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The Current State of Bone Loss Research: Data From Spaceflight and Microgravity Simulators

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ABSTRACT

Bone loss is a well documented phenomenon occurring in humans both in short- and in long-term spaceflights. This phenomenon can be also reproduced on the ground in human and animals and also modeled in cell-based analogs. Since space flights are infrequent and expensive to study the biomedical effects of microgravity on the human body, much of the known pathology of bone loss comes from experimental studies. The most commonly used in vitro simulators of microgravity are clinostats while in vivo simulators include the bed rest studies in humans and hindlimb unloading experiments in animals. Despite the numerous reports that have documented bone loss in wide ranges in multiple crew members, the pathology remains a key concern and development of effective countermeasures is still a major task. Thus far, the offered modalities have not shown much success in preventing or alleviating bone loss in astronauts and cosmonauts. The objective of this review is to capture the most recent research on bone loss from spaceflights, bed rest and hindlimb unloading, and in vitro studies utilizing cellular models in clinostats. Additionally, this review offers projections on where the research has to focus to ensure the most rapid development of effective countermeasures. J. Cell. Biochem. 114: 1001–1008, 2013.

KEY WORDS: BONE LOSS; SPACEFLIGHT; DISUSE; ASTRONAUTS; OSTEOPOROSIS

The 50-year history of humans in space was started by the Russian launch of Yuri Gagarin in April of 1961 and the American launch of Alan Shepherd in May of 1961. Thereafter, a growing number of flights were accomplished (Gemini, Apollo, Apollo-Soyuz, Skylab, Mir, Space Shuttle, and the International Space Station). The impact of such an extreme environment on the human body became apparent. Though bone loss is the most prominent and most well recognized phenomenon, it is only one of many changes the body endures during and after space travel. For example, the neurovestibular system reacts by causing dizziness and increased intracranial pressure, the muscular system experiences atrophy, and the immune system response is weakened [McCarthy, 2005; Kramer et al., 2012]. For the years since the first human-based spaceflight, the field of microgravity research has expanded greatly, shedding some light on common and individual changes. Today, with the International Space Station (ISS) and astronauts from numerous countries including the United States, Japan, the European Union, and Russia, scientists have the ability to decipher long-term effects of microgravity on the human body. This prospective research is critical for the advancement of human-based spaceflight to non-Low Earth Orbit (LEO) missions such as to asteroids, Lagrange points, the Lunar surface, and Mars. Most recently, NASA successfully completed an unmanned robotic mission by landing its largest rover to date on Mars. This accomplishment is perhaps an omen to future human space travel. For such missions to be successful, scientists must improve the understanding of the different effects of space environment on human health and performance to be able to develop effective countermeasures. This requires critical assessment of the current state of the field.

This review is focused on the impacts to the skeletal system, which has been shown to closely mimic osteoporosis. Osteoporosis is a well-known condition in which there is a decrease in bone mass and signs of altered bone microarchitecture. Such skeletal status is vulnerable to fracture upon impact. Susceptibility to fractures could be a limiting factor in sending humans to non-LEO missions without adequate countermeasures. While bone loss in astronauts resembles osteoporosis, the pathology occurs more quickly with exposure to microgravity than during aging. Osteoporosis is classified into two main forms: primary and secondary osteoporosis. Primary osteoporosis is usually associated with aging and decreased gonad function, such as lower levels of estrogen. Secondary osteoporosis is caused by extraneous health problems or environments [Lau and Guo, 2011]. Bone loss in astronauts is primarily related to that of disuse, and thus can be classified as secondary osteoporosis. Maintaining bone mass depends on a balance between bone formation by the

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osteoblast and bone resorption by the osteoclast during normal bone remodeling cycles. These tightly coupled processes of remodeling can be perturbed by spaceflight, resulting in a decrease in bone mass [Lau and Guo, 2011].

Disuse osteoporosis is largely a regional phenomenon in lower limbs because of the vast decrease in weight bearing. In normal conditions, these bones are exposed to mechanical stimulations during daily movement provided by static gravity-induced weight-bearing, opposite and equal ground reaction forces, and dynamic loading generated by muscular contractions during locomotion. As such, exercise is essential for increasing or maintaining bone mass and strength on Earth. For example, 1 year of weight training showed higher bone mineral density (BMD) in the trochanter and femoral neck in postmenopausal women when compared to a control non-weight training group [Milliken et al., 2006]. In spaceflight, exercise has largely been ineffective in counteracting bone loss [Hawkey, 2003; Cavanagh et al., 2005; Davis and Davis, 2012]. However, recent resistive devices have shown promise, when coupled with dietary countermeasures, to attenuate BMD deficits [Smith et al., 2012].

The data resulting from microgravity-based research is generally obtained in four main ways. The first is data from the limited number of actual spaceflight missions. The second approach is using human subjects to induce disuse in a technique called the bed rest model, noted by the 6° tilt down position mimicking the fluid shift seen in spaceflight [Zwart et al., 2007]. The third system is an animal model using the hindlimb unloading method, noted by the elevation of the hindlimbs to cause a similar fluid shift [Gupta et al., 2012]. Lastly, cell-based studies are accomplished through clinostats, namely the Rotating Wall Vessel (RWV) and Random Positioning Machine (RPM) [Pardo et al., 2005; Patel, 2007]. These machines rely on suspension of particles or the constant movement of the gravity vector relative to the cells in such a way that the cells are unable to sense gravity. In this review, key findings in the microgravity-induced bone loss field in response to each of these conditions are consolidated to give researchers the current status of the field.

BONE LOSS RESPONSE IN SPACEFLIGHT

It is well documented that spaceflight induces a decrease in bone mass in both humans and animals after prolonged stay in space. There is a vast amount of individual variation in the data available from human studies as a result of a relatively small sample size, leaving it difficult to draw definitive conclusions. However, a few patterns remain fairly consistent through the years of human-based space travel. Namely, there is an increase in bone resorption and decrease in bone formation. In spaceflight missions aboard the ISS, seven of eight astronauts experienced decreased BMD in the range of 2.5–10.6% in the lumbar vertebrae. All eight astronauts experienced a loss of total BMD in the range of 3–10% in the femur and four of the eight had 1.7–10% loss in the femoral neck [Kozlovskaya and Grigoriev, 2004]. In another study on astronauts from the Skylab missions, there was an increase in bone resorption markers due to exposure to spaceflight for 28–84 days. There was a steady increase in urinary excretion of collagen breakdown products during spaceflight and a recovery after landing [Smith et al., 1998]. Additionally, in another study, astronauts who flew 2–6 months on the ISS showed that in-flight and post-flight vitamin K1 concentrations were unchanged from the preflight mean. Consistently, urinary excretion of γ-carboxyglutamic acid (GLA), which is a measure of vitamin K-dependent protein turnover, did not change in response to flight. Also serum undercarboxylated osteocalcin, which is also a measure of vitamin K function, was generally unchanged in response to flight in these astronauts [Zwart et al., 2011].

There is some data from historical programs such as Mir and the Space Shuttle. In the Mir missions, one cosmonaut experienced a 7.74% decrease in bone mass of the calcaneus by 1 month as well as 2.27% in the tibial trabecular bone, as measured by broadband ultrasound attenuation (BUA) [Collet et al., 1997]. After 6 months exposure to microgravity, another cosmonaut lost 4.5% of trabecular bone and 2.9% of cortical bone in the tibia compared to measurements before launch [Collet et al., 1997]. After 6 months of return to Earth's gravitational field, there was no difference between pre-flight and post-flight bone mass in the cortical bone and still a 2.55% decrease in the trabecular bone mass, suggesting a site-specific partial recovery [Collet et al., 1997]. Both cosmonauts showed a trend toward decreased bone formation markers, including osteocalcin (OCN), bone alkaline phosphatase (ALP), and the C-terminal peptide of pro-collagen type 1 (PICP) during spaceflight. There was an increase in PICP and a decrease in OCN post-flight [Collet et al., 1997]. Additionally, there was a trend towards an increase in two bone resorption markers during flight [Collet et al., 1997]. One of the Mir missions that involved four European astronauts showed no change before, during, and after launch in stress-related hormones, insulin growth factor 1 (IGF-1) and cortisol, in three astronauts while one experienced an increase in cortisol before launch [Caillot-Augusseau et al., 1998]. Collective data on BMD in various locations of the skeleton for 16–18 astronauts presented in Figure 1 [LeBlanc et al., 2000]. These data confirm the changes stated above. In another study, parathyroid hormone (PTH) decreased during flight compared to pre-flight levels in one cosmonaut. Initially during post-flight, PTH remained within

Variable	N	%/Month	SD
BMD Spine	18	-1.06*	0.63
BMD Neck	18	-1.15*	0.84
BMD Troch	18	-1.56*	0.99
BMD Total	17	-0.35*	0.25
BMD Pelvis	17	-1.35*	0.54
BMD Arm	17	-0.04	0.88
BMD Leg	16	-0.34*	0.33
Lean Total	17	-0.57*	0.62
Lean Leg	16	-1.00*	0.73
Lean Arm	17	0.00	0.77
Fat Total	17	+1.79	4.66

*p<0.01

Fig. 1. Average BMD and lean muscle data for 16–18 astronauts for various locations of the musculoskeletal system [LeBlanc et al., 2000].

the normal range and then sharply increased for a short time, eventually returning to normal levels. In another cosmonaut, PTH did not change during flight but increased above normal range after flight and returned to normal levels after 1 week [Caillot-Augusseau et al., 1998]. Additionally, bone resorption can be marked by the breakdown of collagen, including the products N-telopeptide (NTX), pyridinium (PYD), and deoxypyridinoline (DPD). In these cosmonauts, the bone resorption markers, with the exception of pyridinium, were increased during flight compared to pre-flight. These markers decreased post-flight, with the exception of one astronaut who experienced a sharp, unexplainable increase in pyridinium [Caillot-Augusseau et al., 1998].

While long-term spaceflight poses the most imminent danger to astronauts, some short-term spaceflight missions aboard the US Space Shuttle have also induced changes in skeletal remodeling. A mission of 8–15 days induced a 3% decrease in the lumbar vertebrae BMD while the BMD increased in the calvarius post-flight compared to pre-flight [Miyamoto et al., 1998]. Both astronauts experienced elevated urinary calcium excretion after flight compared to pre-flight, which eventually corrected itself, but calcium regulators such as PTH, calcitriol, and calcitonin did not change [Miyamoto et al., 1998]. Elevated bone resorption markers were observed in the urine, and bone formation, as marked by total and bone-specific ALP, decreased in both astronauts 1 week after flight [Miyamoto et al., 1998]. There was no significant change in total BMD over the whole body by X-ray measurements in both astronauts, but a trend towards a decrease in BMD in lumbar spine and increase in the calvarius were observed [Miyamoto et al., 1998]. Perhaps, these observations could be explained by redirection of bone formation due to microgravity and may be related to the cephalic fluid shift associated with spaceflight.

In rodent animal studies, one study investigated the alterations of bone microarchitecture in wild type and pleiotrophin–transgenic mice exposed to microgravity on the ISS for 91 days. Pleiotrophin (PTN) was selected because of its positive effects on bone turnover. The study revealed a bone loss phenotype during spaceflight in both wild type and PTN-transgenic mice in the form of a decrease in trabecular number and an increase in mean separation of trabecular architecture. However, trabecular thickness did not change when compared to controls on Earth [Tavella et al., 2012]. In another study, 17 days of spaceflight altered the biomechanics of femur bones, mostly concentrated on tissue properties rather than bone structure [Vajda et al., 2001]. Spaceflight did not affect maximum stress tolerance of the femur but did decrease the flexural rigidity compared to ground controls [Vajda et al., 2001]. There was no change in cortical bone mass, but endocortical bone resorption was decreased along with a trend towards decreased bone formation [Vajda et al., 2001]. To test whether changes in bone due to spaceflight exposure could impact ossification of new bone, growing rats were exposed to 11 days of spaceflight. There was no change in the width or longitudinal growth rate of the tibial growth plate [Sibonga et al., 2000]. Other studies have shown that 16 days of spaceflight decreased mineral content in the distal section of the femur diaphysis, which correlated to reduced *type I collagen* [Arnaud et al., 2000]. In some of the earliest observations of spaceflight, it was found that after nearly 20 days in space, rats suffered a drastic decrease in periosteal bone formation with no change in bone resorption [Morey and Baylink, 1978]. After 26 days of post-flight, the loss of bone mass was regained [Morey and Baylink, 1978]. This phenomenon was observed in another flight where rats exposed to nearly 19 days of spaceflight experienced an inhibition of periosteal bone formation in the tibial and humeral diaphyses and subsequent correction post-flight [Wronski and Morey, 1983]. These data collectively suggest that spaceflight alters bone remodeling in animal models.

These reported results indicate that regardless of significant individual variability in the astronauts' physiological response to microgravity there is a pattern of an increase in bone resorption and a decrease in bone formation, leading to site-specific loss of BMD. Furthermore, animals exposed to spaceflight also experience bone loss phenotype which proves the adequacy of animal models in addressing different aspects of bone remodeling. This could advance the bone loss research since conducting such studies is much easier and definitely less costly than sending humans in space.

BONE LOSS RESPONSE IN BED REST STUDIES

Bed rest studies are currently the only human-based ground analog to microgravity. Subjects are required to remain in bed at a 6°-degree head-down tilt from weeks to months in time. Subjects perform all daily functions including eating and sleeping in bed, and cameras are placed in discreet places to ensure that subjects do not deviate from the protocol.

A potential countermeasure for microgravity-induced alterations in the cardiovascular system consists of treadmill exercise in a lower body negative pressure (LBNP) chamber. A LBNP environment creates a hypergravity load on the lower body, causing both mechanical and cardiovascular adaptation, and it is used to simulate orthostatic stress by unloading of the arterial and cardiopulmonary baroreceptors [Brown et al., 2003]. This countermeasure has recently been evaluated on bone response in identical twins. In one study using male identical twins, collagen cross-links and serum and urinary calcium concentrations, both measurements marking bone resorption, increased during 30 days of bed rest in non-exercise control subjects compared to the LBNP group [Smith et al., 2003]. There was a smaller increase in pyridinium collagen cross-links above pre-bed rest levels in the LBNP group compared to the increase in the control group. In this study, there was no change in the markers of bone formation in both groups [Smith et al., 2003]. They concluded that LBNP exercise partially mitigated bone loss as marked by decreased bone resorption [Smith et al., 2003]. In a follow-up study, female identical twins were subjected to similar conditions. Bone resorption markers were excreted in both control and LBNP groups throughout the 30 days of bed rest, and bone formation markers showed either no significant change or a tendency towards decreased expression [Zwart et al., 2007]. The exercise group showed less urinary calcium and helical peptide excretion than the control group, and the BMD in the femoral shaft and total hip was not different in the exercise group after bed rest compared to their pre-bed rest values while the control group had decreased BMD [Zwart et al., 2007]. Therefore, LBNP had a smaller

protective effect on bone mass loss in women than men, signifying that the impact of disuse may be gender-specific. In another study, ten healthy males were exposed to 35 days of bed rest and five additional males were control subjects. In this study, subjects experienced decreased muscle strength in the knee and hip, and bed rest caused atrophy in the extensor muscles of the gluteus, thigh, calf, and knee. Bone density was decreased in the proximal tibia and was not recaptured after 4 weeks of recovery time that included exercise. However, muscle mass and strength had partially recovered by exercise after 4 weeks [Berg et al., 2007]. Additionally, vitamin K has long been the subject of effective countermeasure research because of its important role in bone health on Earth. However, it has been show that vitamin K is not effective in spaceflight or human bed-rest studies [Zwart et al., 2011]. In another study, researchers showed that bone loss occurs more rapidly and dramatically in trabecular bone than cortical bone. In a 35 day bed rest trial, bone density was measured 2 weeks after exposure to the head-down tilt and confinement to a bed. There was reduction of bone mass in cancellous areas, with losses of 1% in the distal femur, 3% in the patella, and 2% in the distal tibia, and no changes in the distal radius [Rittweger et al., 2005]. Additionally, bone mass in the distal radius remained unchanged after 56 days and 90 days of bed rest, but bone mass in the distal tibia declined 3.6% and 6% correspondingly. Moreover, decreases of cortical bone thickness and density were below 2% after as long as 90 days bed rest [Rittweger et al., 2005].

In comparison to spaceflight, mineral loss and regional bone loss is similar in both environments. As bone is lost, there is a risk of kidney stones from the increased urinary calcium excretion in both bed rest and spaceflight [Jones, 2005]. The headward shift of blood and other fluids mimics the puffy faces seen in spaceflight, and after approximately 1 day, the body adapts to the increased volume by increased urination, as also observed in spaceflight. Additionally, bed rest subjects develop a mild vertigo, causing nausea and dizziness. Spaceflight microgravity is known to alter the neuro-vestibular system, where sensors in the ears and nerves in the soles of the feet are unbalanced, causing nausea and dizziness [Jones, 2005].

BONE LOSS RESPONSE IN HINDLIMB UNLOADING (HLU)

The rodent HLU animal model has been used to partially mimic aspects of microgravity exposure such as removal of skeletal weight-bearing loads and cephalic fluid shift. The hindlimbs are unloaded while the forelimbs remain physiologically loaded and used as internal controls. The head-down tilt from raising the hindlimbs provides the cephalic fluid shift, mimicking the situation observed in spaceflight. The HLU system applies minimal stress on the animal as noted by normal weight gain and eating habits of acclimated animals compared to controls [Morey-Holton and Globus, 1998].

One HLU study showed that deletion of destrin, an actin depolymerizing factor, resulted in enhanced BMD loss, including impact on trabecular thickness, trabecular number, and bone volume fraction of the femur when compared to control mice. The remodeling of the cytoskeleton through actin dynamics is essential for basic biological processes including bone formation [Shuang et al., 2012]. In another knockout study, bone specific connexin 43 (Cx43) deletion was used to assess impact on bone loss [Lloyd et al., 2012]. Cx43 is the most abundant gap junction protein in bone, and it is an integral component of skeletal homeostasis. The Cx43 knockout caused a baseline osteopenic phenotype in cortical bone including decreased cortical thickness, decreased BMD, and increased porosity with no impact to trabecular bone. After 3 weeks of HLU, wild type mice showed decreases in trabecular bone volume fraction, connectivity density, trabecular thickness, and trabecular tissue mineral density but the effects were attenuated in Cx43-knockout mice [Lloyd et al., 2012]. Additionally, an HLU study showed a small increase in serum calcium and a decrease in 1,25-dihydroxyvitamin D, and the levels returned to control after 5–15 days of unloading [Halloran et al., 1986]. There was no change in PTH in the serum in response to unloading [Globus et al., 1986a; Halloran et al., 1986]. The changes in fat-free bone mass depended on the type of bone, where unloading reduced the weights of both the tibia and vertebrae in the lumbar spine but not the humerus or vertebrae of the cervical spine [Globus et al., 1986b]. These findings suggest a decrease in calcium content of the bone [Globus et al., 1986b; Vico et al., 1995], and the mineralized matrix of the unloaded bones appeared to be more immature than control bones based on density-gradient fractionation studies [Bikle et al., 1987]. The decrease in bone mass was most likely due to a combination of changes in both bone formation and bone resorption. An indicator of bone formation is the radius of the periosteum, and this marker decreased with HLU. However, in these HLU studies, the radius of the endosteum did not change, marking the absence of a change in bone resorption [Globus et al., 1986a; van der Meulen et al., 1995]. HLU decreased the number of osteoblasts in the metaphysis of the tibia after 5 days, but this change was normalized by 14 days [Halloran et al., 1986; Wronski and Morey-Holton, 1987; Machwate et al., 1993]. Subsequently, there was a decrease in trabecular bone volume by 14 days of HLU in multiple independent studies as represented in Figure 2 [Halloran et al., 1986; Wronski and Morey-Holton, 1987; Vico et al., 1991; Machwate et al., 1994; Bloomfield et al., 2002]. These data suggest that HLU-related changes in rodents are partially due to changes in osteoblast function. However, after 14 days of unloading, there was no change in ALP activity [Machwate et al., 1993], but the mRNA levels for *transforming growth factor β-2 (TGFβ2)*, *insulin-like growth factor 2 (IGF2)*, and *osteopontin (OPN)*, all markers of bone formation, decreased in an independent study [Zhang et al., 1995]. There have been conflicting reports regarding HLU effects on osteoclast regulation and activity, depending on animal weight changes. If there was no difference between control and HLU animal weight, there was no change in bone resorption [Halloran et al., 1986; Machwate et al., 1994, 1995]. However, if there was a change in weight, osteoclast activity increased [Wronski and Morey-Holton, 1987; Vico et al., 1991, 1995].

Taken together, despite the variability among independent studies, these data show a pattern of bone loss induced by animal HLU. Comparison with spaceflight data shows that the HLU model

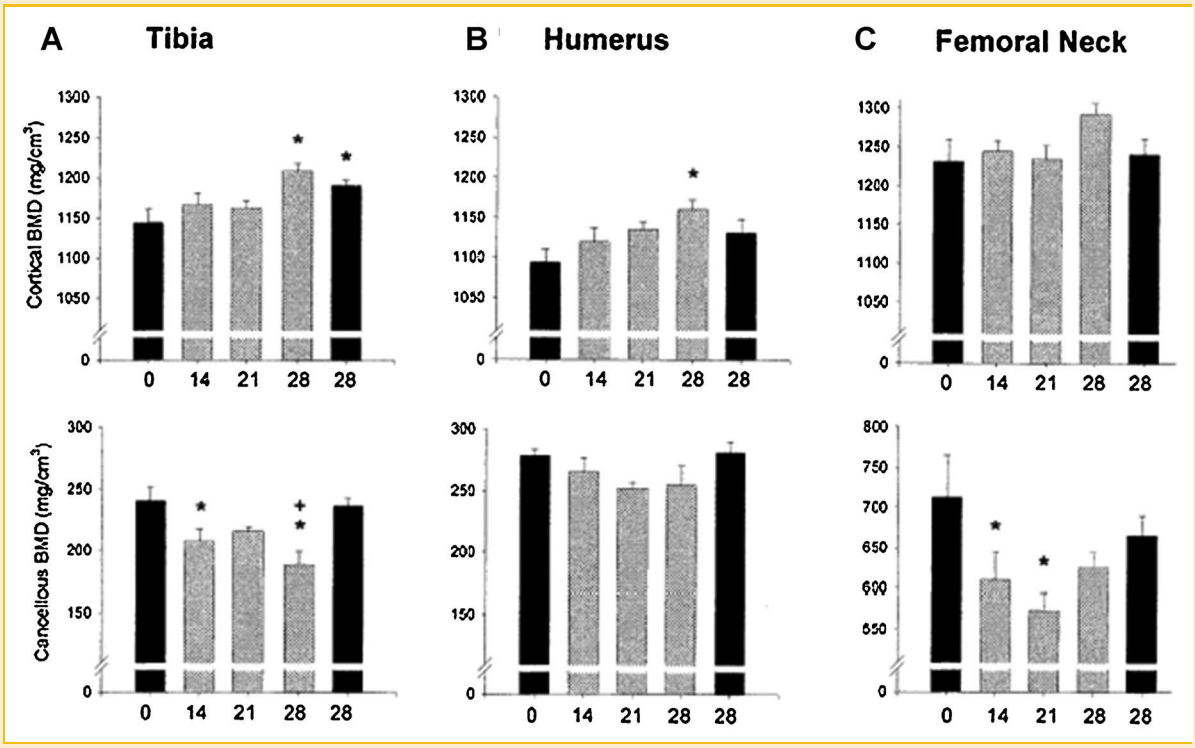

Fig. 2. Effects of HLU on BMD in the cortical and trabecular (cancellous) bone in the tibia (A), humerus (B), and femoral neck (C). *HLU (gray bar) significant compared to 0 day control (black bar), +HLU (gray bar) significant compared to 28 day control (black bar) [Bloomfield et al., 2002].

causes skeletal changes similar to spaceflight, with few differences [Vico et al., 1991; Bikle et al., 1994, 1997]. The model is most accurate and useful in studying the response of bone in short duration spaceflight. Spaceflight and HLU both decrease bone strength [Martin, 1990; Vailas et al., 1990], and reloading triggers bone formation rates to return to control both after stimulus exposure. Additionally, the model induces a similar cephalic fluid shift as observed in spaceflight. Although there is a significant similarity in the functional and structural changes in spaceflight and in HLU, it is important to note that spaceflight unloads the entire body whereas this model only unloads the hindlimbs [Morey-Holton and Globus, 1998, 2002].

BONE LOSS RESPONSE IN CLINOSTATS

A clinostat is a device that rotates around at least one axis with a platform that has a small enough radial distance to minimize centrifugal forces. Gravity still exists around the clinostat, but the gravity vector relative to the biological specimen on the clinostat is changing directions with the rotation. Over time, the gravity vector averages to a net zero force a method called gravity-vector averaging [Hoson, 1997; Huijser, 2000]. The RPM and the RWV are the two most commonly utilized clinostats for ground-based studies using cellular models.

Both the RWV [Ontiveros and McCabe, 2003; Zayzafoon et al., 2004; Ward et al., 2006; Patel, 2007] and the RPM [Nakamura et al., 2003; Pardo et al., 2005; Patel, 2007] have been previously used by various groups to assess the effects of microgravity or disuse on bone cells as well as on other cells and tissue constructs. Various markers of bone formation have been assessed using the two simulators, including *ALP*, *OCN*, matrix mineralization, and *runt homology domain transcription factor* (*runx2*). *ALP*, *OCN*, and matrix mineralization have been shown to decrease after exposure to the RWV in primary mouse calvariae [Zayzafoon et al., 2004], and *ALP*, *runx2*, and *OCN* decreased in human mesenchymal stem cells (hMSC), MC3T3 mouse pre-osteoblasts, and 2T3 mouse pre-osteoblasts compared to static controls [Ontiveros and McCabe, 2003; Zayzafoon et al., 2004; Patel, 2007]. In contrast, in ROS.SMER #14 rat osteoblast cells, RWV induced an increase in *ALP* and *OCN* expression [Rucci et al., 2002], signifying the dependence of clinostat results on species and cell type [Monici et al., 2006]. Additionally, destrin, an actin depolymerizing factor important in cytoskeletal biology, was upregulated in 2T3 cells when exposed to the RWV. Furthermore, RNAi-mediated knockdown of destrin enhanced the microgravity-induced downregulation of osteoblast proliferation and differentiation [Shuang et al., 2012].

Many of these results are comparable to findings obtained with the three-dimensional (3D) clinostat. For example, while cell morphology remains unchanged, alkaline phosphatase activity as a marker of bone formation in vitro decreases in a time dependent manner as shown in Figure 3 [Pardo et al., 2005]. Moreover, studies show that the gene expression for *ALP* and *runx2* as well as mineralization have been shown to decrease with exposure to the 3D clinostat compared to static controls [Nakamura et al., 2003; Yuge et al., 2003; Pardo et al., 2005; Patel et al., 2009]. There are a limited

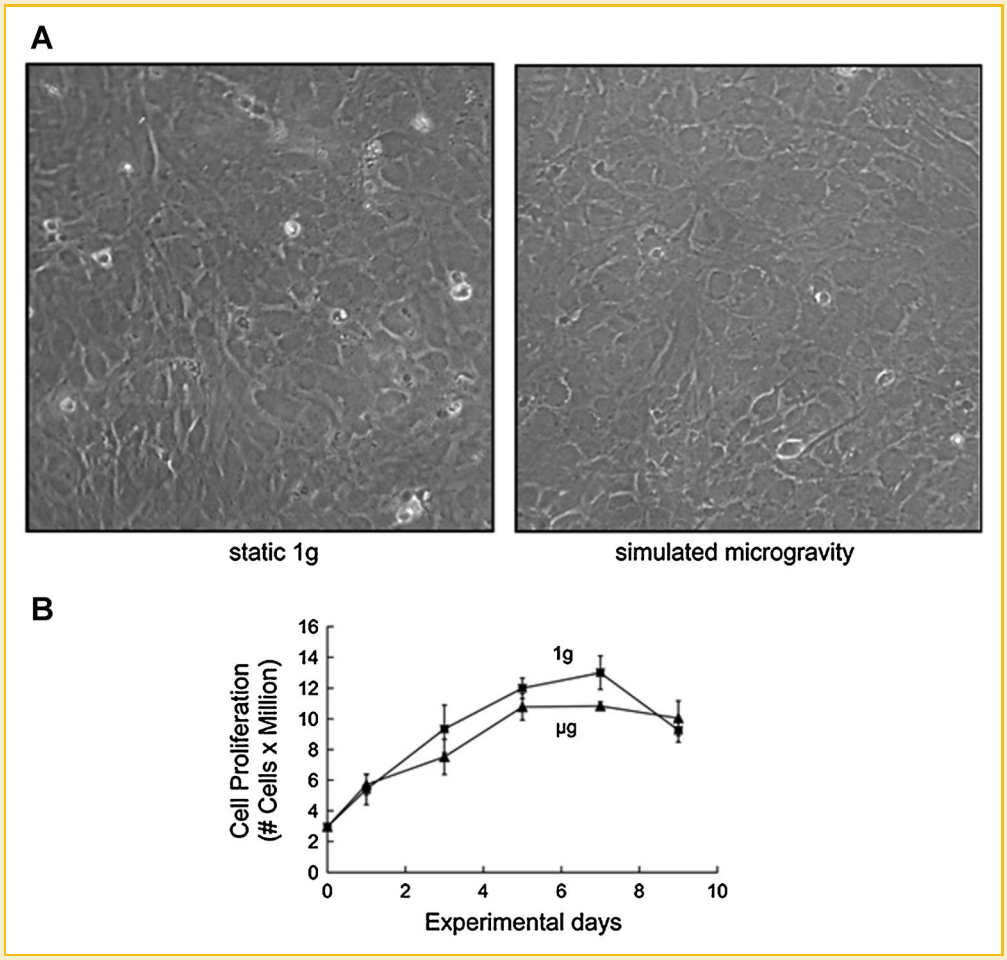

Fig. 3. Simulated microgravity has no significant effect on 2T3 cell morphology and proliferation. On day 0, 2T3 cells grown in OptiCells were placed on the RPM or exposed to the static 1-g condition for 1, 3, 5, 7, or 9 days. Cells were fed every 3 days with fresh medium. A: representative phase-contrast photomicrograph (original magnification, ×100) of 2T3 cells exposed to either control 1-g condition or RPM for 3 days. B: cell proliferation was determined by counting the number of cells in each OptiCell using the Coulter counter as shown in the bar graph (mean ± SE; n = 6) [Pardo et al., 2005].

number of studies on bone resorption and clinostats. One study exposed preosteoclasts to the RPM and found an increase in apoptosis and differentiation into osteoclast-like cells due to RPM exposure compared to static control [Monici et al., 2006]. Using reverse transcriptase-polymerase chain reaction (RT-PCR), they found elevated expression of osteoclast markers, including receptor activator of the nuclear factor κB (RANK) and its ligand (RANKL). Another study performed a gene microarray on osteoclasts subjected to the RWV. The study revealed an increase in osteocastogenesis by twofold. The microarray exposed numerous genes that were upregulated which are critical to osteoclast activity. For example, osteoclast differentiation marked by transcription factors such as c-Jun, MITF, and CREB were increased after exposure to the RWV [Sambandam et al., 2010].

Despite conflicting results, these studies show that the ground-based simulators closely mimic changes observed in astronauts after spaceflight by showing an inhibition of osteoblast differentiation and matrix mineralization and increased differentiation into osteoclast-like cells. These investigations partially validate the use of the clinostat experimental systems for studying changes associated with the exposure to microgravity. In general, accumulated knowledge from spaceflights and experiments on Earth demonstrate certain patterns of skeletal tissue response to real and modeled microgravity that constitute a specific bone loss phenotype, implying possible changes at the genomic level.

CONCLUSION

There is a substantial amount of bone loss research from both actual spaceflights and analog models. The objective of this review was to assess the current status of bone loss studies, consolidate the references to assist researchers in furthering of this field. While countermeasures have focused on exercise and nutrition balance, the reviewed plethora of gene analysis data from clinostat and animal models suggest that the future therapies may be supplemented by pharmacological agents targeting gene expression. Such targeted therapies may provide new and innovative methods that augment bone research in ways necessary for extended missions such as non-LEO destinations. While the United States has ventured

to send astronauts as far as the Moon, it has been 40 years since the last Apollo mission. Since then, pathologies which affect astronauts have become widely apparent and strikingly prohibitive to repeat such a program without adequate countermeasures. Among these maladies, bone loss remains a critical road block to advanced human travel beyond LEO. To endeavor for missions to asteroids and interplanetary destinations, the field must produce remedies that shift the balance between the risk and scientific benefit towards lower risks.

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