THE IMPACT OF SKELETAL UNLOADING ON BONE FORMATION
Daniel D. Bikle, M.D., Ph.D., Takeshi Sakata, M.D., Ph.D. and Bernard P. Halloran, Ph.D.
Veterans Affairs Medical Center and University of California, San Francisco, CA

ABSTRACT
Skeletal unloading leads to decreased bone formation and decreased bone mass. Bone resorption is uncoupled from bone formation, contributing to the bone loss. During space flight bone is lost principally from the bones most loaded in the 1 g environment. Determining the mechanism(s) by which loading of bone is sensed and translated into a signal(s) controlling bone formation remains the holy grail in this field. It seems likely that matrix/cell interactions will underlie much of the mechanocoupling. Integrins are a prime mediator of such interactions. The role for systemic hormones such as PTH, GH and 1,25(OH)2D compared to locally produced factors such as IGF-I, PTHrP, BMPs and TGFβ in modulating the cellular response to load remains unclear. Our studies demonstrate that skeletal unloading leads to resistance to the anabolic actions of IGF-I on bone as a result of failure of IGF-I to activate its own signaling pathways. This is associated with a reduction in integrin expression, suggesting crosstalk between these two pathways. As the mechanism(s) by which bone responds to changes in mechanical load with changes in bone formation is further elucidated, applications of this knowledge to other etiologies of osteoporosis are likely to develop. Skeletal unloading provides a perturbation in bone mineral homeostasis that can be used to understand the mechanisms by which bone mineral homeostasis is maintained, and that such understanding will lead to effective treatment for disuse osteoporosis in addition to preventive measures for the bone loss that accompanies space travel.

Keywords: bone, IGF-I, integrins, MAPK, proliferation, unloading

INTRODUCTION
Bone serves the triple functions of maintaining the internal structural support for the body, housing the hematopoietic system, and providing the reservoir for calcium and phosphate. The focus of this review is on the structural role, and the response of bone to changes in demands of this structural role during periods of unloading. An excellent example of the latter is the response of bone to weightlessness during space flight. Loss of bone during extended space flight has long been a concern that could limit the ability of humans to explore the universe. Under such conditions bone is lost especially in those bones most stressed by gravity prior to flight. On the other hand increased stress on bone in as elite athletes in high impact sports such as gymnastics results in increased bone during training. Although the phenomenon of bone loss or gain with changes in skeletal loading is well established, the mechanisms by which bone senses load and adjusts to it are not so clear. What actually is the stimulus and what are the sensors? What are the target cells? How do the sensors communicate the message into the cells, and by what pathways do the cells respond? What is the role of endocrine factors versus paracrine or autocrine factors in mediating or modulating the response? None of these questions has been answered with certainty, but as will become apparent in this review, we have some clues.

SPACE FLIGHT
Human studies. Much of our information concerning the impact of space flight on bone mineral homeostasis comes from the Skylab flights in 1973 during which the Skylab was occupied by three groups of three astronauts for 28, 59 and 84 days, from Salyut-6 cosmonauts exposed to space flight for 75–184 days, and from two EUROMIR 94 cosmonauts in space for 1 and 6 months. The results show a surprising amount of individual variation. With the small numbers of subjects studied and the marked differences between flights with respect to duration, diet, and use of counter measures, such variation makes it difficult to draw firm conclusions. Nevertheless, some patterns emerge.

During the three Skylab flights in which bone mineral data were obtained, the loss of bone density from the calcaneus was greater in the 84-day flight than in the shorter flights, ranging from +0.7 to -7.9% in the three astronauts (Vogel et al., 1977). Similar losses were observed in the Salyut-6 cosmonauts with total decrements from -0.9 to -19%; the losses were greater in the 140-day to 184-day flights than in the 75-day flight (Stupakov et al., 1984). Broad band ultrasound attenuation (BUA) of the calcaneus of the two EUROMIR cosmonauts was reduced by 7.34 and 13.18%, respectively, for the 1 and 6 month periods (Collet et al., 1997). Their bone densities fell 2.27 and 4.5% in the trabecular bone of the tibia with a 1% gain in the tibial cortex of the cosmonaut in space for one month compared to a 2.94% loss in the cosmonaut in space for 6 months (Collet et al., 1997). Changes in bone density in the radius and ulna of the Skylab astronauts and in the radius of the EUROMIR cosmonauts were minimal (Vogel et al., 1977; Collet et al., 1997). Changes in the lumbar spine of cosmonauts following extended space flight were mixed in that the cancellous bone of the vertebral bodies was minimally affected, but the cortical bone of the posterior processes showed a substantial loss (Oganov et al., 1991). A five-year follow-up study of the Skylab astronauts indicated further loss of bone density from the heel in those who originally had only modest changes immediately postflight, with little recovery in those astronauts who had the greatest decrements postflight (Tilton et al., 1980). Thus, the data suggest that the bones most stressed by gravity (e.g. the calcaneus, tibia), are likely to be most affected by weightlessness, that the loss in bone mineral from such bones may be progressive at least over six months, and that recovery is slow and may not be complete even after five years.
Animal studies. The most widely utilized animal for space flight studies has been the rat. Many of the studies used young, growing male rats flown for rather short durations (1–3 weeks). The rats were either housed separately (in the earlier studies) or in groups of six within an animal enclosure module (AEM) (more recent studies). The limited mobility of animals in the individual modules compared to the AEM added an element of immobilization to that of microgravity, and as will be discussed below led to more profound changes in bone mass and turnover. Most of the data come from assessments of the tibia, although non-weight bearing bones are also affected by spaceflight. One of the flights, Cosmos-926, had an onboard 1 g centrifuge which permitted more direct comparisons between microgravity and normal gravity conditions, although the environment of a short arm centrifuge cannot be considered normal. Sample collection was, in general, limited to the recovery period which varied from hours to days from the return of the animals to earth. As such, the effects of reentry and variable periods of recovery cloud the interpretation of some of the data obtained.

The earliest reported change in bone during space flight was the near cessation of bone growth, as evidenced by the appearance of an extensive arrest line in the periosteum of cortical bone in both the tibia and humerus (Morey et al., 1978; Wronski et al., 1983). The endosteal surface appears to be less affected (Westerlind et al., 1995), although reductions in the amount of bone in the tibial metaphysis and lumbar vertebrae after space flight are well documented (Vico et al., 1991; Vico et al., 1993). Non-weight bearing bones and weight bearing bones at the sites of muscle insertions are less impacted by space flight (Vico et al., 1988), although reductions in bone maturation in the jaw have been noted (Simmons et al., 1992). Subtle disruption in the growth plate is also observed (Montufar-Solis et al., 1992), but determining whether such changes are due to microgravity versus the mechanical stress of landing is difficult. More recent space flights with group housed (AEM) young rats have shown less impressive and often insignificant changes in bone mass, bone strength, or bone formation (Bateman et al., 1998; Lafage-Proust et al., 1998; Wronski et al., 1998), although a study of ovariectomized 12-week old rats housed in the AEM demonstrated decreased bone formation and cancellous bone loss (Cavolina et al., 1997).

Space flight leads to a decrease in osteoblast number and activity (Yagodovsky et al., 1976; Jee et al., 1983; Vico et al., 1988; Turner et al., 1995; Zerath et al., 1996), likely the result of altered differentiation of osteoblast precursors (Garetto et al., 1990). Density fractionation studies suggest altered bone maturation (Simmons et al., 1986). The marker of bone mineralization, osteocalcin, is reduced by space flight (Patterson-Buckendahl et al., 1987), as is its mRNA level (Backup et al., 1994; Bikle et al., 1994a) and that for prepro2[1] collagen (Cavolina et al., 1997), while the mRNA level of the marker of matrix formation, alkaline phosphatase, is increased (Bikle et al., 1994a). These findings are consistent with altered bone maturation. Osteoclast number and activity, on the other hand, are not altered in most studies of bones from space flight animals (Vico et al., 1988; Vico et al., 1991; Zerath et al., 1996). This may reflect an important difference between the young growing rat and the adult human in whom bone resorption does appear to play a significant role in the bone loss of space flight. The net result of these space flight induced changes is weaker bone (Spengler et al., 1983; Patterson-Buckendahl et al., 1987; Shaw et al., 1988; Vailas et al., 1990), which was prevented in animals maintained in the 1 g centrifuge (Spengler et al., 1983).

GROUND BASED MODELS OF SKELETAL UNLOADING

Space flight studies must overcome enormous technical problems, and are necessarily limited in size and frequency. Therefore, ground based models have been developed to evaluate the effects of skeletal unloading. We have used the hindlimb elevation (tail suspension) model because it simulates space flight better than models involving nerve resection, total body immobilization, or leg immobilization with casts or taping of the leg to the body in that it reproduces the fluid shifts seen in space travel, is reversible, and is well tolerated by the animals with minimal evidence of stress as indicated by continued weight gain in younger animals (Globus et al., 1984) and normal levels and circadian rhythms of corticosterone (Halloran et al., 1988). Furthermore, the forelimbs are normally loaded in this model and serve as an internal control for systemic versus local effects caused by skeletal unloading.

A variety of models were tested to simulate the skeletal unloading and fluid shift conditions of space flight. Some of the early models of hindlimb elevation involved a back harness which resulted in substantial bone loss, but was stressful to the animals in that normal grooming and feeding were inhibited. The animals either lost weight or failed to grow during the experiment. The use of the tail traction method, using orthopedic tape to secure the tail to an overhead pulley system allowing substantial freedom of movement around the cage, has led to a model in which the rats continue to gain weight, groom, and show no alterations in levels or circadian pattern of corticosterone (Globus et al., 1984; Wronski et al.,1987; Halloran et al., 1988). We have recently adapted this model to the mouse with success. The forelimbs are normally weighted and can serve as internal controls, although we now appreciate that the bones in the forelimbs are not perfect surrogates for the bones in the hindlimbs (Bikle et al., 1995). Most of the data with this model focus on changes in the cancellous region of the proximal tibia and periosteal bone formation at the tibiofibular junction. However, periosteal and endosteal bone formation rates are not uniform along the bone, and the different sites are affected differently by skeletal unloading (Kodama et al., 1997). In particular, periosteal bone formation decreases from distal to proximal tibia, whereas endosteal bone formation has the opposite
gradient. Periosteal bone formation is much more sensitive to skeletal unloading than endosteal bone formation along the shaft until one reaches the secondary spongiosa of the proximal tibia, which like the periosteum, is quite sensitive to skeletal unloading. The age of the animal is also an important variable. Bone formation in the unweighted tibiae and lumbar vertebrae of six-week old rats is inhibited by five days of unloading, but recovers to nearly normal by 15 days of continued unloading (Globus et al., 1984; Globus et al., 1986); whereas in six-month old rats, bone formation rates are substantially lower prior to unloading, decrease more slowly with unloading, and show no evidence of recovery even after five weeks of continued unloading (Dehority et al., 1999). Paradoxically, mRNA and protein levels of insulin-like growth factor I (IGF-I), which is thought to regulate bone formation, are higher in the bones of older rats (3–24 months) than in the bones of six-week old rats (Bikle et al., 1994c). Thus in the discussion that follows, one must bear in mind the site specific nature of the observations and the possibility that the changes observed with young animals may not be identical to the changes seen in older animals.

Skeletal unloading by the hindlimb elevation method simulates a number of features of space flight in that bone formation, mineralization, and maturation are inhibited (Globus et al., 1984; LeBlanc et al., 1985; Globus et al., 1986; Bikle et al., 1987; Abram et al., 1988; Kidder et al., 1990; Drissi et al., 1999; Sakata et al., 1999; Shiiba et al., 2002), osteoblast number is decreased (Halloran et al., 1986), serum and skeletal osteocalcin levels fall (Patterson-Buckendahl et al., 1989), the ash content of bone decreases (Globus et al., 1984; Globus et al., 1986; Abram et al., 1988), and bone strength diminishes (Shaw et al., 1987; Abram et al., 1988; van der Meulen et al., 1995). When osteoblasts or stromal cells from the bones of the unloaded limbs are cultured in vitro they are fewer in number and/or proliferate more slowly (Machwate et al., 1993; Kostenuik et al., 1997; Sakata et al., 1999), suggesting that the antiproliferative signal from skeletal unloading causes a persistent change in the osteoprogenitor cell population. These cells show a reduction in c-fos mRNA levels consistent with decreased proliferation, an increase in alkaline phosphatase mRNA but decreased osteocalcin mRNA levels and reduced mineralization consistent with an altered differentiation process similar to that seen in bones from flight and hindlimb elevated animals (Kostenuik et al., 1997; Drissi et al., 1999; Sakata et al., 1999). Although bone formation recovers in the younger animals despite continued unloading (Globus et al., 1986; Drissi et al., 1999; Sakata et al., 1999), the bone lost during the initial week was not regained as long as unloading was continued (Globus et al., 1986). If after two weeks of hindlimb elevation the animals are returned to normal weight bearing, bone formation is accelerated until bone mass is restored to normal (Sessions et al., 1989). In contrast to the unweighted bones of the hindlimbs, no significant change in bone mass or bone formation is observed in the humeri, mandible, and cervical vertebrae during hindlimb elevation (Globus et al., 1984). Bisphosphonates can prevent the relative loss of bone during hindlimb elevation (Bikle et al., 1994d; Kodama et al., 1997), but they do not prevent the fall in bone formation (Bikle et al., 1994d) or loss of bone strength (Kodama et al., 1997).

Bone remodeling is regulated by local and systemic factors. Systemic factors such as corticosterone and PTH were not found to change during hindlimb elevation (Halloran et al., 1988; Halloran et al., 1993). However, 1,25(OH)2D3 production and plasma levels fall during the early stages of skeletal unloading, in parallel with the fall in bone formation (Halloran et al., 1986; Sessions et al., 1989). Thus, the hindlimb elevation model simulates at least some of the results from space flight in that 1,25(OH)2D levels fall at least over the first week of weightlessness. During the second week of hindlimb elevation, 1,25(OH)2D3 levels return toward control values as bone formation again increases (Halloran et al., 1986). With the resumption of normal weight bearing, 1,25(OH)2D3 production and bone formation are increased (Sessions et al., 1989). These data suggest a strong link between the skeletal response of bone to unloading and 1,25(OH)2D3 production. However, infusion of 1,25(OH)2D3 fails to prevent the changes in bone formation induced by skeletal unloading (Halloran et al., 1986) indicating that the fall in 1,25(OH)2D levels is not the only perturbation responsible for the cessation of bone formation.

Although changes in PTH and GH are less well documented during skeletal unloading than are the changes in 1,25(OH)2D levels, bone and cartilage cells contain PTH (Goltzman, 1995; Irie and Ozawa, 1996; Kostenuik et al., 1999) and GH receptors (Barnard et al., 1988; Werther et al., 1990), and both PTH and GH are anabolic for bone. Therefore, we examined the ability of these hormones to prevent the fall in bone formation during skeletal unloading. In this we were partially successful in that PTH prevented the loss of cancellous bone volume but did not restore periosteal bone formation to normal or prevent the deficit in overall tibial mass induced by unloading (Halloran et al., 1997). Furthermore, although PTH given in vivo increased the number of bone marrow stromal cells (BMSC) capable of forming bone nodules (Nishida et al., 1994), this effect was not seen in cells obtained from the bones of hindlimb elevated animals (Kostenuik et al., 1999). This resistance to PTH did not appear to be at the level of the PTH receptor, since no changes in the PTH receptor protein or mRNA could be detected (Kostenuik et al., 1999). To examine the effect of GH we (Halloran et al., 1995) used hypophysectomized (HPX) animals. Like PTH, GH was partially effective in reducing bone loss. However, the resistance to GH in the unloaded bones with respect to reversing the inhibition of bone formation caused by skeletal unloading was quite substantial. This resistance was not at the level of the...
GH receptor or GH receptor signaling pathway since GH was at least as effective in stimulating the mRNA levels of IGF-I, alkaline phosphatase, osteocalcin and procollagen in the unloaded bones as in the loaded bones (Bikle et al., 1995).

Although PTH, GH, glucocorticoids, and 1,25(OH)2D may play a role in modulating the response of bone to skeletal unloading, local factors within the bone itself are likely to have the major role in mediating this response. Such factors include blood flow, which is reduced in the bones of the hindlimbs of tail suspended animals (Colleran et al., 2000). However, our attention has been drawn to factors produced by bone which stimulate bone growth include transforming growth factor-β (TGF-β), bone morphogenetic proteins (BMPs), basic and acidic fibroblast growth factor (bFGF, aFGF), and insulin like growth factors (IGF-I, IGF-II). These factors stimulate bone cell and chondrocyte proliferation as well as collagen production and proteoglycan synthesis. Systemic hormones impact on these local factors in that PTH and GH increase whereas cortisol decreases IGF-I production (McCarthy et al., 1990; Canalis et al., 1991), while 1,25(OH)2D3 and glucocorticoids each increase the IGF-I receptor level (Bennett et al., 1984; Kurose et al., 1990).

We have focused on IGF-I because it is abundantly produced by murine bone (Canalis, 1985), is a well studied regulator of bone cell proliferation and differentiation (Baker et al., 1993), and when knocked out the animals show retarded bone growth (Powell-Braxton et al., 1993; Bikle et al., 2001) and bone formation (Bikle et al., 2001) that is readily reverses with exogenous IGF-I (Bikle et al., 2001). We (Bikle et al., 1994) noted that skeletal unloading did not reduce IGF-I and IGF-I receptor mRNA and protein levels. Similar results at least for IGF-I receptor mRNA levels were recently found in rat osteoblasts flown in space (Kumei et al., 2002). When IGF-I was infused into unloaded rats, their unloaded bones (tibiae) did not increase in size unlike the bones of normally loaded animals (Kostenuik et al., 1997), and BFR was not stimulated (Figure 1), although stimulation of BFR in the humerus was equivalent to that seen in the normally loaded rats (Figure 1). Proliferation of bone cells as assessed by bromodeoxyuridine in vivo was depressed in the unloaded bones and failed to respond to IGF-I infusion, unlike the situation in the normally loaded bones (Figure 2). Apoptosis of osteoblasts and osteocytes was increased by skeletal unloading, although IGF-I infusion did not alter this process in either the normally loaded or unloaded animals (Figure 3). This resistance to IGF-I was also seen when the bone cells (BMSC) were studied in vitro. BMSC from the tibiae of hindlimb-elevated rats formed fewer colony forming units in vitro (Kostenuik et al., 1999) (Figure 4), and failed to respond to IGF-I administration with an increase in proliferation (Kostenuik et al., 1999) (Figure 5). Thus, unloaded bone and cells from such bone are resistant to IGF-I. The question is why.

**IGF-I signaling pathways** (Figure 6). The IGF-I receptor is comprised of two alpha and two beta subunits (review in Le Roith et al., 2001). IGF-I binding to the receptor results in activation of its intrinsic tyrosine kinase resulting in phosphorylation of multiple sites within the tail of the beta subunit. This creates multiple docking sites for a variety of endogenous substrates including members of the insulin receptor substrate (IRS) family which associate with IGF-IR via the PTB and SH2 domains, growth receptor binding protein-2 (Grb2) which binds to specific motifs in the IGF-I receptor as well as in IRS, and the p85 subunit of phosphatidyl inositol 3 kinase (PI3K) which binds to other specific motifs within IRS. Shc, when tyrosine phosphorylated in response to IGF-I, binds to the SH2 domain of Grb2, which in turn forms a complex with Sos, a guanine nucleotide exchange factor that mediates GDP/GTP exchange in ras and thus activates it. Ras then activates Raf (MAPKKK), which
Figure 2. Skeletal unloading decreases osteoblast proliferation and blocks the stimulation of osteoblast proliferation by IGF-I in vivo. The rats were either unloaded (hindlimb elevated) or normally loaded for 14 days, and infused with IGF-I or vehicle as described in figure legend 1. 5-bromo-2’-deoxyuridine (BrdU) was infused during the first 7 days. Osteoblast proliferation was evaluated by BrdU incorporation of the osteoblasts in the trabecular bone of proximal tibiae. Representative samples are shown from the rats given IGF-I or vehicle, and unloaded or normally loaded. The arrows point to cells immunostained for BrdU.

Figure 3. Effect of skeletal unloading and IGF-I administration in vivo on apoptosis of osteoblasts and osteocytes. The rats were either unloaded or normally loaded for 7 days, and infused with IGF-I or vehicle during this interval as described in figure legend 1. The determination of apoptosis of osteoblasts (left panel) and osteocytes (right panel) using the TUNEL procedure was performed in the proximal tibial trabecular bone. Data are means ± SD.* Significantly greater than loaded rats (p < 0.01)

Figure 4. Effect of skeletal unloading and IGF-I administration on the colony number in BMOp cells. BMOp cells were isolated from the tibiae and femora of rats that were normally loaded or unloaded and treated with vehicle or IGF-I for 7 days as described in figure legend 1. The BMOp cells were cultured for 12 days in vitro then stained with crystal violet. This figure shows representative cultures. IGF-I stimulated colony formation only in the cells from normally loaded bone; colony formation was markedly reduced in the cells from unloaded bone, and did not respond to the in vivo administration of IGF-I.

Figure 5. Effect of skeletal unloading and IGF-I administration in vitro on 5’-bromo-2’-deoxyuridine (BrdU) incorporation in BMOp cells. BMOp cells were isolated from the tibiae and femora of rats which were normally loaded or unloaded for 7 days. On day 8 of culture, the cells were treated with 10 ng/ml IGF-I or vehicle for 24 hours. During the last 4 hours of treatment, the cultures were labeled with 10 µM BrdU. Data are means ± SD * Significantly greater than paired vehicle cultures (p = 0.037, paired t-test)
Figure 6. The cellular response to IGF-I. IGF-I binds to its receptor stimulating autophosphorylation of specific tyrosine residues, providing docking sites for several cellular proteins including the insulin receptor substrate (IRS-1) and Shc. IRS-1 is also tyrosine phosphorylated providing docking sites for an additional set of proteins including the p85 subunit of phosphatidylinositol 3 kinase (PI3K) and Grb2. PI3K is a multifunctional kinase, but in the depicted pathway it phosphorylates the membrane lipid PIP2 to PIP3, providing a docking site for Akt. This enables the kinase PDK to phosphorylate and activate Akt. Akt then phosphorylates the proapoptotic factor BAD, leading to its degradation—the antiapoptotic pathway. Akt can also enter the nucleus (PI3K may do likewise) where it may phosphorylate transcription factors regulating the transcription of cyclins and cyclin inhibitors in a manner favoring proliferation. The IGF-IR when stimulated by IGF-I also phosphorylates Shc which then activates Grb2 to bind Sos, a GDP/GTP exchange factor capable of activating ras. Ras in turn initiates a serine/threonine phosphorylation cascade leading to phosphorylation and activation of Erk 1,2. These MAPKs enter the nucleus to phosphorylate transcription factors such as Elk and c-jun leading to increased cyclin D1, and reduced cyclin inhibitors p21cip and p27kip, thus promoting proliferation.

BMSC from unloaded bones have normal levels of the IGF-I receptor, but the addition of IGF-I to these cells fails to lead to its autophosphorylation (Figure 7). Similarly, ras was not activated (Figure 7), and ERK1/2 were not phosphorylated (Figure 7) in response to IGF-I in BMSC from unloaded bones, in contrast to that from normally loaded animals. Akt levels were reduced by skeletal unloading (Figure 7), consistent with the increase in apoptosis found in vivo (Figure 3), but seemed to be phosphorylated to comparable degrees by IGF-I in the BMSC from both normally loaded and unloaded animals when the reduced total levels in the unloaded animals were taken into account. These results suggest that the resistance to IGF-I in unloaded bone is primarily a failure of IGF-I to activate its own receptor and stimulate proliferation through the ras/MAPK pathway.
Figure 7. Skeletal unloading blocks the ability of IGF-I to stimulate phosphorylation of IGF-IR, Erk 1,2, and activate ras. The BMSC from the tibiae of hindlimb elevated or control animals were cultured for 7 days, serum deprived for 24 hours, and then exposed to 10 ng IGF-I/ml or vehicle for 5 minutes. The IGF-IR was immunoprecipitated with anti IGF-IR, and the phosphorylated form detected on western analysis with an anti ptyr antibody. Activated ras was determined by co-immunoprecipitation with raf. Total levels of IGF-IR and ras in the lysate and the phosphorylated and total levels of Erk 1,2 and Akt were detected by western analysis with specific antibodies.

At this point we do not know why the IGF-I signaling pathway is disrupted by skeletal unloading. However, based on the work of others (Zheng et al., 1998; Maile et al., 2001) in other cell types showing crosstalk between integrins and growth factor receptors such as IGF-IR, our hypothesis is that skeletal unloading down regulates the integrin signaling pathway and in so doing reduces the responsiveness of bone cells to IGF-I and, perhaps, other growth factors. Consistent with this hypothesis is our finding that the mRNA levels of selected integrins are reduced by skeletal unloading (Figure 8). This hypothesis is currently under investigation in our laboratory.

SUMMARY

Skeletal unloading leads to decreased bone formation and decreased bone mass. Bone resorption is uncoupled from bone formation, contributing to the bone loss. During space flight bone is lost principally from the bones most loaded in the 1 g environment, and some redistribution of bone from the lower extremities to the head appears to take place. Although changes in calcitropic hormones have been demonstrated during skeletal unloading (PTH and 1,25(OH)2D decrease), it remains unclear whether such changes account for or are in response to the changes in bone formation and resorption. Bed rest studies with human volunteers and hindlimb elevation studies with rats have provided useful data to help explain the changes in bone formation during space flight. These models of skeletal unloading reproduce a number of the conditions associated with microgravity, and the findings from such studies confirm many of the observations made during space flight.

Determining the mechanism(s) by which loading of bone is sensed and translated into a signal(s) controlling bone formation remains the holy grail in this field. Such investigations couple biophysics to biochemistry to cell and molecular biology. Although studies with cell cultures have revealed biochemical responses to mechanical loads comparable to that seen in intact bone, it seems likely that matrix/cell interactions will underlie much of the mechanocoupling. Integrins are a prime mediator of such interactions. The role for systemic hormones such as PTH, GH and 1,25(OH)2D compared to locally produced factors such as IGF-I, PTHrP, BMPs and TGF-β in modulating the cellular response to load remains unclear. Our own data demonstrates that skeletal unloading leads to resistance to the anabolic actions of IGF-I on bone as a result of failure of IGF-I to activate its own signaling pathways. Why this is so remains unknown. As the mechanism(s) by which bone responds to changes in mechanical load with changes in bone formation is further elucidated, applications of this knowledge to other etiologies of osteoporosis are likely to develop. Skeletal unloading provides a perturbation in bone mineral homeostasis that can be used to understand the mechanisms by which bone mineral homeostasis is maintained, with the expectation that such understanding will lead to effective treatment for disuse osteoporosis in addition to preventive measures for the bone loss that accompanies space travel.
REFERENCES


