

# MOLECULAR THERAPIES FOR DISUSE OSTEOPOROSIS

Ted A. Bateman

*Department of Bioengineering, Clemson University, Clemson, SC*

## ABSTRACT

Microgravity causes changes in physiological systems that are both detrimental to human health and valuable for biomedical research. Some of the most pronounced and long-term changes occur in skeletal tissue, which experiences a profound and rapid wasting. Finding a countermeasure to the bone atrophy associated with weightlessness is necessary before long-duration human space exploration can be possible. However, these physiological changes can also be exploited as a biomedical model for osteoporosis, offering an extreme environment in which therapeutics can be tested and mechanisms examined. Utilizing space as a biomedical test-bed has been done on several flights: STS-41, 52, 57, 60, 62, 63, 77 and 108, the aims and results of which will be briefly summarized. The rationale for spaceflight serving as a biomedical test-bed is that microgravity exposure (and resulting changes in the spacecraft environment) causes an accelerated model for biomedical disorders experienced, often as a result of the normal aging process, here on Earth. The most common target system for these flights was skeletal, with the goal of mimicking osteoporosis, but immune dysfunction, wound healing and muscle atrophy were also studied. Most recently (STS-108, December 2001), the biotechnology company Amgen examined the ability of osteoprotegerin (OPG) to mitigate the osteoporosis caused by microgravity. OPG is a protein that is critical to the differentiation and activation of bone resorbing osteoclasts. Amgen is developing OPG as a treatment for osteoporosis and the bone loss associated with metastatic bone cancer. Over the 12-day flight, the mice experienced a decline in bone strength (15-20% relative to ground controls) that was greater than that of ground-based disuse models. The mechanical testing data was complimented by serum, mRNA and histological analyses that indicated a decline in bone formation and an increase in bone resorption in addition to an inhibition of mineralization. OPG mitigated the decline in mechanical strength by preventing the increase in resorption and maintaining mineralization.

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## INTRODUCTION

Osteoporosis is a disease that is defined generally as a reduction in the amount of bone mass leading to fractures after minimal trauma. Clinical diagnosis is made from bone densitometry scans demonstrating a density 2.5 standard deviations below the mean peak bone mass of Caucasian women. It is often called a 'silent disease' because patients do not recognize symptoms until a fracture occurs. It is the increased risk for fracture that is of primary concern and the consequences are generally underestimated by society.

There are 1.5 million osteoporotic fractures every year ([www.nof.org](http://www.nof.org)). The most common sites are at the

vertebra, wrist and femoral neck. A femoral neck, or hip fracture, in people over 50 years of age leads to a significantly increased risk of death. The reduction in quality of life after even a minor fracture can be considerable and permanent.

More than 44 million Americans, nearly 80% of whom are women, are at risk for osteoporosis. Half of all women, and a quarter of all men over the age of 50 will have an osteoporotic fracture in her/his remaining life. Osteoporosis is generally under diagnosed, in part because reliable therapies have only recently been available. The bisphosphonate alendronate (Fosamax, Merck) was approved to treat osteoporosis in 1997. Several other therapies have since been approved and others are in various stages of development.

Osteoporosis has multiple pathologies. The most common cause, and the reason why four out of five people with osteoporosis are women, is the natural decline in hormone levels post-menopause, especially estrogen. This results in a sharp, but temporary decline in bone mass, lasting typically 5-7 years. Low testosterone levels can also cause osteoporosis in men (Gruenewald and Matsumoto, 2003). Metabolic disorders of the thyroid and parathyroid glands can cause osteoporosis (Burman, 1997; Rodan, 2002). Poor diet and lack of exercise during development can lead to a less than optimum 'peak bone mass', resulting in a greater risk for osteoporosis decades later (Entrala-Bueno et al., 2003).

Osteoclasts remove old, damaged bone and osteoblasts replace it with new, better bone. The activity of these two cell types is generally coupled. Through cytokine signaling, a direct increase or decrease in the activity level of one cell type will lead to an indirect change in the other cell type in the same direction. There are, however, some pathologies for osteoporosis that uncouple osteoclast and osteoblast activity resulting in a particularly serious decline in bone mass and strength. An example of this is glucocorticoid (steroid) induced osteoporosis, where both osteoclast activity is increased and osteoblast activity is reduced (Nishimura and Ikuyama, 2000).

## DISUSE OSTEOPOROSIS IN HUMANS

Another example of uncoupled osteoclast and osteoblast activity occurs in disuse osteoporosis. The unloading of bone results in the physiological application of Wolff's Law. Simply stated, Wolff observed that mechanical stresses dictate the architecture of bone. The osteoblast and osteoclast activities that occur with bone remodeling change when the forces placed on the skeleton change (Frost, 2001). One example of disuse osteoporosis is bone loss in astronauts.

Disuse osteoporosis is not restricted to spaceflight, but can also be caused by extended periods of bed rest and spinal cord injury/nerve damage. The extent of disuse osteoporosis caused by long-term space flight has not

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\* Correspondence to: Ted A. Bateman

Department of Bioengineering, Clemson University  
501 Rhodes Research Center, Clemson, SC 29678

Email: [bateman@clemson.edu](mailto:bateman@clemson.edu)

Phone: 864-656-0180; FAX: 864-656-4466

been demonstrated with the current, relatively short duration space flights. The degree of bone loss experienced by spinal cord injury (SCI) patients may be our best indicator of the potential quantity of bone loss for an astronaut exposed to micro- and reduced gravity on a multi-year trip to Mars and back. SCI causes declines in total skeletal mass of >30% and localized declines in bone mineral content of 50% in load bearing bones have been reported, leading to increased risk for fractures, even though the patients are generally immobile (Frisbie, 1997).

The microgravity environment of space deleteriously affects the skeleton, resulting in long-term demineralization of load-bearing bones, for example, the os calcis (Tilton et al., 1980; Rambaut and Johnston, 1979). International Space Station (ISS) studies confirm the results from Skylab studies showing vertebral and proximal femur bone mineral loss ranging from 0.9-1.6% per month. Increased serum and urine markers for bone resorption have been consistently observed (Caillot-Augusseau et al., 1998; Smith et al., 1998; Smith et al., 1999). Bone formation may also be reduced in spaceflight, though not as definitively (Smith et al., 1999), causing an uncoupling effect on formation and resorption. This combination does not result in greater levels of serum calcium (clinical hypercalcemia), but it does lead to increased calcium excretion in urine and feces (Whitson et al., 1997). Additionally, calcium absorption by the gut is reduced during spaceflight, resulting in a negative calcium balance of ~250mg/day (Rambaut and Johnston, 1979; Smith et al., 1999). Rambaut and Johnson extrapolated some of the Skylab results and estimated a loss of ~25% of skeletal calcium (300g) in a year. An extrapolation of the negative calcium balance determined by Smith et al. suggests a more modest loss of 100g of calcium in a year, which still is considerably more than that demonstrated in bed-rest studies (Smith et al., 1998). What is of more concern is that the recovery period following spaceflight that is estimated to be 2-3 times longer than the period of microgravity exposure (Smith et al., 1999; Tilton et al., 1980).

#### **ANIMAL MODELS FOR DISUSE OSTEOPOROSIS**

Flying laboratory animals in space is important to supplementing data collected from astronauts to characterize the effects of microgravity on physiological systems. A considerable body of research shows that a lack of weighted loading produces a number of effects on the rat skeletal system. Although growing rats exposed to spaceflight experience a reduction in bone formation (Morey and Baylink, 1978; Spengler et al., 1983), there is little effect on resorption. In rats exposed to spaceflight, skeletal changes include a significant decrease in bone formation in the tibia (Jee et al., 1983; Vico et al., 1988), with similar alterations of bone formation observed in humerus trabecular and periosteal bone (Vico et al., 1988). This reduced bone formation results in compromised mechanical properties, including diminished femur torsional strength (Spengler et al.,

1983) and lowered humerus and tibia flexural strength and stiffness (Shaw et al., 1988).

In order to further increase the amount of spaceflight-related bone research, ground-based models for the skeletal unloading component of spaceflight have been developed. Antiorthostatic (back-harnessing, hindlimb suspension) unloading of rats was designed (Morey, 1979) as a means for modeling important aspects of spaceflight in an experimental model. Later modified for use in mice (Simske et al., 2003; Simske et al., 1992), this model has been used extensively in both rats and mice (Morey-Holton and Globus, 2002). Nerve damage models are also used to cause disuse osteoporosis (Bateman et al., 2001; Kodama et al., 1999; Simske et al., 1994; Wang et al., 2001a), resulting in a bone formation/resorption balance that may be more similar to what astronauts experience in microgravity. [Portions of this section from (Simske and Bateman, 1996)].

#### **SPACEFLIGHT AS A BIOMEDICAL TEST BED**

Because of the accelerated nature of the osteoporosis caused by spaceflight, it has the potential to be an ideal, controlled environment to test therapeutics for terrestrial osteoporosis. However, until human spaceflight becomes routine, experimentation in humans (except in the context of developing countermeasures) is not likely. Nonetheless, spaceflight biomedical models for human diseases do hold promise with rodents, and have been used on several occasions.

These first disease model experiments were performed by the Center for Cell Research (CCR) at Penn State University, a NASA Center for the Commercial Development of Space (CCDS). On STS-41, CCR partnered with the biotechnology company, Genentech (South San Francisco, CA), to examine the effects of human growth hormone (hGH) on skeletal and muscle properties (Cronin et al., 1992; Jiang et al., 1993; Turner, 1995). This was a short flight (four days) and neither spaceflight nor GH had an effect on skeletal properties, while spaceflight did result in a decline in soleus mass. A proprietary bone protein being developed as a treatment for osteoporosis was the target of an experiment sponsored by Merck (Whitehouse Station, NJ) on STS-52 (Backup et al., 1994; Turner et al., 1995). The CCR also flew rats on STS-57 to examine the combined effects of platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) on wound healing (Davidson et al., 1999). These investigators concluded that spaceflight inhibits the ability of these growth factors to promote wound healing. Another experiment with the STS-57 rats demonstrated site specific differences in expression of skeletal mRNA for TGF-beta during spaceflight (Westerlind and Turner, 1995).

On STS-62, in addition to basic-science investigations (Day et al., 1998; Kraemer et al., 2004), the combined effect of spaceflight and ovariectomy was examined. Results suggested that estrogen modifies the skeletal response to spaceflight (Cavolina et al., 1997b). The

combined effects of a naturally occurring osteoporosis, such as post-menopause or age related osteoporosis, and spaceflight on the skeletal system is particularly applicable and important to examine. They are unavoidably combined with spaceflight-induced osteoporosis in astronauts and should be characterized in long-duration studies.

The NASA Research Partnership Center (formerly CCDS) BioServe Space Technologies, at the University of Colorado, has coordinated the examination of commercial molecular therapeutics on STS-60, STS-63, STS-77 and STS-108. The first three flights were sponsored by Chiron Corporation (Emeryville, CA) and the most recent flight by Amgen Inc. (Thousand Oaks, CA). Experiments on both STS-60 and STS-63 evaluated the ability of interleukin-2 to mitigate immune system deficiencies caused by spaceflight (Chapes et al., 1999b). On STS-77, insulin-like growth factor-I and the immuno-skeletal complex were evaluated (Bateman et al., 1998; Chapes et al., 1999a; Pecaut et al., 2000). On STS-108, the first flight in which adult mice were examined, the efficacy of the anti-resorptive protein osteoprotegerin to mitigate spaceflight-induced bone loss was studied (Bateman et al., 2002; Kostenuik et al., 2002). Other systems were studied with placebo treated mice (Gridley et al., 2003; Harrison et al., 2003; Pecaut et al., 2003). The results from this flight will be discussed briefly in this paper.

#### **MOUSE AND RAT MODELS IN SPACE**

Some recent experiments have shown few effects of spaceflight on rat bone (Bateman et al., 1998; Wronski et al., 1998). It has been hypothesized that young, group-housed rats may not experience the same reduction in bone formation (Bikle and Halloran, 1999; Morey-Holton et al., 2000; Wronski et al., 1998) that has been experienced (1) in young singly-housed rats (Morey and Baylink, 1978) or (2) in older (12-18 weeks old) group-housed rats (Arnaud et al., 1992; Cavolina et al., 1997a). This is a reasonable hypothesis, particularly when one considers that rats in the Animal Enclosure Module (AEM) are relatively crowded. This crowding allows normal social activity to load the skeletal system in space, and AEM housed ground controls to be hypoactive, effectively unloading the skeletal system. In young rats, which gained nearly 50% in body mass during the period of spaceflight (Bateman et al., 1998; Wronski et al., 1998), the pre-programmed bone growth combined with the social activity-induced loading may override factors that influence bone formation in older rats. It is logical to eventually fly adult animals on ISS for longer durations. However, until the hardware is available for ISS research, short-duration Shuttle flights will have to form our basis for pre-clinical spaceflight models for disuse osteoporosis.

Spaceflight experiments with rodents have almost exclusively been done with rats. With the exception of STS-90, where neonatal mice were examined (Hayes, 1999) by sacrificing the dams three and six days into the flight, STS-108 was the only other flight in which mice

have been flown on the Space Shuttle. This is primarily because of concerns about the more odiferous pheromones in mouse urine compared to rats, the details of which can be read elsewhere (Dalton et al., 2003)

Some of the benefits of flying mice in space are not immediately obvious. On earth, it is generally not a concern how much oxygen laboratory animals consume, or how much carbon dioxide they produce as waste. However, in the closed environment of any spacecraft, calculating these resources is very important. Mice require less volume and mass (including food and water consumed), produce less metabolic heat, consume less oxygen, require fewer lithium hydroxide canisters be flown to remove carbon dioxide and call for less crew time (for food and water replacement). In fact, mice need lower quantities of all resources than rats and allow larger sample sizes (n value). The main drawback to their use is their greater odor compared to rats. Improved sensitivity of analysis techniques, such as serum chemistry, have largely eliminated the need for the larger sample aliquots that rats provide. Additionally, genetic techniques are generally more advanced for mice (Gunter and Dhand, 2002).

Which species is better for spaceflight experimentation? The answer is that both are fundamentally important. Research and countermeasure development on ISS will be lacking until both species are available. For example, rats are generally a better model for toxicological and behavioral studies, whereas mice are the standard model in the fields of immunology and radiation. It is not simply a matter of modifying existing models for the other species, but in many cases, one may mimic human physiology better than the other. Within a given field, one species may be preferable over another depending on the biomedical disorder. For example, rats are likely a better model for post-menopausal osteoporosis (because the estrogen response is more similar to that of humans) which, combined with spaceflight-induced osteoporosis, is important to understand (Turner, 1999). The combination of spaceflight and age related osteoporosis is equally important, and relevant for both genders. Models for Type II osteoporosis have been characterized in both mice and rats (Banu et al., 2002; Ferguson et al., 2003). However, the rat model appears to be specific to male Sprague-Dawley (SD) rats (Wang et al., 2001b), and not viable in the smaller F344 rat. Mature male SD rats are large, greater than 500 grams in mass, limiting the number of rats per habitat to four or five (based on pre-flight biomass limitations of 1800 grams for Animal Enclosure Hardware and 2400 grams for the Advanced Animal Habitat), rather than eight (AEM) to twelve (AAH) mice. Therefore, the benefits of much greater statistical power (and fewer consumable resources) in mice make them a better, more practical species for spaceflight examinations of age related osteoporosis.

#### **COMMERCIAL BIOMEDICAL TESTING MODULE**

Space Shuttle flight STS-108 (December 2001) was the first opportunity to analyze the skeletal system of space-

flown mice. The payload, Commercial Biomedical Testing Module (CBTM-01), sponsored by the biotechnology company Amgen, had the goal of examining the efficacy of the protein osteoprotegerin (OPG) to prevent the expected spaceflight-induced osteopenia. OPG is a naturally circulating protein that prevents osteoclastic differentiation, development and survival by serving as a decoy receptor to RANK ligand (Hsu et al., 1999; Lacey et al., 1998; Simonet et al., 1997; Takahashi et al., 1999; Yasuda et al., 1998). The 24 flight mice (half treated with OPG) were ten weeks old at launch. This age was chosen since large changes in development are limited (the mice were expected to gain approximately 5% body mass in two weeks) but measurable bone formation could occur during the course of the experiment. Ground controls included both vivarium and AEM (flight hardware) housed mice.

A brief summary of the results are as follows (Bateman et al., 2002; Kostenuik et al., 2002). Spaceflight caused a decline in femur elastic strength of 15-20% compared to AEM and vivarium housed placebo controls. OPG treatment in spaceflight mice nearly reversed this decline in strength. This spaceflight-induced decline in femoral strength was caused by three mechanisms: reduced bone formation, increased bone resorption, both affecting bone structural properties, and an inhibition of mineralization, affecting bone material properties.

The decline in bone formation in placebo treated spaceflight mice was observed by quantitative histomorphometry, serum chemistry and mRNA expression. Bone formation rates were reduced 40-60% compared to AEM and vivarium controls. Serum levels of alkaline phosphatase and mRNA expression of osteocalcin in the diaphysis of humeri were also reduced. OPG, because of osteoclast/osteoblast coupling, also had the effect of reducing bone formation.

The increase in bone resorption does not appear to be as clear as the decline in bone formation. Serum levels of tartrate resistant acid phosphatase (TRAP) were increased compared to both sets of ground controls, while mRNA expression of RANK ligand was significantly increased compared to vivarium controls, but not AEM controls. Messenger RNA expression of OPG was not changed by spaceflight. OPG largely reversed the increase in bone resorption.

Spaceflight did not have a significant effect on the organic constituent of femur mass. Spaceflight did, however, have a large effect on the mineral component of bone. This effect was large enough that the percent mineral composition at the femur epiphysis (greater component of trabecular bone) was significantly less in placebo treated spaceflight mice compared to baseline controls. This suggests that spaceflight affected a decline in bone material properties compared to preflight conditions.

Data from non-skeletal tissues were also collected from the placebo treated mice to examine the effects of spaceflight on other physiological systems. Changes to the immune system are summarized in a Journal of

Applied Physiology commentary (Sieck, 2003), followed by the complete articles (Gridley et al., 2003; Pecaut et al., 2003), identifying spaceflight induced reductions in interleukin-2 and the development of anemia. Atrophy of calf muscle tissue was also examined (Harrison et al., 2003), identifying a fiber type dependent reduction in cross-sectional area of 15-30% and declines of citrate synthase activity in spaceflight mice. Other tissues are currently being examined, including microarray analysis of quadriceps (the University of Colorado, Clemson University and the Medical University of South Carolina), kidney and liver (Tulane University, NASA Ames Research Center and Loma Linda University) and morphological examination of the vestibular system (The Jackson Laboratories). Other tissues are available for analysis.

## CONCLUSION

The scientific community will have exciting, new opportunities later this decade as animal research becomes a reality on the ISS. Though the emphasis of ISS research is changing to support the new exploration initiative with countermeasure development, there are still opportunities for commercial examination of molecular therapies and basic research. These three components of pre-clinical study in space (countermeasure development, commercial biomedical model exploitation and basic science) are not mutually exclusive. The development of countermeasures for bone loss and other biomedical disorders caused by spaceflight can (and should) involve the commercial developers of the therapies. These companies have an understanding of their compounds that, when applied to a unique population (otherwise healthy astronauts), will be invaluable. They often have analysis tools developed specifically for a particular disease and therapy. Basic research is fundamental to explaining the novel physiological changes caused by spaceflight. It is not enough to simply find a countermeasure for a functional change, but the root molecular causes of the functional changes need to be fully characterized too so that therapies may be continually modified and improved: today's basic research is tomorrow's applied research. In total, if future opportunities are maximized to their full potential, spaceflight experiments with rodents can provide not only an opportunity to test countermeasures, but also a platform to test therapeutics in an accelerated, extreme environment and identify the molecular causes for the changes. On STS-108, an osteoporosis caused by reduced bone formation, an increase in bone resorption and an inhibition of mineralization provides three mechanisms to be identified and exploited. Alterations to the immune and skeletal muscle systems were also identified to be caused by spaceflight in mice, with other tissues yet to be examined.

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## REFERENCES

- Arnaud, S. B., Fung, P., Popova, I. A., Morey-Holton, E. R. and Grindeland, R. E. (1992). Circulating parathyroid hormone and calcitonin in rats after spaceflight. *J. Appl. Phys.* 73, 169S-173S.
- Backup, P., Westerlind, K., Harris, S., Spelsberg, T., Kline, B. and Turner, R. (1994). Spaceflight results in reduced mRNA levels for tissue-specific proteins in the musculoskeletal system. *Am J Physiol* 266, E567-73.
- Banu, J., Wang, L. and Kalu, D. N. (2002). Age-related changes in bone mineral content and density in intact male F344 rats. *Bone* 30, 125-30.
- Bateman, T. A., Dunstan, C. R., Lacey, D. L., Ferguson, V. L., Ayers, R. A. and Simske, S. J. (2001). Osteoprotegerin ameliorates sciatic nerve crush induced bone loss. *Journal of Orthopaedic Research* 19, 518-523.
- Bateman, T. A., Morony, S., Ferguson, V. L., Simske, S. J., Lacey, D. L., Warmington, K. S., Geng, Z., Tan, H. L., Shalhoub, V., Dunstan, C. R. et al. (2002). Osteoprotegerin mitigates spaceflight-induced changes in mouse bone mass, density and mineral composition. *Journal of Bone and Mineral Research* 17, S136-S136.
- Bateman, T. A., Zimmerman, R. J., Ayers, R. A., Ferguson, V. L., Chapes, S. K. and Simske, S. J. (1998). Histomorphometric, physical, and mechanical effects of spaceflight and insulin-like growth factor-I on rat long bones. *Bone* 23, 527-35.
- Bikle, D. D. and Halloran, B. P. (1999). The response of bone to unloading. *J Bone Miner Metab* 17, 233-44.
- Burman, K. D. (1997). Thyroid disease and osteoporosis. *Hosp Pract (Off Ed)* 32, 71-3, 78-85; discussion 85-6.
- Caillot-Augusseau, A., Lafage-Proust, M. H., Soler, C., Pernod, J., Dubois, F. and Alexandre, C. (1998). Bone formation and resorption biological markers in cosmonauts during and after a 180-day space flight (Euromir 95). *Clin Chem* 44, 578-85.
- Cavolina, J. M., Evans, G. L., A., H. S., Zhang, M., Westerlind, K. C. and Turner, R. T. (1997a). The effects of orbital spaceflight on bone histomorphometry and messenger ribonucleic acid levels for bone matrix proteins and skeletal signaling peptides in ovariectomized growing rats. *Endocrinology* 138, 1567-1576.
- Cavolina, J. M., Evans, G. L., Harris, S. A., Zhang, M., Westerlind, K. C. and Turner, R. T. (1997b). The effects of orbital spaceflight on bone histomorphometry and messenger ribonucleic acid levels for bone matrix proteins and skeletal signaling peptides in ovariectomized growing rats. *Endocrinology* 138, 1567-76.
- Chapes, S. K., Simske, S. J., Forsman, A. D., Bateman, T. A. and Zimmerman, R. J. (1999a). Effects of space flight and IGF-1 on immune function. *Adv Space Res* 23, 1955-64.
- Chapes, S. K., Simske, S. J., Sonnenfeld, G., Miller, E. S. and Zimmerman, R. J. (1999b). Effects of spaceflight and PEG-IL-2 on rat physiological and immunological responses. *J Appl Physiol* 86, 2065-76.
- Cronin, M., Battersby, J., Hancock, W., Schwall, R. and Clark, R. (1992). Delivery of recombinant human growth hormone to rats during exposure to microgravity on NASA Space Shuttle Discovery. *Physiologist* 35, S51-2.
- Dalton, P., Gould, M., Girten, B., Stodieck, L. and Bateman, T. A. (2003). Preventing annoyance from odors in spaceflight: a method for evaluating the sensory impact of rodent housing. *J Appl Physiol*.
- Davidson, J. M., Aquino, A. M., Woodward, S. C. and Wilfinger, W. W. (1999). Sustained microgravity reduces intrinsic wound healing and growth factor responses in the rat. *Faseb J* 13, 325-9.
- Day, J. R., Frank, A. T., O'Callaghan, J. P. and DeHart, B. W. (1998). Effects of microgravity and bone morphogenetic protein II on GFAP in rat brain. *J Appl Physiol* 85, 716-22.
- Entrala-Bueno, A., Iglesias, C. and de Jesus, F. (2003). Diet and physical activity: a healthful binomial. *Eur J Clin Nutr* 57 Suppl 1, S63-5.
- Ferguson, V. L., Ayers, R. A., Bateman, T. A. and Simske, S. J. (2003). Bone development and age-related bone loss in male C57BL/6J mice. *Bone* 33, 387-98.
- Frisbie, J. H. (1997). Fractures after myelopathy: the risk quantified. *J Spinal Cord Med* 20, 66-9.
- Frost, H. M. (2001). From Wolff's law to the Utah paradigm: insights about bone physiology and its clinical applications. *Anat Rec* 262, 398-419.
- Gridley, D. S., Nelson, G. A., Peters, L. L., Kostenuik, P. J., Bateman, T. A., Morony, S., Stodieck, L. S., Lacey, D. L., Simske, S. J. and Pecaute, M. J. (2003). Genetic models in applied physiology: selected contribution: effects of spaceflight on immunity in the C57BL/6 mouse. II. Activation, cytokines, erythrocytes, and platelets. *J Appl Physiol* 94, 2095-103.
- Gruenewald, D. A. and Matsumoto, A. M. (2003). Testosterone supplementation therapy for older men: potential benefits and risks. *J Am Geriatr Soc* 51, 101-15; discussion 115.

- Gunter, C. and Dhand, R. (2002). Human biology by proxy. *Nature* 420, 509.
- Harrison, B. C., Allen, D. L., Girten, B., Stodieck, L. S., Kostenuik, P. J., Bateman, T. A., Morony, S., Lacey, D. and Leinwand, L. A. (2003). Skeletal muscle adaptations to microgravity exposure in the mouse. *J Appl Physiol* 95, 2462-70.
- Hayes, N., Nowakowski, (1999). Cell proliferation, nucleotide metabolism and cell death in the developing telencephalon are affected by space flight. *Soc Neurosci Abstr* 25, 256.
- Hsu, H., Lacey, D. L., Dunstan, C. R., Solovyev, I., Colombero, A., Timms, E., Tan, H. L., Elliott, G., Kelley, M. J., Sarosi, I. et al. (1999). Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. *Proc Natl Acad Sci U S A* 96, 3540-5.
- Jee, W. S. S., Wronski, T. J., Morey, E. R. and Kimmel, D. B. (1983). Effects of spaceflight on trabecular bone in rats. *Am J Physiol* 244, R310-R314.
- Jiang, B., Roy, R. R., Navarro, C. and Edgerton, V. R. (1993). Absence of a growth hormone effect on rat soleus atrophy during a 4-day spaceflight. *J Appl Physiol* 74, 527-31.
- Kodama, Y., Dimai, H. P., Wergedal, J., Sheng, M., Malpe, R., Kutilek, S., Beamer, W., Donahue, L. R., Rosen, C., Baylink, D. J. et al. (1999). Cortical tibial bone volume in two strains of mice: effects of sciatic neurectomy and genetic regulation of bone response to mechanical loading. *Bone* 25, 183-90.
- Kostenuik, P. J., Bateman, T. A., Morony, A., Warmington, K. S., Geng, Z., Simske, S. J., Ferguson, V. L., Dunstan, C. R. and Lacey, D. L. (2002). OPG prevents relative osteopenia and deficits in skeletal strength in mice during a 12 day spaceflight. *Journal of Bone and Mineral Research* 17, S209-S209.
- Kraemer, W. J., Mastro, A. M., Gordon, S. E., Koziris, L. P., Bush, J. A., Volek, J. S., Staron, R. S., French, D. N., Sharman, M. J., Jemiolo, B. et al. (2004). Responses of plasma proenkephalin peptide F in rats following 14 days of spaceflight. *Aviat Space Environ Med* 75, 114-7.
- Lacey, D. L., Timms, E., Tan, H. L., Kelley, M. J., Dunstan, C. R., Burgess, T., Elliott, R., Colombero, A., Elliott, G., Scully, S. et al. (1998). Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 93, 165-76.
- Morey, E. R. (1979). Spaceflight and bone turnover-correlation with a new rat model of weightlessness. *Bioscience* 29, 168-172.
- Morey, E. R. and Baylink, D. J. (1978). Inhibition of bone formation during space flight. *Science* 201, 1138-41.
- Morey-Holton, E. R. and Globus, R. K. (2002). Hindlimb unloading rodent model: technical aspects. *J Appl Physiol* 92, 1367-77.
- Morey-Holton, E. R., Halloran, B. P., Garetto, L. P. and Doty, S. B. (2000). Animal housing influences the response of bone to spaceflight in juvenile rats. *J Appl Physiol* 88, 1303-9.
- Nishimura, J. and Ikuyama, S. (2000). Glucocorticoid-induced osteoporosis: pathogenesis and management. *J Bone Miner Metab* 18, 350-2.
- Pecaut, M. J., Nelson, G. A., Peters, L. L., Kostenuik, P. J., Bateman, T. A., Morony, S., Stodieck, L. S., Lacey, D. L., Simske, S. J. and Gridley, D. S. (2003). Genetic models in applied physiology: selected contribution: effects of spaceflight on immunity in the C57BL/6 mouse. I. Immune population distributions. *J Appl Physiol* 94, 2085-94.
- Pecaut, M. J., Simske, S. J. and Fleshner, M. (2000). Spaceflight induces changes in splenocyte subpopulations: effectiveness of ground-based models. *Am J Physiol Regul Integr Comp Physiol* 279, R2072-8.
- Rambaut, P. C. and Johnston, R. S. (1979). Prolonged weightlessness and calcium loss in man. *Acta Astronautica* 6, 1113-1122.
- Rodan, G. A. (2002). Bisphosphonates and primary hyperparathyroidism. *J Bone Miner Res* 17 Suppl 2, N150-3.
- Shaw, S. R., Vailas, A. C., Grindeland, R. E. and Zernicke, R. F. (1988). Effects of a 1-wk spaceflight on morphological and mechanical properties of growing bone. *Am J Physiol* 254, R78-83.
- Sieck, G. C. (2003). Highlighted Topics: Commentary. *J Appl Physiol* 94, 2084-2084.
- Simonet, W. S., Lacey, D. L., Dunstan, C. R., Kelley, M., Chang, M.-S., Lüthy, R., Nguyen, H. Q., Wooden, S., Bennett, L., Boone, T. et al. (1997). Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 89, 309-319.
- Simske, S. J. and Bateman, T. A. (1996). Targeted cytokine therapy for prevention of spaceflight-induced bone changes. *NRA 96-HEDS-04*, 6-7.
- Simske, S. J., Ferguson, V. L. and Bateman, T. A. (2003). Mice and osteoporosis research. In *Recent Research Developments in Biotechnology and Bioengineering Vol. 5*, (ed. S. Pandalai), pp. 97-127. Kerala, India: Research Signpost.
- Simske, S. J., Guerra, K. M., Greenberg, A. R. and Luttgies, M. W. (1992). The physical and mechanical effects of suspension-induced osteopenia on mouse long bones. *J Biomech* 25, 489-99.
- Simske, S. J., Schmeister, T. A., Fleet, M. L., Broz, J. J., Gayles, E. C. and Luttgies, M. W. (1994). An experimental model for combined neural, muscular, and skeletal degeneration. *J. of Neuromusculoskeletal Systems* 2, 116-123.
- Smith, S. M., Nillen, J. L., Leblanc, A., Lipton, A., Demers, L. M., Lane, H. W. and Leach, C. S. (1998).

- Collagen cross-link excretion during space flight and bed rest. *J Clin Endocrinol Metab* 83, 3584-91.
- Smith, S. M., Wastney, M. E., Morukov, B. V., Larina, I. M., Nyquist, L. E., Abrams, S. A., Taran, E. N., Shih, C. Y., Nillen, J. L., Davis-Street, J. E. et al. (1999). Calcium metabolism before, during, and after a 3-mo spaceflight: kinetic and biochemical changes. *Am J Physiol* 277, R1-10.
- Spengler, D. M., Morey, E. R., Carter, D. R., Turner, R. T. and Baylink, D. J. (1983). Effects of spaceflight on structural and material strength of growing bone. *Proc Soc Exp Biol Med* 174, 224-8.
- Takahashi, N., Udagawa, N. and Suda, T. (1999). A new member of tumor necrosis factor ligand family, ODF/OPGL/TRANCE/RANKL, regulates osteoclast differentiation and function. *Biochem Biophys Res Commun* 256, 449-55.
- Tilton, F. E., Degioanni, J. J. and Schneider, V. S. (1980). Long-term follow-up of Skylab bone demineralization. *Aviat Space Environ Med* 51, 1209-13.
- Turner, R. T. (1995). Effects of short-term spaceflight and recombinant human growth hormone (rhGH) on bone growth in young rats. *Aviat Space Environ Med* 66, 763-9.
- Turner, R. T. (1999). Mice, estrogen, and postmenopausal osteoporosis. *J Bone Miner Res* 14, 187-91.
- Turner, R. T., Evans, G. L. and Wakley, G. K. (1995). Spaceflight results in depressed cancellous bone formation in rat humeri. *Aviat Space Environ Med* 66, 770-4.
- Vico, L., Chappard, D., Palle, S., Bakulin, A. V., Novikov, V. E. and Alexandre, C. (1988). Trabecular bone remodeling after seven days of weightlessness exposure (BIOCOSMOS 1667). *Am J Physiol* 255, R243-7.
- Wang, H., Ferguson, V. L., Simske, S. J. and Bateman, T. A. (2001a). Comparison of two nerve damage disuse models in mice: Sciatic nerve crush and sciatic neurectomy. *Journal of Bone and Mineral Research* 16, S484-S484.
- Wang, L., Banu, J., McMahan, C. A. and Kalu, D. N. (2001b). Male rodent model of age-related bone loss in men. *Bone* 29, 141-8.
- Westerlind, K. C. and Turner, R. T. (1995). The skeletal effects of spaceflight in growing rats: tissue-specific alterations in mRNA levels for TGF-beta. *J Bone Miner Res* 10, 843-8.
- Whitson, P. A., Pietrzyk, R. A. and Pak, C. Y. (1997). Renal stone risk assessment during Space Shuttle flights. *J Urol* 158, 2305-10.
- Wronski, T. J., Li, M., Shen, Y., Miller, S. C., Bowman, B. M., Kostenuik, P. and Halloran, B. P. (1998). Lack of effect of spaceflight on bone mass and bone formation in group-housed rats. *J Appl Physiol* 85, 279-85.
- Yasuda, H., Shima, N., Nakagawa, N., Yamaguchi, K., Kinosaki, M., Mochizuki, S., Tomoyasu, A., Yano, K., Goto, M., Murakami, A. et al. (1998). Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci U S A* 95, 3597-602.

