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Calcium Metabolism of Bone in Microgravity: An Investigation and Simulation of Bone Demineralization in Space

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SENIOR THESIS APPROVAL

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"Calcium Metabolism of Bone in Microgravity:
An Investigation and Simulation of Bone Demineralization in Space"

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Overview of the Space Environment

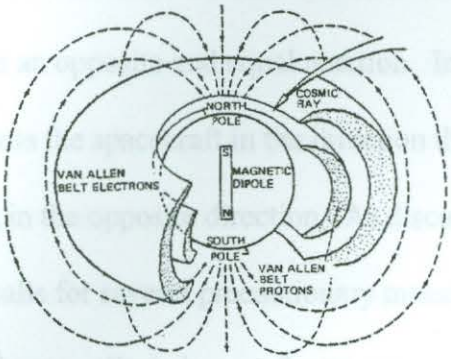
Extended space exploration poses many physiological problems that must be overcome before humanity can reach past the boundaries of Earth's orbit. The farthest a human has been able to travel is to the moon and back, and currently astronauts are confined to orbiting the Earth. While many astronauts and cosmonauts have remained aboard the space station Mir for months, their stay was not without detrimental effects upon their return. Currently, the International Space Station and missions to Mars are the projects involving humans in space at the National Aeronautics and Space Administration (NASA).

The upper boundary of the atmosphere is defined as the "area where collisions between air molecules become so infrequent as to be immeasurable."[14] This exists approximately 700 km above sea level. Without the atmosphere to filter out the sunlight, space is about 25% brighter than on Earth. This not only causes brightening of objects in the sun's view, but also creates darker shadows where the sun is not directly shining. Despite the difference, no alterations in visual performance have been recorded.[4]

Radiation poses a problem for extended spaceflights because it has the potential to ionize the molecules in living cells. Ionizing radiation comes in the forms of x-rays, gamma rays, electrons, protons, neutrons, alpha particles, and heavy ions. There are three main sources of ionizing radiation. Galactic cosmic radiation originates from supernovas and consists of protons and alpha particles. Trapped-belt radiation exists in the Van Allen belts around the Earth. The Earth's magnetic field traps these particles in two belts that begin at approximately 480 km and 9660 km above the Earth, respectively. The belts consist of protons and electrons but do not pose a threat to the Space Shuttle because it maintains an orbit below the belts. *(Figure 1)* The

third and most hazardous radiation originates from solar flares. These particles are mainly protons, but some alpha particles have been detected.

Figure 1. Van Allen radiation belts
(from Space Physiology and Medicine, Ch. 2, fig. 2-6)



The absence of gravity provides an obstacle that must be overcome. In actuality, gravity does exist from particles and other objects, but these particles are quantitatively insignificant. The term microgravity is used to describe the environment of space and the resulting weightlessness that ensues on all objects. The gravitational force of Earth is 9.8 m/s^2 (or 1 G). Outside the Earth's atmosphere, gravity's effect is significantly reduced.

At launch, the gravitational forces (G's) are significantly increased, causing moderate stress on the human body. This force is well within physiological limitations, usually at 3-4 G's chest to back, but sometimes reaching 8-9 G's. The seats of a spacecraft are reclined almost perpendicular to the axis of acceleration, which aids in negating such force.[6]

After the astronaut has reached the microgravity environment, there is a period of relearning that must occur since humans are accustomed to living in gravity. Thanks largely to automation, humans now have become more of a supervisor and information manager of instruments and experiments. This still requires, however, the successful completion of control,

memory, sensory, and motor functions.[4] Reorientation is necessary because, in space, there are no ups and downs. In fact, the Space Shuttle flies upside-down from an Earth perspective, causing the crew to naturally want to orient themselves to keep Earth upright.

An additional adjustment involves Newton's third law of motion, which simply states that for every action, there is an opposite and equal reaction. In space, if an astronaut tosses a wrench, the tool will fly across the spacecraft in the direction thrown and the astronaut will notice that he/she is moving in the opposite direction. As discussed later in the survey of spacecraft, action-reaction calls for several precautionary measures.

While all seems well externally in human functioning in microgravity, internally the human body is undergoing many physiological changes in response to the loss of gravitational stress. The two chief effects of weightlessness are translocation of the fluids within the body and diminished physical activity. Prolonged spaceflight results in many specific homeostatic imbalances that will be discussed later.

Re-entry into the Earth's atmosphere reapplies the force of gravity to the body. Increased G's are felt in the head to foot plane, but are tolerable. Upon return, it takes several days for the body to return to homeostasis in terms of the normal gravity environment.

Survey of Spacecraft Support Systems

Supporting human life in space means that all necessities for living must be taken aboard the spacecraft. In addition, numerous life-support systems must be installed to counteract the physiological alterations of microgravity as much as possible. Current technology limits what can done about the absence of gravity and resulting weightlessness, but the internal environments of spacecraft are designed to be very similar to Earth.

On Earth, the pressure of the atmosphere is 14.7 psi and is composed of 78% nitrogen, 21% oxygen, 1% argon, and 0.04% carbon dioxide. The Space Shuttle maintains 14.7 psi, 80% nitrogen, and 20% oxygen; whereas spacecraft made during the beginnings of the space program had 100% oxygen (Gemini and early Apollo missions) or even required spacesuits to be worn (Mercury missions). Temperature is controlled at 18-27 °C (64-81 °F).[15]

The requirements of a spacecraft's atmosphere include:

- a partial pressure of oxygen that is neither hyperoxic nor hypoxic
- minimize the possibility of atmospheric explosion
- provide sufficient cooling for electronics
- minimize the threat of decompression sickness
- ability to maintain structural integrity
- compensate for gas leakage[15]

There are extreme physiological implications that may result in abnormal pressurization. When free gas in tissues is subjected to changes from external pressure resulting in pain and tissue injury, the condition is called barotrauma. This is prevented in spacecraft by reducing cabin pressure from 14.7 to 10.2 psi before extravehicular activity (EVA). Decompression sickness occurs when the sum of partial pressures of gases in the tissue exceeds the ambient pressures of those gases. Oxygen does not significantly cause bubbles in tissue, so this ailment is alleviated by breathing pure oxygen before decompression. The partial pressure of oxygen in the Space Shuttle is kept at 3.2 ± 0.25 psi; very near the Earth's value of 3.06 psi at sea level. When external pressure drops so rapidly that the lungs (and therefore the body) simply explode, explosive decompression occurs.

Carbon dioxide is removed from the air by lithium hydroxide canisters, since the atmosphere is enclosed, and is maintained at a partial pressure of 0.15 psi. Absolute humidity, the actual partial pressure of water vapor, is kept between 0.12 and 0.27 psi.

Estimated water consumption for each crewmember per day shows that 2.6 kg are used for food and beverage, 5.5 kg are used for hygiene, and up to 50 kg are used for housekeeping.[15] Systems for showering, washing, and laundering have yet to be perfected because the water flow cannot be adequately controlled. Urine is collected in bags and dumped in space, while feces is collected, vacuum-dried, and returned to Earth for disposal.

To counter the effects of microgravity, systems were designed to allow astronauts to effectively exercise. Weightlifting cannot be utilized in space, but elastic resistance training can. Simple devices involving stretchable material are used to create resistance in the muscles. A treadmill is mounted in the spacecraft, along with restraints to keep the astronaut from flying off the machine. Walking on the treadmill requires downward and rearward forces, stimulating the muscles of the astronaut. Treadmill activity also maintains aerobic conditioning, and therefore the ability to perform physical activity.[4]

The ergonomics of space require precautions that ensure the safety of the crew and the prevention of accidents. Velcro-like material is applied to the floors and soles of shoes in places where a technician must remain steady. All switches are made for easy manipulation because astronauts tend to overshoot their target. With decreased physical activity, flight plans usually have astronauts perform complex tasks first, before fatigue sets in.

The survey of spacecraft, thus far, has detailed systems of nominal functioning. In the event of an emergency, however, precautionary measures have been taken. For example, in the

event of booster failure during launch, the crew can separate from the booster via a module or ejection seats. (The Space Shuttle does not have an ejection system.) If pressure fails, a pressure suit can be donned and sections of the spacecraft can be sealed off.

Human Physiology in Space

Mercury and Gemini missions of the early 1960's did little in the way of informing scientists about the complications of microgravity. These missions were simply designed to prove a human could be put into space and that certain spacecraft instruments and procedures were functional. Of course, the Apollo program set for its goal landing a man on the moon, but it was during these missions that scientists began examining specific abnormalities reported from astronauts of earlier missions.

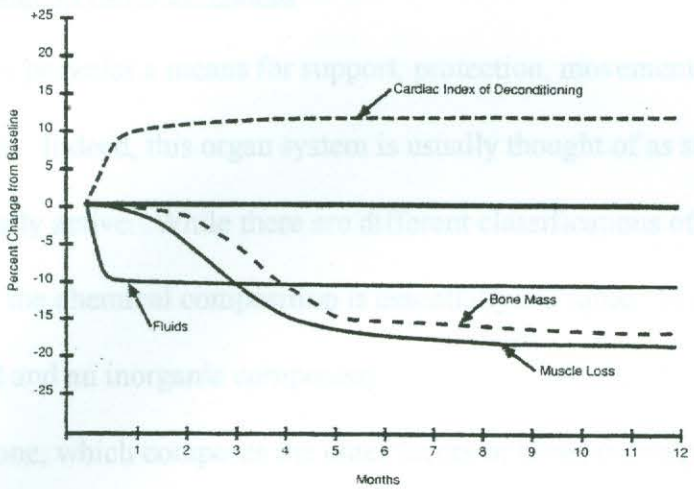
When the Apollo program ended in 1972, the next mission, Skylab, had as its distinct purpose, the observation and experimentation of creating a habitable environment in space. During these missions, which lasted up to 84 days, motion sickness, reduced cardiovascular efficiency, and bone mineral loss were observed. Further analysis was provided by the Russian space station Mir, launched in 1986. Humans were supported in space for missions lasting up to one year. A variety of physiological difficulties were reported, depending on the astronaut's lifestyle aboard Mir.[13]

The actual effect of microgravity is believed to originate in the fluid shift. The shift causes a decreased response of gravity receptors by an unknown mechanism, causing body fluid to swell around the head and chest, away from the legs.[9] This causes the brain to believe the body needs to rid an apparent excess of fluids from the body.

Aside from the overall effects of fluid imbalance and diminished physical activity,

extended space living can have dramatic effects on any system of the body. Current research is being conducted that is investigating neurovestibular dysfunction, cardiovascular deconditioning, hematologic and immunologic changes, bone mineral loss, muscular deconditioning, and metabolic and endocrinologic changes. Prolonged spaceflight results in decreases of blood volume, red blood cell mass, muscle strength, cardiac output, and increased loss of calcium in bone. *(Figure 2)* All these effects tend to peak a few weeks into flight except for loss of calcium, which continues to increase for several months.[6]

Figure 2. Change in Homeostasis Over a Course of Time
(from Space Physiology and Medicine, Ch. 11, fig. 11-1)



Results of experimentation on various physiological responses are not completely collected in a controlled manner. There are a variety of complications that may produce unusable or erroneous data. Sample size is extremely limited (there are only one hundred or so astronauts), as well as scientists' ability to directly observe the experiment in space. Systems in the spacecraft used as countermeasures interfere with pure analysis since the very problem often being observed is also being prevented by mission protocols.

Data on the physiological shifting seen in flight assumes that the body is in homeostasis

before flight. Some systems respond immediately to the environmental change. Other systems continually respond throughout the duration of the flight. A new steady state is usually reached for most physiological functions around four to six weeks into flight.[9]

Of the major cardiovascular, muscular, skeletal, and hormonal changes, the loss of bone density and subsequent weakness of bone tissue is of particular importance. If any extended mission to is to be successful, scientists must develop countermeasures that minimize the apparent loss of bone mass in humans. First, an understanding of basic bone physiology is required to investigate alterations in bone mass.

Physiology of the Human Skeletal System

The skeleton provides a means for support, protection, movement, mineral storage, and blood cell formation. Indeed, this organ system is usually thought of as static, while in fact bones are dynamically active. While there are different classifications of bone (i.e., long, short, flat, and irregular), the chemical composition is essentially the same. In general, there is an organic component and an inorganic component.

Compact bone, which composes the outer layers of bone, is composed of 30% of this organic matrix and 70% of the inorganic salts, mainly in the form of calcium compounds. The major salt form is hydroxyapatite, which contributes to bone hardness. Contributing to bone strength, the matrix includes the collagen fibers that extend along the lines of tension and stress, and the ground substance, which is made of compounds such as proteoglycans, chondroitin sulfate, and hyaluronic acid.

Calcium serves a number of physiological roles, including transmission of nerve impulses, muscle contraction, blood coagulation, and cell division. In humans, there is about

1200-1400 g of calcium, but only 9-11 mg per 100 ml of blood exists in the ionic form to serve these functions.[11] In addition, this ionic calcium that flows through the bloodstream exists in three forms. 40% is combined with plasma proteins and is therefore nondiffusible. 10% is combined with other compounds but is diffusible. 50% of calcium is totally diffusible and in its free ionic form.[7]

Calcium is not readily absorbed in the intestine because it is usually taken in as an insoluble salt. Vitamin D, in its active 1,25-dihydroxycholecalciferol form, significantly increases intestinal absorption of calcium. Extreme doses of vitamin D have been shown to cause bone absorption, but smaller quantities promote calcification.[7]

When extracellular calcium levels are altered, research shows that the levels return to normal in a short time. This phenomenon is indicative of an exchangeable form of calcium in the body. A small portion comes from permeable cells in the liver and intestine. The overwhelming majority, however, comes from calcium in bone. The major form of the exchangeable calcium is $CaHPO_4$.[7]

Bone-forming cells, known as osteoblasts, stimulate secretion of collagen molecules and ground substance. Together, these compounds form osteoid. Osteoid is collagen-like, with the difference being that calcium can precipitate in osteoid. When calcium and phosphate levels become high enough, hydroxyapatites form, hardening the osteoid. As the hardening continues, osteoblasts become trapped and are now called osteocytes.

A unique aspect of bone is that it is continually being reformed in response to stresses from the environment on the body. As explained, osteoblasts contribute to deposition of bone tissue. In contrast, bone is reabsorbed by cells called osteoclasts. Osteoclasts secrete proteolytic

enzymes that digest the matrix and metabolic acids that demineralize the salts to soluble forms. The rate of deposition and resorption are essentially equal in homeostasis. Osteoclasts can absorb an area 1 mm in diameter and several millimeters long in a period of three weeks.[7] Meanwhile, osteoblasts are continually depositing bone tissue in concentric rings known as osteons. Osteons are formed around the blood vessels that network throughout the bone.

There are great advantages to this system of remodeling. First, remodeling allows the body to adjust where it needs added strength in response to stress. Likewise, the shape of the bone can be rearranged over time, reflecting where bone is most stressed. Third, new matrix can replace older, weak matrix to ensure optimal support. Bone is deposited in proportion to the compressional load, while the stress determines the shape, a concept that is known as Wolff's law.[7] Remodeling, according to Wolff's law, is accomplished through calcification and osteoblast activity. Observations supporting Wolff's law are provided by the fact that protrusions exist where there are muscle attachments, and long bones tend to be thickest midway along the shaft where the most stress is applied.[11]

A leading hypothesis of the mechanism for Wolff's law states that there are piezoelectric effects that influence remodeling. Compression is thought to create a slight negative potential to that area, while creating a positive potential in the place of no stress. The negative potential attracts osteoblasts and therefore contributes to bone formation.[7]

The body regulates the remodeling system by controlling ionic calcium levels. Two hormones produced by different endocrine glands are dedicated to this purpose. The major hormone is parathyroid hormone (PTH), appropriately named since it is secreted from the parathyroid gland. PTH causes increased absorption of calcium into the extracellular fluid and

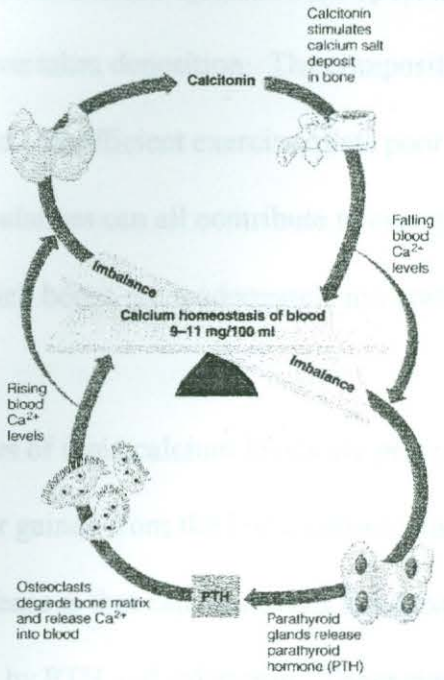
decreased excretion of calcium by the kidneys. There are three mechanisms by which PTH does its job. First, PTH acts by turning on calcium pumps in the bone and/or stimulates osteoclast activity and formation. Second, PTH increases the absorption of calcium in the distal convoluted tubules of the kidney. Third, PTH increases the formation of 1,25-dihydroxycholecalciferol from vitamin D.

The release of PTH is accomplished by a negative feedback mechanism. When there is the slightest decrease in ionic calcium levels, PTH is stimulated. In contrast, when the levels of ionic calcium rise above normal levels, PTH is inhibited.

If ionic calcium levels fall below 6 mg per 100 mL blood, hypocalcemia results, causing spontaneous discharging of the nervous system from ion imbalance. This can also cause dilatation of the heart, enzyme impairment, altered membrane permeability, and impaired blood clotting. Hypercalcemia occurs if ionic calcium levels rise above 12 mg per 100 mL blood. Here, the nervous system is depressed, constipation occurs, and altered sinus rhythms are observed. Lethal levels of ionic calcium are reached below 3 mg per 100 mL blood and above 17 mg per 100 mL blood.[7]

The second hormone and its role of controlling calcium levels was only discovered in the last 20 years. Named calcitonin for its effects, this hormone is made in the parafollicular cells of the thyroid gland. Its effects are just the opposite of PTH. *(Figure 3)* Calcitonin inhibits bone resorption and encourages calcium deposition. Like PTH, calcitonin acts in three ways, each with different reaction times. Immediately, there is a decrease in osteolytic activity, which results in the favoring of calcium mineralization in bone. A transient effect is to increase osteoblast activity. Finally, the prolonged effect is to decrease osteoclast activity.

Figure 3. Regulation of Calcium Metabolism
(from Human Anatomy & Physiology Ch. 6, fig. 6-11)



None of these pathways of calcitonin directly involve altering the levels of ionic calcium in the bloodstream. Indeed, research has shown that calcitonin weakly affects ionic calcium levels because PTH will override any effects of calcitonin.[7] In other words, if calcitonin were to make calcium levels decrease in the blood by stimulating their return to bone salts, PTH would be activated to return the ionic calcium levels to what they were had calcitonin never acted.

Only a 10% increase in ionic calcium in blood is required for calcitonin to become active. This is a faster response than PTH, but the response is also short term. Therefore, PTH is the primary means of regulating calcium levels. This means that the body will mainly remove calcium from bone to increase ionic calcium levels in the presence of PTH, or keep calcium salts intact and excrete more calcium through the kidney in the absence of PTH. Calcitonin opposes the expression of the PTH activity by stimulating bone formation, not by changing ionic calcium

levels.

The major imbalance of the skeletal system is osteoporosis, which refers to a group of diseases in which resorption overtakes deposition. The composition of the bony matrix stays the same, but bone mass is reduced. Insufficient exercise, diets poor in calcium, abnormal vitamin D receptors, and hormone imbalances can all contribute to osteoporosis. Another major bone disease is osteomalacia, in which bones are inadequately mineralized, weakening the stability of the bone tissue.

Homeostatic imbalances of ionic calcium levels are protected by two lines of defense. As much as 0.3 g can be lost or gained from the body, causing disorders like hypo- and hypercalcemia. The first line of defense is that calcium exists in an exchangeable form. The second is hormone regulation, namely by PTH and calcitonin. These signals will be stimulated within three to five minutes of altered levels. Overall, PTH and vitamin D provide the ultimate control over calcium serum levels.[7]

Bone Metabolism in Microgravity

During the Skylab missions, average loss of calcium over the 84-day mission was 25 g of the total body calcium.[5] The first countermeasures involved exercises using equipment like the treadmill. However, there was still a substantial amount of mineral loss from the bone, especially in the ankle, femur, and pelvis. Fortunately, lost calcium begins to return when the astronaut reenters gravity on Earth, but it may not return to pre-flight levels. In fact, nine Skylab astronauts had their bone densities measured five years after their return from a mission, with statistically significant losses observed.[1]

A variety of techniques are used to assess bone density. Photon absorptiometry is used to

calculate bone mineral mass before and after flights. X-ray tomography is used to specifically target the vertebral mineral density. Dual-energy absorptiometry is used to determine if exercise is an effective countermeasure. So far, these instruments have led scientists to believe that recovery does take as long a period as did loss, and "weightless" exercise proves inefficient.[10]

A variety of protocols were implemented to curb the deterioration of bone minerals. Diet, as well as urinary and fecal output, were all monitored. During a 30-day stretch, calcium was readily lost from the body, plateauing after the 30-day mark. After 28 days, calcium lost in urine rose to 150 mg/day and calcium lost in feces reached as high as 300 mg/day after 84 days. *(Figure 4)*

An estimated 0.8% of the total body calcium was lost from astronauts on the 84-day Skylab mission.[10] At that rate, 25% of the body's calcium would be lost in one year's time. This calcium loss translates into a 1-2% loss of bone density per month in microgravity.[8] It was obvious to scientists that the rate of absorption overtook the rate of deposition. In essence, in its process of remodeling, the skeleton recognizes that it no longer needs to maintain the integrity it had under the influence of gravity. (This concept is supported if observed in terms of Wolff's law.) The unanswered question was why this occurred.

There are various hypotheses, most of which have not provided enough data to suggest adequate countermeasures. The leading thought is that the loss is caused by down-regulation of PTH and/or the active form of vitamin D. Since the rate of absorption is greater, ionic calcium levels in the bloodstream increase. This signals the parathyroid gland to stop secreting PTH. Without PTH, the body loses calcium through the kidneys because resorption is not being stimulated. Further, calcium is lost in the feces because vitamin D is not being converted to its

active form, so less calcium is absorbed through the intestine. Because of this, the body becomes dependent on the skeleton, rather than the diet, for calcium.[8] *(Figure 5)*

Figure 4. Calcium Balance in Urine and Feces
(from Space Physiology and Medicine, Ch. 17, fig. 17-1)

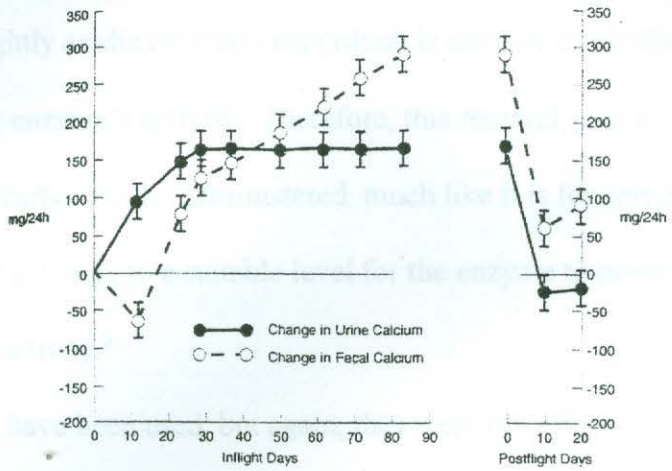
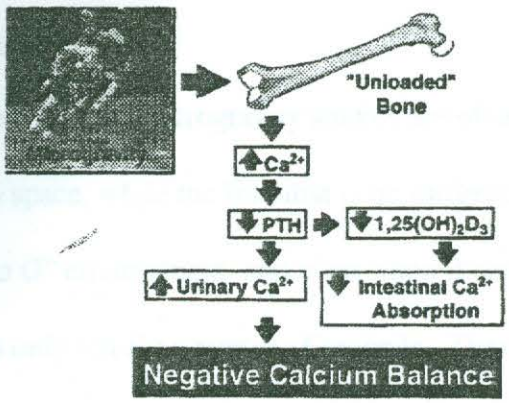


Figure 5. Results of Microgravity on Bone Metabolism
(used by permission from "Perspective on the Impact of Weightlessness on Calcium and Bone Metabolism, fig. 3)



A second, although less known, hypothesis involves the body's use of nitric oxide, NO. Recent data on osteocyte behavior shows that osteocytes sense the stresses of gravity via stretch-activated calcium channels. When these membrane channels open, intracellular calcium levels increase, activating the enzyme nitric oxide synthase, which produces NO. NO is presented to

osteoblasts via proteins, effectively down-regulating osteoclasts and the effects of PTH. The NO-synthase enzyme is regulated by pH and calcium levels. Without the stimulus of gravity to open these calcium channels, NO production is simply regulated by pH levels. Mild acidemia has been observed in cells whose stretch receptors are no longer activated, possibly from the lack of NO. The slightly acidic environment (which is actually clinically insignificant) is enough to inhibit the enzyme's activity. Therefore, this research concludes that dietary alkali, such as potassium bicarbonate, be administered, much like it is for certain cases of osteoporosis. This would bring the pH back to a suitable level for the enzyme to produce NO and NO to stimulate osteoblast activity.[3]

Supplements have been used, but again, they were not effective. Bone morphological proteins, growth hormone, vitamin D, and PTH were all used during experiments, but demineralization was still reported.[1]

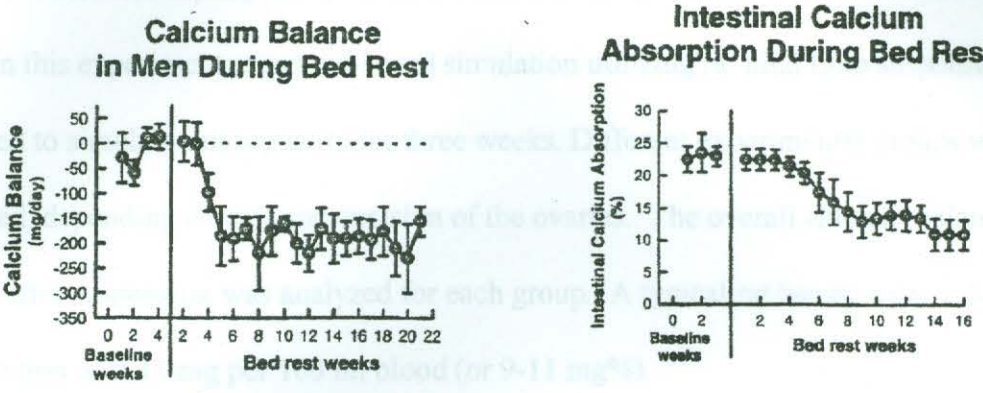
Ground-based Simulations

As previously stated, data on microgravity studies are often difficult to collect. In most cases, the experiment is in space, while the scientist is on the ground. On Earth itself, it is very difficult to produce a "zero G" environment, and even when it can be done (i.e., NASA's "vomit comet"), weightlessness is only felt for a matter of seconds. There are two sufficient models of microgravity: bed rest studies and hind-limb immobilization.

Volunteers who agree to bed rest studies do so for periods up to 28 weeks. Interestingly, similar results were obtained from bed rest individuals and astronauts. Analysis has been conducted on the lower limbs, namely the calcaneus bone. Losses up to 1% per week were reported during a 28-week study. *(Figure 6)* Exercise, static compression, low body negative

pressure, calcitonin, and phosphates were all utilized during testing but not one proved effective. Negative calcium balance was somewhat diminished, however, in an experiment that introduced biphosphonates into the volunteers' bodies.[8]

Figure 6. Bed Rest Studies as a Ground-Based Simulation of Microgravity
(used by permission from "Perspective on the Impact of Weightlessness on Calcium and Bone Metabolism" fig. 2&4)



The first uses of animal apparati were unsuccessful at creating an ideal microgravity situation. Such models included confinement to small cages, limb casting, induced paralysis, and surgical tenotomy. These models failed to meet the criteria for an acceptable model. For the experiment to be sufficient, the following conditions must be met:

- muscular atrophy
- headward fluid shift
- animal's ability to function normally through front limb use
- unloading of rear limbs without paralysis
- weight balance
- validation with spaceflight data[1]

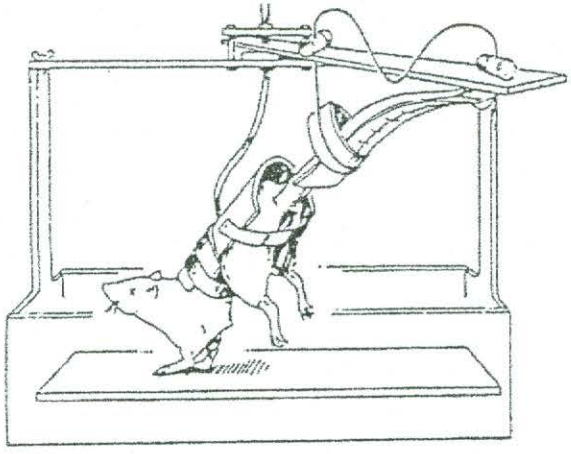
The first suspension models utilized an orthopedic casting material bonded to a rat, effectively tilting their head down 30 degrees. The rats did not meet the weight balance

criterion, which was attributed to the stressful situation. To provide a more comfortable environment, apparati involving plaster of Paris or orthopedic traction tape were developed. In all, the first week of suspension became an accurate simulation of the first week spent in microgravity for rat models. The one difference is that mineralization in rats after experiments occurred much more rapidly than in astronauts returning to Earth.

In this experiment, a ground-based simulation utilizing rat hind limb suspension was conducted to simulate microgravity for three weeks. Different experimental groups were suspended, depending on diet and excision of the ovaries. The overall calcium balance in serum samples after suspension was analyzed for each group. A typical rat has an ionic calcium concentration of 9-11 mg per 100 ml blood (or 9-11 mg%).

The suspension model used in this experiment was maintained at the University of Arkansas for Medical Sciences (UAMS) in the Physiology graduate department. *(Figure 7)* The rats are classified as *Sprague dawley*. The apparatus used to suspend the rats was certified by NASA as an acceptable model.

Figure 7. Example of a Suspension Apparatus



Bone mineral densities (BMDs) were analyzed before and after suspension by researchers at UAMS. This data, while not conducted in this specific experiment, was utilized to determine if there were any significant changes in calcium levels as a result of loss in bone mineral density.

The purpose of this experiment is simply to reproduce and verify the results that ionic calcium serum levels increase due to the unloading of bone minerals in the rat skeleton. Further analysis in other experiments is needed to confirm urinary and fecal output, vitamin D levels in serum, and the effects of medicinal countermeasures such as alkali or vitamin supplements.

Procedure

Each rat specimen (*Sprague dawley*) was mounted on the suspension apparatus at UAMS. (See *figure 7.*) They were suspended for a period of three weeks. Each rat was weighed before suspension and just before termination. There were four sets of experimental groups, divided by their diets and whether the ovaries were removed. The groups were labeled as high soy, ovariectomized; regular, ovariectomized; high soy-intact; regular-intact. The control rats, which were not suspended, were kept in cages. The same classification was used on the control rats as for the suspended rats.

Bone mineral densities (BMD's) were taken by UAMS researchers before and after suspension on the groups of rats using a Lunar Corporation Piximus Bone Density instrument. The left and right femur, as well as the spine, of each rat was measured for BMD values.

Serum samples were taken from the rats upon termination. Each specimen had its blood removed by direct injection into the heart. The blood was centrifuged for 15-20 minutes at 8000 rpm. The subsequent serum samples were immediately refrigerated for later analysis.

Calcium content in each serum sample was analyzed using a Perkin-Elmer atomic absorption (AA) spectrophotometer. Protocols for the AA called for the use of lanthanum to precipitate out of solution any phosphate compounds that could interfere with analysis. A solution with a 1:50 dilution of sample to lanthanum was required. For use in this particular AA, a small amount of solution was needed, so 40 μL of serum was diluted with 1.97 mL lanthanum.

The calcium-magnesium hollow cathode lamp was set to record at 422.7 nm. Five dilutions were made for each sample. Each solution was analyzed and an average value was obtained. Additionally, during each measurement, the AA was set to record seven values and

average them before giving a final reading.

The AA was standardized using solutions made at calcium concentrations of 1 mg/L and 3 mg/L. The instrument was evaluated on its accuracy at measuring the known standards after each five samples were analyzed. If the value drifted more than 0.02 mg/L, the instrument was re-standardized.

To obtain the mg% of calcium for each rat, the average of the measurements was calculated and used in the following formula:

$$(\text{sample conc. mg/L}) * (50) / (1000\ \text{L/ml}) * (100) = \text{Ca mg\%}$$

BMD results were analyzed to obtain the percent difference of BMD in each rat. The mean and standard deviation of each group was calculated. The BMD and serum calcium level of each rat was analyzed together to determine the link between a change in calcium levels and the loss or gain of bone minerals.

Results

The first group of serum samples received from UAMS researchers were of rats that had no treatments. This group was used to determine the ability of the AA to record calcium concentration. This group was labeled as "standard," with the following results:

ID	Exp. Group	Ca Conc. (mg/L)	Ca mg%
#1	standard	1.88	9.40
#2	standard	1.99	9.95
#3	standard	2.15	10.75
#4	standard	2.10	10.50

The mean of the Ca mg% was calculated to be 10.15 mg%.

The second set of samples received were suspended for a period of three weeks. Some rats in this group had their ovaries removed and some were on special diets. The weight of each rat before and after suspension was recorded. This is also the group that the BMD's were recorded. The following table shows the weight balance in each rat, as well as the experimental procedures performed on each rat. "HS-ovx" classifies rats that received high soy diets and were ovariectomized. "Reg-ovx" signifies rats that received regular diets and were ovariectomized. "Reg-intact" represents those rats that received regular diets and had their ovaries intact.

ID	Group	Weight(i) (g)	Weight(f) (g)	∆ Weight (g)
1S	HS-ovx	329.60	295.70	-33.90
2S	HS-ovx	303.70	276.70	-27.00
3S	HS-ovx	322.60	299.20	-23.40
4S	Reg-ovx	312.40	282.90	-29.50
5S	Reg-intact	308.90	223.90	-85.00
6S	Reg-intact	271.00	257.30	-13.70

For these same rats (1S-6S), ionic calcium concentration was analyzed using their serum samples. Each sample was measured up to five times, depending on the amount of serum available. The results are tabulated in the following chart.

ID	Group	#1 (mg/L)	#2 (mg/L)	#3 (mg/L)	#4 (mg/L)	#5 (mg/L)	Mean	Ca mg%
1S	HS-ovx	1.63	1.70	1.72	1.59	1.54	1.64	8.18
2S	HS-ovx	1.92	2.02	2.09			2.01	10.05
3S	HS-ovx	1.54	1.68	1.65	1.52	1.52	1.58	7.91
4S	Reg-ovx	1.38	1.43	1.27	1.43	1.40	1.38	6.91
5S	Reg-intact	1.67	1.82	1.72			1.74	8.68
6S	Reg-intact	1.37	1.40	1.52	1.47	1.45	1.44	7.21

The mean of the HS-ovx Ca mg% was calculated to be 8.71 mg%. The reg-ovx rat, being the only one analyzed, was 6.91 mg%. The Reg-intact group had a mean of 7.95 mg%.

Further experiments were conducted throughout the semester on several more groups of rats. However, BMD's were not obtained for this particular suspension. The data in the following tables reflect the calcium levels obtained through AA analysis. (Note: "LS-intact" represents rats that received a low soy diet and had their ovaries intact.)

ID	Group	#1 (mg/L)	#2 (mg/L)	#3 (mg/L)	#4 (mg/L)	#5 (mg/L)	Mean	Ca mg%
7S	HS-ovx	1.57	1.57	1.48	1.47	1.44	1.51	7.53
8S	HS-ovx	1.29	1.27	1.25	1.16	1.24	1.24	6.21
9S	HS-ovx	0.86	1.08	1.06	1.07	0.95	1.00	5.02
10S	HS-ovx	1.29	1.54	1.35	1.38	1.45	1.40	7.01
11S	LS-intact	1.52	2.46	2.47	1.68	1.45	1.92	9.58
12S	LS-intact	2.57	2.53	1.40	1.64	3.30	2.29	11.44
13S	LS-intact	1.47	1.91	1.67	1.55	2.33	1.79	8.93

For rats 11S-13S, there was an error in the feeding schedule for these rats. Instead of high soy, they were given low soy diets. The mean of calcium concentration for rats 7S-10S was calculated to be 6.44 mg%, while the mean for the LS-intact group (11S-13S) was 9.98 mg%.

The final group of rats were used as controls. These rats were not suspended. The next table gives the results for their analysis.

ID	Group	#1 (mg/L)	#2 (mg/L)	#3 (mg/L)	#4 (mg/L)	#5 (mg/L)	Mean	Ca mg%
1C	HS-ovx	1.14	1.50	1.34	1.52	1.48	1.40	6.98
2C	LS-intact	1.67	1.63	1.63	1.58	1.65	1.63	8.16
3C	LS-intact	1.35	1.52	1.75	1.70	1.63	1.59	7.95
4C	LS-intact	1.54	1.81	1.61	1.59	1.76	1.66	8.31
5C	reg-intact	1.53	1.64	1.61	1.61	1.52	1.58	7.91
6C	reg-intact	1.52	1.63	1.59	1.66	1.55	1.59	7.95
7C	reg-intact	1.65	1.64	1.57	1.55	1.54	1.59	7.95

The mean of the control HS-ovx is simply the one measurement taken. The mean calcium concentration of the LS-intact control group was calculated to be 8.14 mg%. The reg-intact control group had an average of 7.94 mg%.

BMD data was obtained from the researchers at UAMS. Each rat, labeled with ID numbers 1S through 6S, had its right femur, left femur, and spine analyzed before and after suspension. Using an electronic spreadsheet, the difference in bone mineral content (BMC), not BMD, was calculated, and from that data, the percent difference was obtained. The mean and standard deviation from each group was obtained from statistical analysis. *Table 1* arranges these data into two groups, one for the femurs and one for the spines. Graphical analysis shows the mean percent difference in BMC for each group (*graph 1*).

The data in *Table 1* shows the BMC before and after suspension. The data was grouped into the same treatment groups classified for the serum calcium study (i.e., HS-ovx, reg-ovx, reg-intact.) Further, femur and spine data were separated.

The final value was subtracted from the initial value of BMC to give the difference. The percent difference was calculated by dividing the difference by the initial value and multiplying by 100. The mean percent difference was calculated by averaging the initial and final values. A negative value reflected a loss in BMC and a positive value represented some gain.

The standard deviation for each group was calculated using the electronic spreadsheet features.

Table 1-Statistical analysis of bone mineral content for femurs and spines before(i) and after(f) suspension

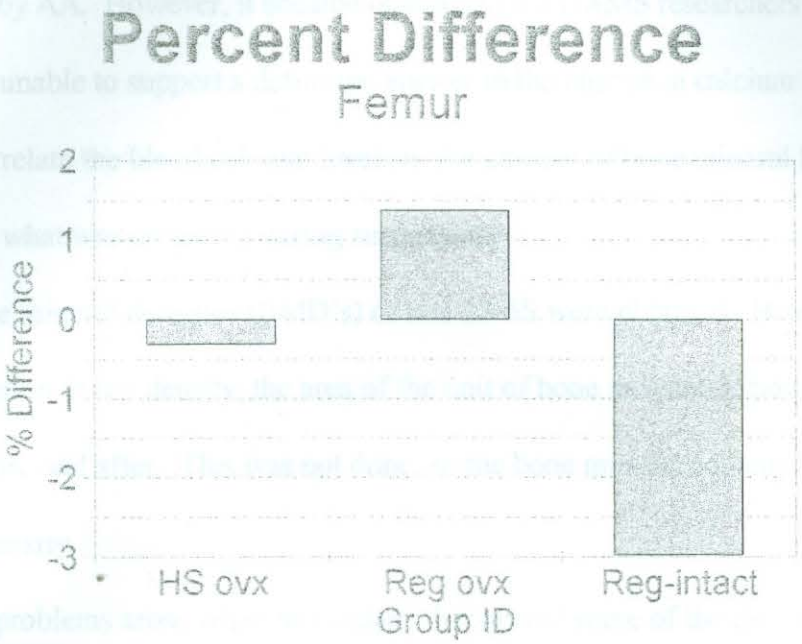
ID	Group	ROI	BMC (i)	BMC (f)	Δ BMC	% Δ
1S	HS-ovx	right leg	0.464	0.486	0.022	4.741
		left leg	0.472	0.465	-0.007	-1.483
2S	HS-ovx	right leg	0.452	0.471	0.019	4.204
		left leg	0.467	0.446	-0.021	-4.497
3S	HS-ovx	right leg	0.528	0.507	-0.021	-3.977
		left leg	0.513	0.512	-0.001	-0.195
		AVG:	0.483	0.481	-0.001	-0.311
4S	Reg-ovx	right leg	0.434	0.434	0.000	0.000
		left leg	0.438	0.450	0.012	2.740
		AVG:	0.436	0.442	0.006	1.376
5S	Reg-intact	right leg	0.537	0.578	0.041	7.635
		left leg	0.572	0.566	-0.006	-1.049
6S	Reg-intact	right leg	0.606	0.550	-0.056	-9.241
		left leg	0.536	0.558	0.022	4.104
		AVG:	0.571	0.554	-0.017	-2.977

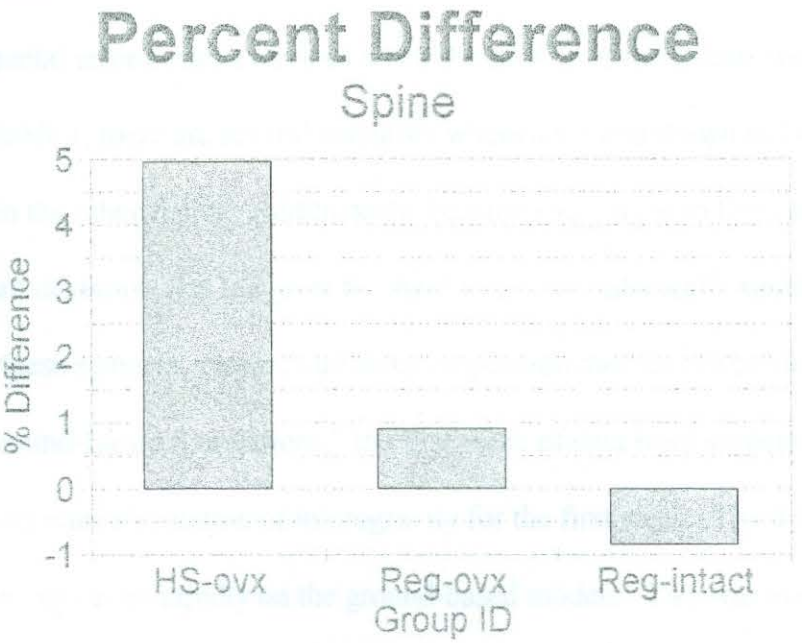
	ROI	Mean	Std Dev
HS ovx	femur	-0.311	3.954
Reg ovx	femur	1.376	1.937
Reg-intact	femur	-2.977	7.328

ID	Group	ROI	BMC (i)	BMC (f)	Δ BMC	% Δ
1S	HS-ovx	spine	0.460	0.461	0.001	0.217
2S	HS-ovx	spine	0.379	0.377	-0.002	-0.528
3S	HS-ovx	spine	0.450	0.515	0.065	14.444
		AVG:	0.430	0.451	0.021	4.965
4S	Reg-ovx	spine	0.438	0.442	0.004	0.913
5S	Reg-intact	spine	0.531	0.551	0.020	3.766
6S	Reg-intact	spine	0.526	0.497	-0.029	-5.513
		AVG:	0.528	0.524	-0.004	-0.851

ID	ROI	Mean	Std Dev
HS-ovx	spine	4.965	8.437
Reg-ovx	spine	0.913	
Reg-intact	spine	-0.851	6.562

Graph 1-Mean percent difference in bone mineral contents for a) femurs and b) spines of suspended rats





Discussion

When the experiment first began, the only data to be collected was the serum calcium levels obtained by AA. However, it became obvious to the UAMS researchers that this data alone would be unable to support a definitive answer to the change in calcium levels. It became necessary to correlate the blood calcium levels to the amount of bone mineral lost or gained in order to predict what was occurring during suspension.

The bone mineral densities (BMD's) of rats 1S-6S were obtained. However, to make an accurate description of the density, the area of the unit of bone measured should be the same when taken before and after. This was not done, so the bone mineral content (BMC) was used instead of the density.

Further problems arose when researchers discovered some of the rats were able to slip or chew off their suspension apparati. Unable to determine how long a rat was off the model, much of the data retrieved had to be discarded.

Experimental error became obvious when the BMC's of the femurs were calculated. As can be seen in *Table 1*, there are several instances where a rat was shown to lose BMC in one femur but gain in the other femur. Additionally, because there were so few variables to work with, the standard deviation was too great for there to be any statistically significant data.

Despite these setbacks, research utilizing suspension models can be successful. As discussed in "Ground-Based Simulations," the first week of data from suspension has been shown to be an accurate simulation of microgravity for the first week. The difference is that mineralization occurs more rapidly on the ground-based models. This was evidenced in the rats that were able to fall off their apparati.

The group used as standards had a mean calcium level of 10.15 mg%, a value within the normal range of 9-11 mg% calcium in serum. This provided the evidence needed to show the AA could properly measure calcium in serum samples.

The first set of experimental samples actually decreased from the standard value, which was the opposite of what was expected to occur. However, given the experimental groups of rats used for the specific UAMS experiment (i.e., ovariectomized rats, soy diets), this was taken in stride until controls were obtained.

Further experimental rats were analyzed. These were supposed to be similar to the first group obtained, but an error in the feeding schedule actually gave researchers a new group to study. Instead of a high soy diet, a low soy diet was fed to rats 11S-13S, as well as 2C-4C.

The control rats received similar treatments to their counterparts, the difference being that the controls were not suspended. The following chart compiles the experimental and control data.

Group	Control Value (mg%)	Experimental Value (mg%)
HS-ovx	6.98	8.71
Reg-ovx	no data available	6.91
Reg-intact	7.94	7.95
LS-intact	8.14	9.98

Unfortunately, because the deviation for the BMC data was so large, this data could not be accurately correlated with the change in calcium serum levels as UAMS researchers needed. The relationship hypothesized was that any change in BMC greater than 2% would show a

change in calcium serum levels. At first glance, it was obvious this link could not be made. For instance, for the femurs of reg-intact, there was a -2.977% difference in BMC, yet calcium levels remained consistent.

Discarding the BMC data, the serum calcium levels could still be used to predict what occurred during suspension. The HS-ovx group did have an increase in serum calcium, supporting the hypothesis of this experiment. Likewise, the LS-intact group's serum calcium increased. A control sample was not obtained for reg-ovx. There was no significant change in the reg-intact group.

Data from various surveys of astronauts shows that the results in this experiment are reasonable, despite the complications. It should be noted that these case studies are of humans, while the ground-based suspension was on rats. Therefore, only predictions can be made to assist in setting up additional experiments.

Three groups of astronauts were analyzed for their bone mineral density during the Skylab program. The groups lived in orbit for 28, 59, and 84 days respectively. Diet was regulated up to 20 days before flight and 20 days after flight. Blood, urine, and fecal samples were collected throughout the duration of the experiments. Researchers found that serum calcium and phosphorus increased for the nine astronauts analyzed. BMD was reported at an average 4-7% loss in the Skylab missions. The observation was that bone resorption exceeded formation, increasing the serum calcium levels, which in turn, deregulated the parathyroid gland to secrete less PTH.[1]

This experiment attempted to show the same relationship as the Skylab research. Diets were controlled, blood samples were taken, and BMD's were analyzed. Despite the inability to

use the BMC data in this experiment, it is still predicted that a loss of bone mineral density increased serum calcium levels in the rats.

A case study on a female astronaut was done by the National Space Development Agency of Japan, who collaborated with NASA to collect medical baseline data on a short flight project. BMD, blood, and urine were analyzed for this research. Interestingly, the female subject had serum levels that decreased. A male, also analyzed for this project, had no significant changes. Both subjects had increased urinary calcium up to one week after re-entry. BMD changes were only noted on weight bearing bones. Researchers concluded that calcium balance depends on calcium intake, absorption, excretion, and hormone regulation.[12]

While the excised ovaries were not actually variables for this experiment, it is interesting to note that the "ovx" rats had a lower serum calcium level than the "intact" rats. The mean of the two "ovx" groups was 7.81 mg%, while the "intact" group's average was 8.97 mg%. This suggested there was less demineralization in the "ovx" rats, which is contrary to what might be thought to occur. Given the relationship between menopause (which for this example will be taken as the same as having ovaries removed) and osteoporosis, it would be predicted that the calcium serum would be higher in "ovx" rats than in "intact." Again, the experimental errors may have obstructed this data.

While the goal of this experiment was simply to reproduce data known about bone mineralization and spaceflight, many years of research are necessary to fully understand and prevent bone loss in space. A Research Roundtable was organized, in conjunction with NASA, to define research strategies to prevent or minimize bone loss. The American College of Sports Medicine, who played the central role on this Roundtable, suggested that these activities be a

part of astronaut fitness:

- motor skills to maintain posture and locomotor function
- heavy resistance training for lower extremities and trunk
- impact exercises that strain the bone
- aerobic activity to maintain cardiovascular conditioning.[2]

Further, the Roundtable suggested these research strategies:

- determination of rates and magnitude of bone loss and recovery, including whether individual variation affects these values
- defining the endocrine profile on long term flights, including an investigation of reproductive hormones and mechanical stress on bone loss
- development of noninvasive techniques to measure bone strength, including the characterization of strain related to specific movement and cellular signals that relate to bone loss
- determination to what extent astronauts have adequate intake of calcium, phosphorus, vitamin D, magnesium, and protein necessary for bone structuring[2]

The hypothesis of this experiment was that calcium serum levels increase as a result of bone demineralization. While there were many setbacks, the research showed that there was an increase in serum calcium of suspended rats. The BMC data was to provide a reason for this increase, but the data proved to be insufficient. While a rat model cannot be a true simulation of what occurs in humans during spaceflight, this experiment provides a strong basis to begin further research. Additional experimentation is necessary to study the effects of hormone regulation and any countermeasures to prevent excessive bone loss in spaceflight.

References

1. Arnaud, Sara Bond; Morey-Holton, Emily R. "Skeletal Responses to Spaceflight" Advances in Space Biology and Medicine. Volume 1, 1991.

2. Baldwin, Kenneth M.; White, Timothy P.; et al. "Musculoskeletal adaptations to weightlessness and development of effective countermeasures." Medicine and Science in Sports and Exercise. Volume 10, 1996.

3. Burke, Thomas J.; Landauer, James A. "A Proposed Cause for and Prevention of Bone and Muscle Wasting in Microgravity" Aviation, Space and Environmental Medicine. July 1998.

4. Edgerton, Victor R.; Greenisen, Michael C. "Human Capabilities in the Space Environment" Space Physiology and Medicine. Third Edition. Lea & Febriger, 1993.

5. Goode, Anthony. "Musculoskeletal change during spaceflight: a new view of an old problem" British Journal of Sports Medicine. June 1999.

6. Guyton, Arthur C. Textbook of Medical Physiology. Ch. 44, "Aviation, Space, and Deep Sea Diving Physiology" W.B. Saunders Co., 1986.

7. Guyton, Arthur C. Textbook of Medical Physiology. Ch. 79, "Parathyroid Hormone, Calcitonin, Calcium and Phosphate Metabolism, Vitamin D, Bone and Teeth" W.B. Saunders Co., 1986.

8. Holick, M.F. "Perspective on the Impact of Weightlessness on Calcium and Bone Metabolism" Bone. May 1998.

9. Huntoon, Carolyn Leach; Nicogossian, Arnauld E.; Sawin, Charles F. "Overall Physiological Response to Space Flight" Space Physiology and Medicine. Third Edition. Lea & Febriger, 1993.

10. Leblanc, Adrian D.; Schneider, Victor S.; Taggart, Linda C. "Bone and Mineral Metabolism" Space Physiology and Medicine. Third Edition. Lea & Febriger, 1993.

11. Marieb, Elaine N. Human Anatomy and Physiology. Chapter 6, "Bone and Skeletal Tissues." Benjamin/Cummings Science Publishings, 1998.

12. Miyamoto, Akira; et al. "Medical Baseline Data Collection on Bone and Muscle Change with Space Flight." Bone. May 1998.

13. Nicogossian, Arnauld E.; Pool, Sam L.; Uri, John J. "Historical Perspectives" Space Physiology and Medicine. Third Edition. Lea & Febriger, 1993.

14. Nicogossian, Arnauld E.; Robbins, Donald E. "Characteristics of the Space Environment" Space Physiology and Medicine. Third Edition. Lea & Febriger, 1993.

15. Powell, Michael R.; Sauer, Richard L.; Waligora, James M. "Spacecraft Life Support Systems" Space Physiology and Medicine. Third Edition. Lea & Febriger, 1993.

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