term disuse, remobilized diaphyses undergo expansion at the periosteal envelope, resulting in increased cross-sectional dimensions. Small amounts of periosteal expansion can readily offset the deleterious effects of markedly greater amounts of endocortical bone loss on the mechanical strength of long bones.

Conclusions:

- Long bones in older adult animals can completely recover their whole bone mechanical integrity after long-term disuse osteoporosis.
- They do so through a compensatory increase in their cross-sectional geometry rather than through restoration of bone mass.
- Despite the high structural stiffness of the recovered bones, the tissue modulus of the recovered bone remained lower than normal, suggesting an alteration may occur in the material-level properties of bone tissue formed during recovery.
- Nevertheless, these studies argue that bones in older adult animals will adapt their structure to restore mechanical integrity and overcome the mass defect that remains after long-term disuse osteoporosis.

3. CHANGES IN TRABECULAR BONE ARCHITECTURE AFTER LONG-TERM DISUSE OSTEOPOROSIS

Methods: Changes in trabecular bone architecture were studied from distal metacarpal epiphyseo-metaphyseal regions. Distal metacarpals were scanned using a micro-computed tomography system. Images were acquired at a voxel resolution of 22 μm . Computer-based stereological techniques were used to determine the bone fraction (Tb.V/TV) and mean intercept lengths in each of the principal orthogonal (anatomical) planes (IS-MIL, AP-MIL, ML-MIL) and the degree of anisotropy for the full 3-D cancellous bone data set for each bone. Data are reported as mean \pm S.D. Analysis of variance with post-hoc testing using the Mann-Whitney U-test were used to examine difference among and between groups, respectively. Data are reported as mean \pm S.D.

Results: Trabecular bone loss occurred throughout the experimental period, as shown by decreases in Tb.V/TV (Fig 1). In contrast, trabecular bone architecture, as indexed by mean intercept lengths, was unchanged for the first 12 weeks of immobilization. However, after 26 weeks of immobilization, intercept lengths were increased by more than two-fold over baseline ($p < .02$, Fig 2a-c). The same pattern of change was seen in the MIL for each of the orthogonal axes, indicating that bone loss processes occurred equally on all trabecular surfaces. Accordingly, consistent with the uniform increase in MIL for all orthogonal planes, long-term disuse osteoporosis did not result in any changes in the degree of anisotropy of trabecular bone (Fig 3).

Fig 1: Tb.V/TV

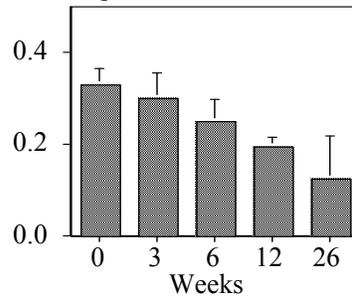


Fig 2a: IS-MIL

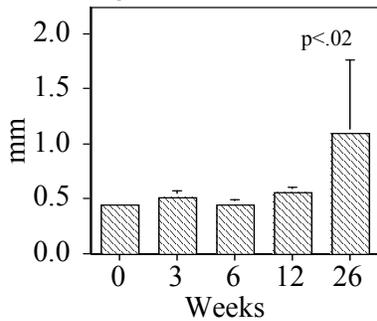


Fig 2b: AP-MIL

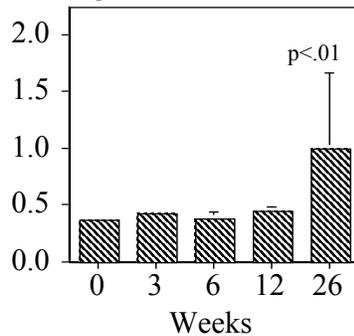


Fig 2c: ML-MIL

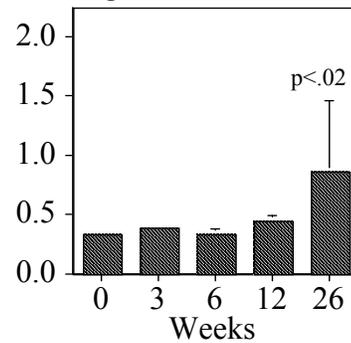
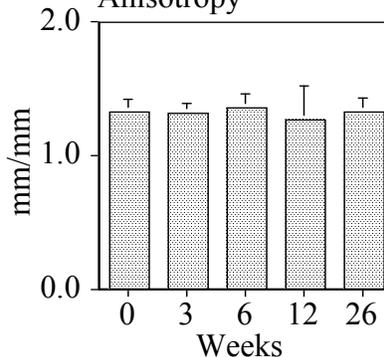


Fig 3: Degree of Anisotropy



Discussion: Perforation of elements and loss of trabeculae have been shown to be key feature of the evolution of osteoporosis. Decreased trabecular number occurs late in the development of disuse osteoporosis in the canine skeleton. Changes in mean intercept lengths seen after long-term immobilization in the current study correspond well to the timing of this loss of trabecular elements. However, whether this bone loss occurs preferentially among longitudinally versus transversely oriented trabecular elements remains unclear. Similarly, whether differential loss of trabeculae results in net changes in the orientation of remaining trabecular element is not known.

The results of the current study show that disuse osteoporosis develops through a uniform symmetric enlargement of existing pores. This is equivalent to the observation that the sequence of trabecular element deletions during age-related bone loss in humans is uniformly random. Recent studies suggest that trabecular bone orientation appears to be stable in post-menopausal bone loss, supporting the idea of a uniform symmetric enlargement of existing pores. The anisotropy ratio (longest/shortest MIL, Fig. 3) was unchanged by disuse in the current study, and

the orientation of the principal axes was not changed. Accordingly, despite the loss of trabecular elements with long-term disuse, the overall plan of orientation remains unchanged, consistent with a uniformly random model for trabecular element deletion.

Data for aging in the human spine and the current mechanical disuse data both conform to a uniformly random model of trabecular element loss. This suggests that the pattern of cancellous bone created during growth governs trabecular pattern changes under many conditions of bone loss. Thus, the developmental influences giving rise to trabecular pattern formation may be key in determining the architectural and mechanical consequences of eventual bone loss. The current data support this idea and suggest that *prevention* (as opposed to treatment) of osteoporosis may depend upon changes in lifestyle which optimize bone during growth and development and maintain bone integrity during aging.

4. HOW DOES CANCELLOUS BONE RECOVER FROM LONG-TERM DISUSE?

Disuse results in not only a dramatic loss of bone mass, but also changes in bone architecture. In cancellous bone, these changes result from a combination of thinning, perforation and ultimately complete loss of trabecular elements. Bone mass that is lost during immobilization can substantially recover with restoration of loading. However, whether bone architecture can recover after long-term disuse is unknown. In the current study, we examined both the bone mass and microarchitectural bases of cancellous bone recovery from long-term disuse osteoporosis.

Methods: Right forelimbs of 5-7 years old retired breeder Beagle dogs (N=32) were immobilized (IM) with a jacket-type plastic splint, placing elbow flexed at 90 degrees and the carpal joint volar-flexed slightly. Immobilization lasted for either 3 or 6 months periods. At the end of which time, the animals were either sacrificed (3IM and 6IM), or remobilized (RM = allowed to resume normal weight-bearing) for an additional 12 months (3IM-12RM and 6IM-12RM). Nonimmobilized age-matched animals were served as control. Before sacrifice, all animals received double fluorescent-labels injections. Changes in cancellous bone were examined using histomorphometry on undecalcified, MMA embedding, longitudinal sections from the distal metacarpal metaphyses. Bone architectural changes were assessed from trabecular bone volume (%Tb.Ar/T/Ar), trabecular number (Tb.N) and trabecular width (Tb.Wi). Osteoclastic activity was assessed from eroded surface (%Er.Pm) and osteoblastic activity assessed from labelled surface (%L.Pm), mineral appositional rate (MAR) and surface-based bone formation rate (BFR/B.Pm). Differences among groups were tested using ANOVA with post-doc testing using Fisher's analysis; significance is reported at $p < 0.05$.

Results: Immobilization: At 3 months after IM, there was a marked reduction (-38%) of cancellous bone mass (%Tb.Ar/T.Ar) (Fig.1), accompanied by a significant decrease in trabecular thickness (Tb.Wi, -45%) (Fig. 2) and trabecular number (Tb.N, -17%) (Fig.3). Bone formation (L.Pm/B.Pm, MAR and BFR/B.Pm), and resorption indices (E.Pm/B.Pm) were significantly higher in 3IM group compared to control group (Table 1). Continued loss of bone mass at 6 months of IM occurred through loss of trabeculae (Tb.N decreased 23% compared to control), without further decrease in Tb.Wi.

Remobilization: In both 3IM-12RM and 6IM-12RM groups, bone mass recovered to control levels indicating that bone mass can recover even in older animals.. This recovery occurred through thickening of the existing trabeculae. Bone microarchitecture did not recover from long-term disuse osteoporosis; trabecular number remained markedly lower in both 3IM-12RM (-12%, $p < 0.09$) and 6IM-12RM (-25%, $p < 0.002$) groups when compared to the control group (Fig.3).

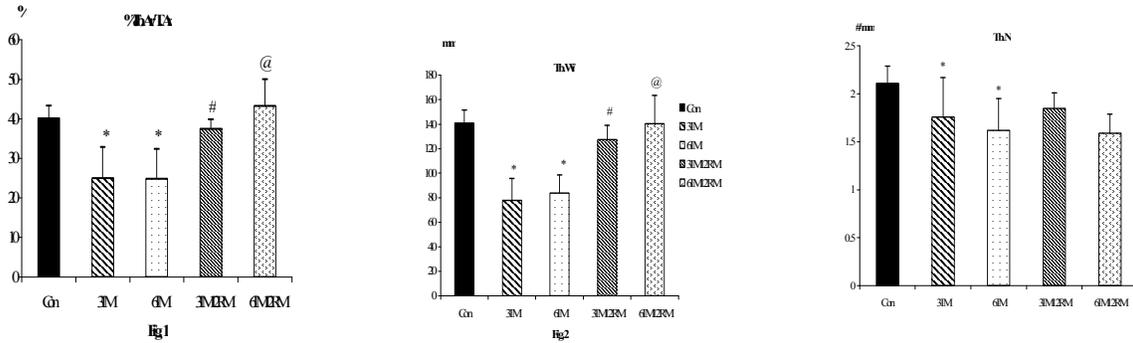


Fig.1: Cancellous bone mass;
Fig.2: Trabecular thickness;
Fig.3: Trabecular number;
Con: control; IM: immobilization;
RM: remobilization;
* $P < 0.05$ vs. control;
$P < 0.05$ vs. 3IM;
@ $P < 0.05$ vs. 6IM.

Table. 1. Dynamic measurement in distal metacarpals

Groups	%L.Pm %	MAR μm	BFR/B.Pm $\mu\text{m}^2/\square\mu\text{m}/\text{d}$	%Er.Pm %	Ac.f cycle/yr
Control	1.1 ± 1.1	0.4 ± 0.4	0.2 ± 0.4	1.6 ± 0.6	0.1 ± 0.1
3IM	7.1 ± 5.9*	0.9 ± 0.1*	2.5 ± 2.2*	8.5 ± 3.1*	0.4 ± 0.3*
3IM12RM	1.3 ± 0.8#	0.1 ± 0.3#	0.1 ± 0.2#	1.9 ± 0.8#	0.0 ± 0.0#
6IM	3.4 ± 2.5	0.7 ± 0.4	0.9 ± 0.6	3.6 ± 2.5	0.2 ± 0.2
6IM12RM	1.8 ± 1.3	0.0 ± 0.0@	0.0 ± 0.0	1.2 ± 0.5@	0.0 ± 0.0

Data are presented as means ± SD; IM, immobilization; RM, remobilization; * $p < 0.05$ vs. control; # $p < 0.05$ vs. 3IM; @ $p < 0.05$ vs. 6IM; L.Pm, labeled perimeter; MAR, mineral apposition rate; BFR/B.Pm, bone formation rate; E.Pm, eroded perimeter; Ac.f, activation frequency.

Discussion: The present study shows that bone cancellous bone mass can recover from long-term IM in older dogs for 3 months reduced cancellous bone mass at 38% in the distal metacarpal metaphyses. This bone loss resulted from a significant decrease in Tb.Wi and Tb.N. Prolonging the IM period to 6 months, there was no further decrease in Tb.Wi, but a continued decline in Tb.N. These findings indicated that long-term IM results in not only a loss of bone

mass, but also the deterioration of bone microarchitecture. Similarly, IM in younger or older dogs for 12 to 16 weeks, induced cancellous bone loss in the same manner.

The Tb.Ar in both 3IM-12RM and 6IM-12 RM restored to the control level, indicating that bone mass can completely recover even in older animals. This recovery occurred by thickening of the existing trabeculae. In the study of 32 weeks RM following 28 weeks IM by Jaworski et.al, which demonstrated that cancellous bone mass was only recovery by 66% at younger dogs (1-3 yrs) and 60% at older dogs (7-8 yrs). Actually, the period of RM in the previous study was shorter than that in the present study. Also, IM in 6 month-old rats for 6 weeks following by 6 weeks remobilization, cancellous bone mass cannot completely recover. Taken together, these studies suggested that cancellous bone mass is able to partially or completely recover depended on the duration of IM and RM as well as the age of animals. However, Tb.N remained lower in both 3IM-12RM and 6IM-12 RM compared to the control group, indicating that loss of the entire elements of trabeculae is not able to be restored even though the weight-bearing loading is resumed. Studies in rats showed that 20 weeks RM following 18 weeks IM can partially restore bone mass, but can not restore the trabecular numbers. Thus, these studies suggested that remobilization is capable to recover bone mass, but the microarchitecture of bone is not possible to restore after long-term disuse. The dynamic histomorphometry showed bone formation, bone turnover and resorption were significantly higher in 3IM compared to the control level. Similarly, previous studies demonstrated that 12 weeks IM in old dog and 16 weeks IM in young dog induced high bone turnover. These studies suggested that disuse cancellous bone loss is a “high turnover” type process, which similar to the post-menopausal osteoporosis.

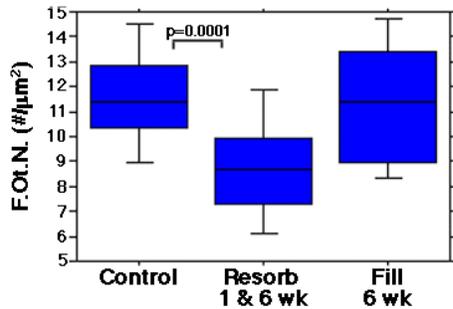
5. OTHER CHANGES IN BONE AFTER LONG-TERM IMMOBILIZATION

Osteocyte viability

In order to assess whether changes in osteocyte integrity result from loss of the normal mechanical loading of bone, cortical bone tissues from dogs in our ongoing NASA-funded experiments were examined.

Methods: Metacarpal diaphyses from dogs forelimbs immobilized for up to 1.5 months were studied, as this time period corresponds to the onset of significant increases in intracortical resorption in unloaded dog forelimbs. Diaphyses were stained in basic fuchsin, which stains osteocyte nuclei and the proteoglycans that line the lacunar and canalicular walls in vital bone. Loss of basic fuchsin staining of osteocytes and canaliculi under fluorescence confocal microscopy corresponds to loss of osteocyte viability in regions of bones. Sections were examined using confocal microscopy with long wavelength (568 nm) to excite basic fuchsin fluorescence. Osteocyte integrity was assessed in bone surrounding osteoclastic resorption spaces in immobilized tissue and compared to non-immobilized control bone.

Results: Osteocyte integrity in unloaded bone was found to be dramatically impaired in regions surrounding osteoclastic resorption spaces. There was loss of normal osteocyte staining, pyknotic nuclei and the canalicular system was no longer evident. Osteocyte in non-remodeling areas of immobilized bone as well as in control bone appear normal. The number of intact osteocytes around resorption spaces was significantly lower than in non-resorbing control bone.



Osteocyte integrity around resorbing and infilling BMU's, shown as fluorescent osteocyte number (F.Ot.N, # ce;;s/mm²)

These data show that unloading of bone 1) is associated with regional impairment of osteocyte viability, and that 2) osteoclastic resorption occurs in these regions of impaired osteocytes. In adjacent areas of bone which were not being remodeled, normal osteocyte, lacunar and canalicular staining were observed. It is noteworthy that not all osteocytes in unloaded bone become impaired by the withdrawal of normal mechanical loading, suggesting either that not all cells are equally affected by the withdrawal of loading. The reasons for this are not currently known. Nevertheless, in areas undergoing resorption, there is a conspicuous change in osteocyte integrity. Moreover, there appear to be similarities between the situation observed after unloading, and the co-localization of osteoclastic resorption and impaired viability after fatigue loading. These data are consistent with the hypothesis that the normal response by osteoclasts to osteocytes that have become injured or outlived their usefulness is focal resorption of the bone and those osteocytes.

Discussion: Changes in osteocyte integrity are consistently associated with sites of bone resorption following disuse. Subsequent to immobilization, there is a dramatic increase in the activation of new resorption sites. After disuse, osteocyte integrity in bone surrounding intracortical resorption spaces is reduced approximately 30% in comparison with controls.

In previous investigations, loss of osteocyte viability was colocalized with bone resorption after overuse, *i.e.* fatigue. These studies gave support for a targeting mechanism by which osteoclasts identify areas of bone to be removed after matrix injury. Similarly, in our study, loss of osteocyte integrity was shown to be highly associated with sites of resorption following disuse. Furthermore, osteocyte integrity was shown to increase in time as the number of infilling BMU's increased.

These observations are consistent with a mechanism in which immobilization results in a reduction to fluid flow through bone tissue. The ensuing compromise to molecular transport and exchange causes loss of cell viability and triggers the remodeling response. Resorption serves as a mechanism for maintaining the viability of the tissue, by removing nonviable osteocytes as well as increasing transport to "deprived" areas. The reestablishment of molecular transport and exchange may, in turn, trigger the apposition of new bone on the inner surface of the resorption cavity. The co-localization of osteocyte integrity and bone resorption after disuse, as well as the previous observation of this co-localization after overuse, is indicative of a common pathway for the signaling and timing of resorption activity within bone tissue.

SUMMARY OF KEY FINDINGS IN RELATION TO CRITICAL PATH ROADMAP QUESTION:

2.19 Is bone loss reversible and within what time frame: Can geometry and architecture return to baseline as well as BMD?

In cortical bone:

- Long bones in older adult dogs do not recover their baseline bone mass after long-term disuse osteoporosis.
- Long bones can completely recover their whole bone mechanical integrity after long-term disuse osteoporosis.
- They do so through compensatory increases in their cross-sectional geometry rather than through restoration of bone mass.
- Despite the high structural stiffness of the recovered bones, the tissue modulus of the recovered bone remained lower than normal, suggesting that alterations occur in the material-level properties of bone tissue formed during recovery.
- Nevertheless, these studies argue that bones in older adult animals will adapt their structure to restore mechanical integrity and overcome the mass defect that remains after long-term disuse osteoporosis.

In cancellous bone:

- Cancellous bone mass can recover from long-term disuse osteoporosis
- Cancellous bone microarchitecture changes are irreversible, such recover occurs through thickening of remaining trabeculae.
- Failure to restore bone microarchitecture may have important implications for determining whether bone can recover adequate mechanical integrity after recovery from osteoporosis.

Presentations

- Programmatic loss of cancellous bone in disuse osteoporosis, Presented Annual meeting of the Orthopaedic Research Society
- Fluorescence provides a measure of local tissue age and remodeling history in compact bone.
- Adult cortical bone recovers from long-term disuse osteoporosis by changing its architecture.
- Cortical bone recovery from disuse osteoporosis, Presented at the BioAstronautics workshop, Galveston, TX, 2001.
- Permeability characteristics of different molecular tracers in loaded and unloaded bone. Presented at the 47th Annual meeting of the Annual meeting of the Orthopaedic Research Society, San Francisco, CA, 2001
- Loss of osteocyte integrity co-localizes with bone resorption following disuse. Presented at the 48th Annual meeting of the Annual meeting of the Orthopaedic Research Society, Dallas, TX, 2002
- Bone mass does not adequately predict variations in bone fragility: A genetics approach Presented at the 48th Annual meeting of the Orthopaedic Research Society, Dallas, TX, 2002

- Remobilization restores cancellous bone mass but not microarchitecture after long-term disuse in older adult dogs Presented at the Annual meeting of the American Society of Bone and Mineral Research, September, 2002, San Antonio TX
- Clement M, Akkus A, Hernandez CJ, Jepsen KJ and Schaffler MB Can adult long bones recover their mechanical integrity after long-term disuse osteoporosis? To be presented at the 49th Annual meeting of the Orthopaedic Research Society, New Orleans, LA, 2003.

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Knothe Tate M.L. and M.B. Schaffler Permeability characteristics of different molecular tracers in loaded and unloaded bone. *American Journal of Physiology* (submitted), 2002.

Trainees Supported

Undergraduates:

Aaron Rabinovich, Wayne State University
Marc Glotkowski, University of Michigan

Graduate students

Tamir A Bloom, Mount Sinai School of Medicine
Mariza Clement, Mount Sinai School of Medicine
James Celestin, Mount Sinai School of Medicine

Post-docs

Shijing Qiu, MD, PhD
Melissa Knothe Tate, PhD
Ozan Akkus, PhD
Christopher Hernandez, PhD
Chaoyang Li, MD