The effect of microgravity on osteoblast metabolism

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Abstract

Several reports have shown the deleterious effects of weightlessness on astronauts. Among the pathological conditions recorded, those involving the skeleton are dramatic, characterised by a decrease of bone mass and by bone demineralization, eventually leading to osteoporosis. This is consistent with the notion that mechanical loading is critical for the maintenance of a correct bone mass, since it has an anabolic effect by activating bone formation and inhibiting bone resorption. Space flight experiments, as well as ground-based studies performed using different models of simulated microgravity, demonstrated that bone loss could, at least in part, be due to a decrease in bone formation by osteoblasts, the cells of the bone tissue devoted to build the bone matrix. Interestingly, it seems that osteoblasts themselves are directly sensitive to the reduced gravity force, which in turn acts by impairing their differentiation and function, as demonstrated by a decrease of the expression of the osteoblast master gene runx2 and of the specific osteoblast marker ALP, along with a decrease of the production of the bone matrix proteins osteocalcin, collagen 1 alpha 1 and osteopontin. Consistently, weightlessness also induced a reduction of osteoblast life-span and an increase of apoptosis. Based on this evidence, there is the need to more deeply investigate the molecular mechanisms underlying weightlessness-induced bone loss, in order to identify new molecular targets for alternative therapies, useful to counteract the deleterious effects of weightlessness in astronauts as well as to cure pathological conditions of reduced bone mass on earth.

Key Words: osteoblast, microgravity, bone remodelling, bone loss

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Studies from space flights over the past three decades have demonstrated the deleterious effects of weightlessness on humans. Now it is well known that weightlessness induces several pathological conditions in astronauts, including immune dysfunctions [50], cardiovascular problems [53], muscle atrophy [51] and, not last, severe skeletal alterations leading to a decrease of bone mass and to bone demineralization [3,4,7,10,54]. One of the first studies showing the severe impact of weightlessness on calcium metabolism came from Parfitt, who demonstrated a total calcium loss average of 18 gr in astronauts after 84 days space flight, due to an increased urinary calcium excretion [41], together with a decrease of intestinal calcium absorption [48]. Further data collected from long-term space flights demonstrated that astronauts can lose as much bone mass in the proximal femur in 1 month as postmenopausal women on earth lose in 1 year [8,10].

Bone is a dynamic tissue subjected to a continuous renewing during the life of each individual by the process of bone remodelling. This process relies on the correct function of two principal cells of the bone tissue: the osteoclasts, multinucleated cells that destroy the bone matrix and the osteoblasts, having osteogenic functions. The osteocytes, another important cell type arising from osteoblasts, are also involved in the remodelling process as they have a mechano-sensorial function [37] and participate to the mineral homeostasis [52]. A correct balance between bone resorption and osteogenic functions is mandatory to maintain a constant bone mass [9,63]. Due to the importance of this balance, several local and systemic factors act for a fine regulation of the bone remodelling process. Indeed, a prominent role in this regulation is accomplished by the mechanical loading, which has an anabolic effect by activating bone formation and inhibiting bone resorption [22,43]. In contrast, during space flights bone tissue is not charged by the weight of the body and this lack of loading is responsible of a loss of bone mass similar to that observed in patients with spinal cord injuries causing immobilization.

Results obtained in human and rodents after space flights indicated that bone mass reduction is not uniformly distributed throughout the skeleton, but is related to the type of bone, i.e. trabecular versus

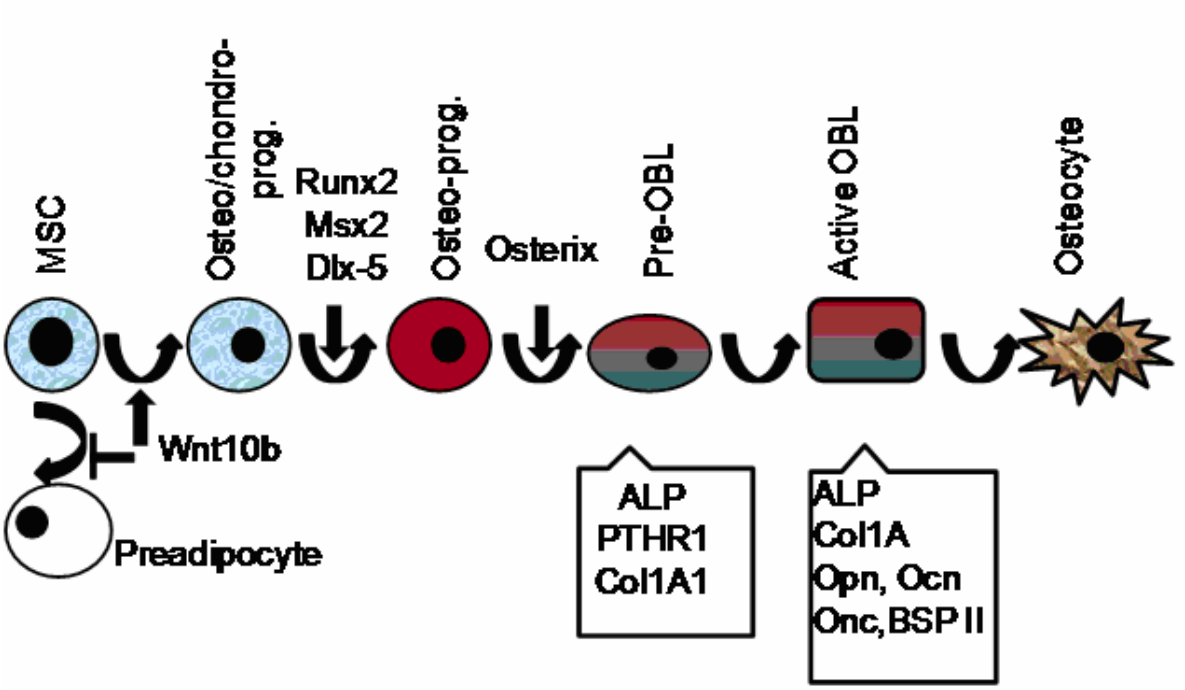

Fig. 1 *Schematic representation of the process of osteoblastogenesis. The Osteoblast arises from a multipotent mesenchymal stem cell (MSC) which, in the presence of proper stimuli, such as the Wnt signals, is committed toward an osteo/chondro-progenitor (osteo/chondro-prog.), followed by an osteoprogenitor cell (Osteo-prog.), a pre-osteoblast (pre-OBL) expressing Alkaline phosphatase (ALP), PTH receptor 1 (PTHR1) and Collagen 1 alpha 1(Col1A1), and an active, mature osteoblast (OBL) which secretes bone matrix proteins, including osteocalcin (Ocn), osteopontin (Opn), osteonectin (Ocn) and bone sialoprotein II (BSPII). Once deposed the bone matrix, a mature osteoblast may differentiate into an osteocyte.*

compact bone, and to the bone sites, i.e. weight- and non-weight-bearing bones [58]. Indeed, histomorphometric studies evidenced bone loss occurring first in weight-bearing bones and later in less weight-bearing bones [58]. Although space flight-induced bone loss is reversible, it has been estimated that the time required to recover the lost bone during the post flight period is greater than the mission length.

Space flight experiments, as well as ground-based studies performed using different models of simulated microgravity, demonstrated that bone loss could be at least partially due to a decrease in bone formation by osteoblasts, whose differentiation and function are negatively affected [5-11,29,39].

With regard to the potential effect of microgravity on bone resorption, results are not so clear. It has been described an increase of bone resorption markers, such as urinary calcium and collagen cross-links levels, in astronauts during 6 months space flight [49]. In contrast, histomorphometric data obtained from tibias of rats exposed to seven days of weightlessness during a space flight, showed a markedly reduction of trabecular bone volume relative to control littermates, while resorption activity remained unchanged [15,57]. Finally, bed rest immobilization studies showed a significant increase of bone resorption markers [21].

Our previous work evidenced that conditioned media harvested from primary osteoblasts cultured for 5 days under simulated microgravity were able to increase in vitro osteoclast formation and activity, by a mechanism involving the RANKL/RANK pathway [45].

Based on this evidence, we can assume that bone loss occurring after weightlessness is the result of an uncoupling of osteoclast and osteoblast activities.

Effects of weightlessness on osteoblasts

Osteoblast formation and function

Osteoblasts are the cells of the bone tissue devoted to build the bone matrix. As described above, they work in concert with the osteoclasts, which are multinucleated cells, having a hematopoietic origin, that resorb the bone matrix, to fulfil the bone remodelling process.

Osteoblasts arise from mesenchymal stem cells (MSCs), which are multipotent cells that, following a specific programme of gene expression, may give rise to different tissue-specific cells, including osteoblasts, chondrocytes, fibroblasts, myocytes and adipocytes

[18,61]. The first step of osteoblastogenesis is the commitment of MSCs towards an osteo/chondro-progenitor, driven by the Wingless-int (Wnt) 10b factor, which also inhibits pre-adipocyte differentiation [2] (Figure 1). This commitment is accomplished by the activation of pro-osteogenic transcription factors, such as runt-related transcription factor 2 (runx2), its downstream gene osterix (osx), distal-less homeobox 5 (dlx-5), and by the suppression of the adipogenic transcription factor CCAAT enhancer binding protein α (C/EBPα) and the peroxisome proliferator-activated receptor γ (PPAR γ). Conversely, high levels of Wnt signalling, together with the presence of runx2, further promote osteoblastogenesis at the expense of chondrocyte differentiation [17]. The transcription factor runx2 plays a key role in skeletal development as it is a master gene for osteoblast differentiation [12,24,38]. Indeed, in runx2 null mice there is no bone formation, and chondrocytes of cartilage templates fail to undergo hypertrophy [13].

As the pre-osteoblasts cease to proliferate, a key signalling event occurs for the development of the large cuboidal differentiated osteoblasts (Figure 1). These active osteoblasts are highly enriched in Alkaline Phosphatase (ALP) and secrete bone matrix proteins, such as collagen I and non-collagenous proteins, including osteocalcin, osteopontin, osteonectin and bone sialoprotein II. As a rule, ALP and the type 1 parathyroid receptor (PTH1R) are early markers of osteoblast progenitors that increase as osteoblasts mature and deposit matrix, but decline as osteoblasts become osteocytes, whereas osteocalcin is a late marker that is up-regulated only in post-proliferative mature osteoblasts [35] (Figure 1).

As already mentioned, the principal function of the osteoblasts is to synthesize the proteins of the bone matrix and to attend the process of calcification. Osteoblasts also play a crucial role in osteoclast biology by expressing and/or secreting key molecules that in turn regulate osteoclastogenesis and bone resorption [63]. Among them, the RANKL (receptor activator of nuclear kappa B ligand) a trans-membrane cytokine expressed by osteoblasts, which interacts with its receptor RANK on the pre-osteoclast surface, thus triggering osteoclastogenesis. Another key molecule secreted by osteoblasts which interfere with the RANKL/RANK pathway is osteoprotegerin (OPG) a decoy receptor for RANKL [47], which has an osteo-protective role. Indeed, OPG is a secreted protein having the same structure of the extracellular domain of RANK so that it binds to RANKL avoiding its interaction with RANK, with a consequent inhibition of osteoclastogenesis.

Effects of microgravity on osteoblast differentiation and function

As stated above, biochemical data from astronauts and histomorphometric analysis of rat bones maintained under microgravity conditions indicated that the reduced bone mass is the result of decreased bone formation in association with normal or increased bone resorption [7]. As far as the impairment of bone formation is concerned, substantial evidence tells us that it is due to a decreased osteoblast differentiation, eventually leading to an impairment of bone matrix formation and mineralization. Interestingly, other studies, investigating the effect of microgravity on osteoblasts, indicated that osteoblasts themselves are directly sensitive to the reduced gravity force.

Histomorphometric analysis performed on long bones of rats placed in orbit for 7 days evidenced a decreased periosteal bone formation, together with a reduction of osteoblast size and osteoblast surface and number in the lumbar vertebra [59]. One of the first evidence of a direct effect of microgravity on osteoblasts came from the experiments of Hughes-Fulford and Lewis [23]. In particular, MC3T3-E1 osteoblast cell lines, launched on the STS-56 shuttle flight and maintained at a microgravity condition of 10^{-6} to 10^{-9}xg, showed a significant reduction of cell growth, together with a reduced glucose utilization, and an abnormal morphology, with a reduction of stress fibre number, relative to 1xg control osteoblasts.

Next, Carmeliet and colleagues evidenced an impairment of the MG-63 human osteosarcoma cell line differentiation in response to systemic hormones and growth factors under microgravity [5]. Indeed, in MG-63 cells subjected to microgravity the levels of ALP, collagen I alpha1 and osteocalcin mRNAs were significantly reduced [6,36]. The use of a three dimensional clinostat device, able to mimic a microgravity condition on earth, confirmed the data obtained during space flights related to osteoblast differentiation. In particular, Yuge and colleagues showed a delay in ALP expression and nodule formation in osteoblasts grown in 3D clinostat relative to 1xg control, together with a decrease of the expression levels of runx2 and osteocalcin [62]. Other bone matrix proteins, such as ostepontin, and the alpha-tubulin were also found to be decreased under simulated microgravity [29].

A suppression of osteoblast differentiation through decreased runx2 and AP-1 activities was also observed in MC3T3-E1 osteoblasts by Ontiveros and McCabe, using the Rotating Wall Vessel (RWV) system [39]. Microgravity also affected osteoblast production of growth factors, such as the Insulin-like Growth Factor-I (IGF-I), which was reduced, while its receptor was increased [28]. Higher mRNA and protein levels of the IGF Binding Protein (IGFBP)-3 were also observed in osteoblasts maintained for 5 days under weightlessness conditions [27]. An increase of glucocorticoid receptors [27], of Prostaglandin E2 (PGE2) and of inducible prostaglandin G/H synthase-2 (PGHS-2) production was also observed under microgravity [26].

Interestingly, ROSSMER#14 osteosarcoma cells [44] and mouse calvarial osteoblasts (manuscript submitted for publication) cultured in RWV showed upregulation of interleukin-6 (IL-6) mRNA, thus suggesting a role of this cytokine in stimulating an increased osteoclastogenesis. A recent work showed that, beside the well known role of microgravity in reducing osteoblast differentiation, this condition also increased adipogenesis of human MSCs, as evidenced by an increase of the PPARγ2, which is known to be important for adipocyte differentiation, together with an increase of adipsin, leptin, and glucose transporter-4 in response to microgravity [64].

Effect of microgravity on osteoblast cytoskeleton.

It is well known that the disruption of the actin-cytoskeleton abolishes the cell response to stress, thus indicating that the cytoskeleton is involved in cellular mechanotransduction. One of the first documentations of an alteration of cytoskeleton organization under microgravity came from Sarkar and colleagues [46], who showed a disorganization of actin cytoskeleton together with a perinuclear distribution of the integrin beta 1. Similar results were also observed by Guignandon and colleagues [19] who reported the ability of microgravity to impair osteoblast integrin-mediated cell adhesion and to induce a disorganization of the cytoskeleton, associated with disassembling of vinculin spots and of phosphorylated proteins within focal contacts. Finally, studies conducted on human MSCs cultured under modelled microgravity showed a disruption of F-actin stress fibres, and a consequent increase of the G-actins. Consistently, the activity of RhoA, a small GTPase involved in the regulation of actin stress fibre formation, and the subsequent phosphorylation of cofilin were significantly reduced by modelled microgravity [32].

Effect of microgravity on osteoblast life-span

Besides the clear effect of microgravity on osteoblast differentiation above described, another phenomenon that could indirectly contribute to the impairment of bone formation is the reduction of osteoblast life-span.

Experiments with rats subjected to 14 days of hind limb unloading showed a reduction of proliferation of osteoprogenitors (osteoblast colony forming units; CFU-O), together with a decrease of ALP-positive colony forming units (CFU-AP) [1].

Moreover, simulated microgravity inhibited rat bone marrow Mesenchymal Stem Cell proliferation (rBMSC), cells being arrested in the G(0)/G(1) phase of cell cycle, while growth factors such as IGF-I, markedly stimulated rat bone marrow MSC proliferation in normal gravity, but had only a slight effect in simulated microgravity [11].

As the effect of microgravity on apoptosis is concerned, a study from Sarkar and colleagues demonstrated an increase of the osteoblast-like ROS 17/2.8 cell apoptosis under simulated microgravity [46]. Moreover, by employing the NASA-approved RWV bioreactor and the rat osteoblast-like cell ROS.SMER#14, we also observed an enhancement of apoptosis along with a reduced proliferation under microgravity [44].

Effect of microgravity on osteoblast transcriptional regulation

Recent microarray gene profiling analysis performed on osteoblasts subjected to microgravity raised a large amount of data which could represent a good starting point to more deeply investigate the mechanisms involved in weightlessness-induced bone loss and to identify new targets for the development of alternative therapies.

The first whole genome profiling analysis came from Pardo's group, working on 2T3 preosteoblasts cells subjected to simulated microgravity for 9 days using the Random Positioning Machine (RPM). The data obtained from this group confirmed a reduction of genes related to osteoblast differentiation including Alp, runx2, osteomodulin, and PTHR1 under simulated microgravity [40]. Similar results were observed by the same group using 2T3 preosteoblasts but employing an alternative model of simulated microgravity, the RWV Bioreactor [42]. Indeed, comparison of the RWV data to the RPM microarray study showed 14 mechanosensitive genes that changed in the same direction.

A further contribution in this field was given by Xing and colleagues [60], who performed a whole gene profiling analysis on mechanically loaded tibiae in a mouse model. Among the 22,000 transcripts analysed, 346 were differentially expressed in the loaded bones compared to the controls.

The list of differentially expressed genes includes genes involved in cell growth, differentiation, adhesion, proteolysis, as well as signalling molecules of receptors for growth factors, integrin, ephrin B2, endothelin, and adhesion G protein coupled receptor. Among the biological network regulated by the loading condition, they found the fibronectin and pleiotrophin signalling molecules, whose function is important in regulating periosteal bone formation and resorption in response to four-point bending.

Our group has recently assessed a global transcriptome analysis of mouse calvarial osteoblasts grown for 5 days at unit gravity or under modelled microgravity (0.008xg) in the RWV bioreactor (manuscript submitted for publication). Interestingly, elaboration of gene profiling data evidenced a set of regulated genes which include genes involved in osteoblast differentiation, function, and osteoblast-osteoclast cross-talk, as well as new genes not previously correlated with bone metabolism. The latter could contribute to a better understanding of the mechanisms underlying bone mass regulation and to

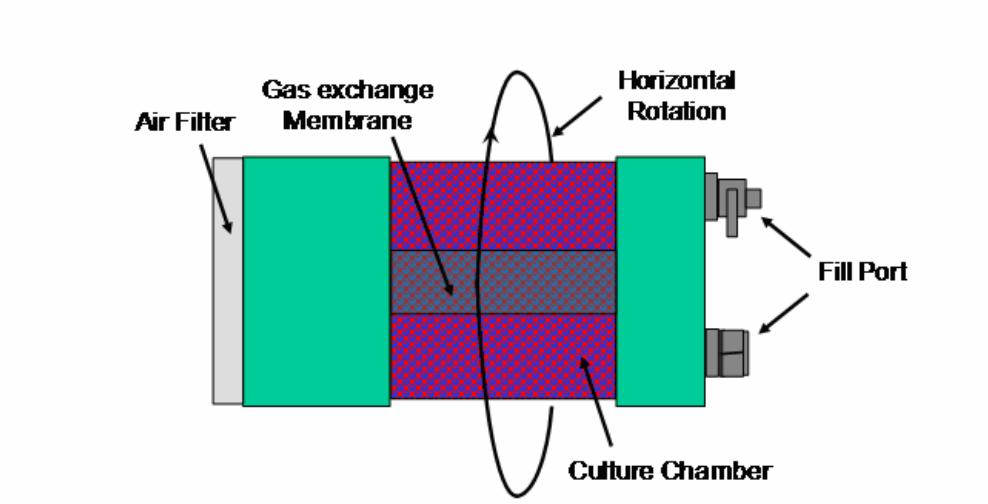

Fig. 2 *Schematic picture of the SLTV of the RWV bioreactor. This bioreactor consists of a cylindrical culture chamber containing an inner co-rotating cylinder with a gas exchange membrane through which an air pump draws incubator air. Cells and liquid media are placed in the space between the inner and the outer cylinders and the device is rotated about its horizontal axis.*

the identification of new targetable molecules for the development of alternative therapies to cure bone loss.

Finally, matching these data with the previously described gene profiling sets we identified a common cluster of genes, whose regulation was consistently in opposite direction when loading and unloading conditions were compared. This set includes genes coding for ECM constituents, for growth factor receptors, for proteins involved in collagen fiber assembly and genes marker of chondrogenic/ osteoblastic cells in bone formation.

On-ground models to mimic the effect of microgravity on bone cells

Bed-rest immobilization.

The effects of immobilization on bone mass and bone remodelling in patients with spinal cord injuries are known to simulate weightlessness-induced bone changes in astronauts. One of the first studies of bed rest was performed in the former Soviet Union (i.e. USSR) by recruiting twenty healthy male volunteers subdivided in the following groups: controls, including subjects who underwent a normal ambulatory life, versus subjects who were maintained in complete immobilization for 120 days. Among the latter group, some subjects received treatment with potassium diphosphonate, to counteract the expected bone loss due to immobilization [55].

Cellular activity measurements showed that in completely immobilized men, the mineralization rate was lower than in controls without change in osteoid parameters; in contrast, osteoclastic parameters were increased.

Further studies demonstrated that already 24 hours of immobilization by head-down tilt bed rest were sufficient to induce a significant rise in osteoclast activity in health subjects, as demonstrated by an increase of bone resorption markers [21].

Finally, in a 17 weeks horizontal bed rest study, alendronate treatment was able to prevent Bone Mineral Density (BMD) loss in all regions except the calcaneus, although the pyridinoline markers and fecal calcium remained elevated and net calcium balance was negative [31].

Rat tail-suspension model.

This is one of the first animal experimental models employed to mimic space flight-induced bone loss on earth. Briefly, rats are hind limb unloaded by tail traction, while the forelimb remain loaded, providing an internal control to distinguish between the local and the systemic effects. Unloaded rats gain weight at the same rate as control rats and have equal levels of glucocorticoids, suggesting minimal conditions of stress, while, relative to controls, they showed reductions in trabecular bone osteoblast number, mineral apposition rate and volume, cortical periosteal mineralization rate, total bone mass, calcium content, and maturation of bone mineral [33,58]. These results are in agreement with the data recorded in rats subjected to space flight, validating this important unloading model on earth.

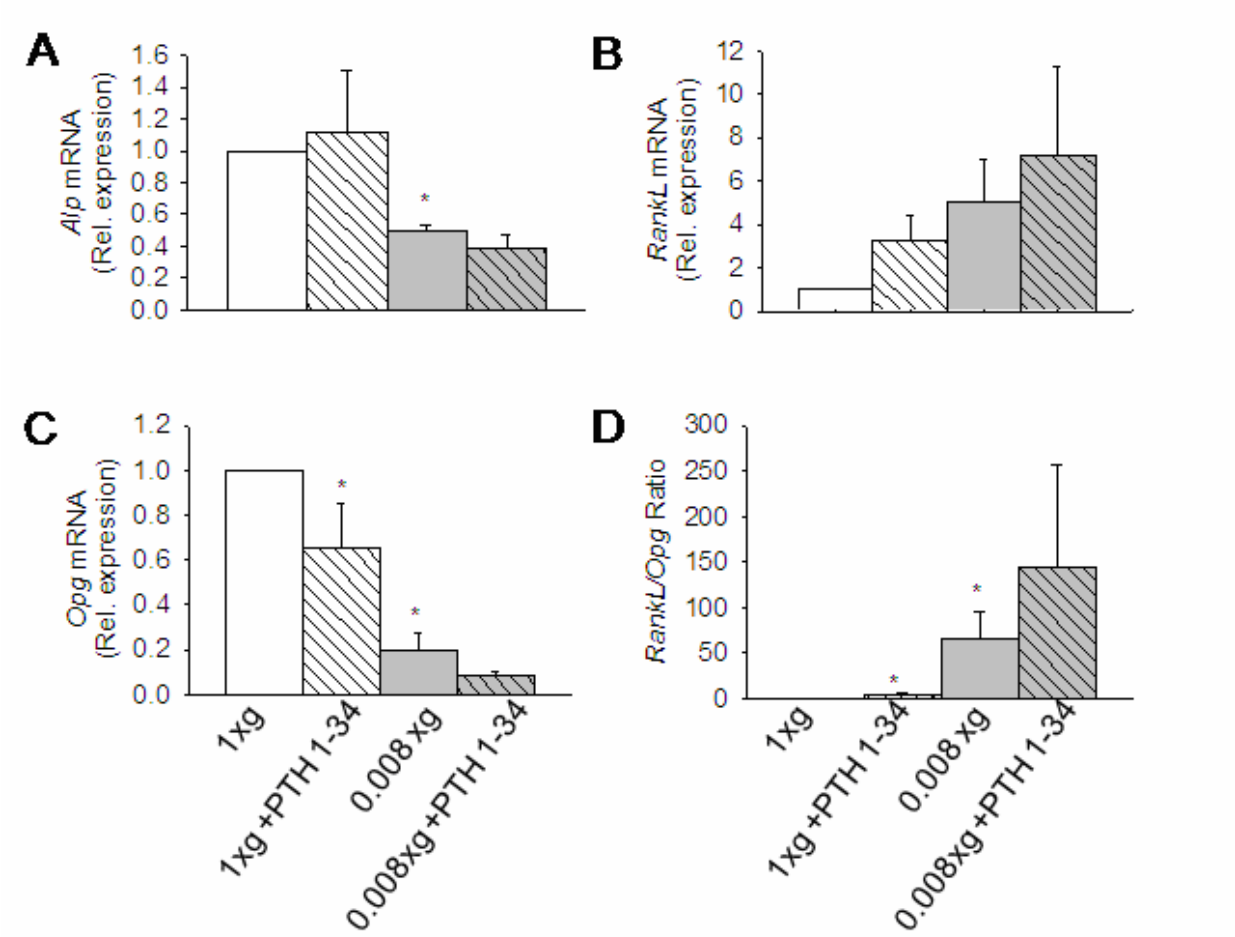

Fig. 3 *Effect of PTH 1-34 on osteoblast gene expression. Primary osteoblast cultures were grown for six days at unit gravity (1xg) or under simulated microgravity (0.008xg) in the presence of vehicle or 25 nM hPTH 1-34. RNAs were then extracted and reverse transcribed. cDNAs were subjected to comparative real time PCR for Alp (A), RankL (B) and Opg (C). (D) Evaluation of the RankL/Opg ratio. Data are the mean ± SEM of three independent experiments (*p<0.05 vs. 1xg).*

On-ground in vitro cell culture systems

Since the year 1997, several works aimed at investigating the effect of microgravity on bone cells have been conducted using bioreactors that allowed a three-dimensional in vitro culture of different cell types, including oteoblasts and osteoclasts, and at the same time were able to simulate a condition of reduced gravity. Among them there is the RWV, a NASA-developed, ground-based, microgravity-simulating system (USA Synthecon Inc., Houston, TX) consisting of a station rotation base and a power supply which allow the horizontal rotation of a culture vessel in which cells are cultured. Two kinds of culture vessels are available, the HARV, used to culture both suspension and anchorage-dependent cells, and the SLTV, for microcarrier cell culture and explanted tissue cultures. The latter is a bioreactor consisting of a cylindrical growth chamber that contains an inner co-rotating cylinder with a gas exchange membrane [14,16,42,44,45], as shown in Figure 2. Cells and liquid culture media are placed in the space between the inner and the outer cylinders, and the assembled device is rotated on its horizontal axis with very low fluid shear forces. Usually, this device requires the use of microcarrier beads, particularly for cultures of adhesive cells, such as the osteoblasts, which form 3D aggregates. Cells attached to microcarriers are in suspensions and rotate inside the vessel as a solid body. Moreover, the horizontal rotation of the vessel subjects the cells to a constant randomization of the normal gravity vector, thus mimicking a state of simulated free fall. Several data provided with these devices showed the ability of microgravity to impair osteoblast differentiation and function [29,36,40,42,62] and to stimulate the ability of osteoblasts to induce osteoclast formation [45]

Therapeutical strategies to prevent weightlessness-induced bone loss

Calcium and vitamin D supplementation

One of the effects of space flights is a reduction of serum calcitriol levels, with consequences in calcium adsorbtion [65]. However, studies on dietary supplementation with calcium and vitamin D3 in astronauts failed to show prevention of the development of osteoporosis [65].

Intermittent treatment with PTH

It is well known that PTH, when administered in an intermittent way, has an anabolic effect on bone mass. A work from Halloran and colleagues [20] demonstrated that although cancellous bone volume was preserved in PTH-treated, unloaded animals, PTH did not restore periosteal bone formation to normal, nor did it prevent the deficit in overall tibial mass induced by unloading. Similar results were observed by Moriyama [34], showing that in rats subjected for 14 days to tail suspension, intermittent treatment with PTH significantly increased cancellous bone volume/tissue volume (BV/TV) relative to the tail-suspended control group, while it failed to prevent cortical bone loss.

We tested in vitro a potential effect of PTH intermittent treatment (25 nM) on primary osteoblast cultures growing for 6 days at 1xg or under simulated microgravity using the RWV bioreactor. We evaluated ALP mRNA expression as a marker of osteoblast differentiation, which was significantly reduced by simulated microgravity (Figure 3A). In contrast, treatment of osteoblasts with PTH was not able to revert ALP reduction induced by simulated microgravity. We also investigated a potential indirect effect of PTH on osteoclastogenesis through regulation of RankL and Opg expression in osteoblasts. As shown (Figure 3B-C) a trend to an increase of RankL together with a significant decrease of Opg mRNAs were observed under simulated microgravity (0.008xg) relative to 1xg. Treatment with PTH did not significantly affect RankL expression neither at 1xg nor at 0.008xg (Figure 3B), whereas it significantly reduced Opg mRNA at unit gravity, with only a trend of reduction of Opg at 0.008xg (Figure 3B-C). Evaluation of RankL/Opg ratio showed a significant increase at 0.008xg *vs*. 1xg, in agreement with our previous data [45], and although to a lower extent, after treatment with PTH in osteoblasts cultured at 1xg, whereas the same treatment failed to significantly affect RankL/Opg ratio under microgravity (Figure 3D).

Taken together, these results indicate that the anabolic treatment in weightlessness condition may be problematic as osteoblasts may become refractory to agents positively influencing their function at unit gravity. In addition, our results suggest an exacerbated deleterious effect of PTH on osteoblast-mediated osteoclastogenesis in microgravity, further making the treatment with this agent unfeasible to cure bone loss caused by space flights.

Bisphosphonates

A work from Kodama group [25] tested the effect of inhibition of bone resorption by the bisphosphonate pamidronate on BMD and strength of hind limb bones in tail-suspended growing rats. Results demonstrated that pamidronate treatment increased secondary cancellous bone but did not restore normal growth-induced periosteal bone apposition and bone strength. In contrast, a bed rest study lasting 17 weeks involving 21 subjects subdivided in a control group and a group treated daily with alendronate (10 mg/day) showed that this treatment, compared to controls, prevented BMD loss in all regions except the calcaneus, although the pyridinoline markers and fecal calcium remained elevated and net calcium balance was negative [31].

Conclusions

It is well known that weightlessness during space flight causes osteoporosis, with a range of loss of bone mineral density in the whole body and legs between 0.3-0.4 % per month [30], together with an increased risk of fractures in later life. Moreover, although space flight-induced bone loss is reversible, it has been estimated that the time required to recover the lost bone during the post flight period is greater than the mission length.

A constant bone mass relies on a correct balance between the osteogenic function by osteoblasts and bone destruction by osteoclasts. This balance, that underpins the bone remodelling process, is under the control of many factors, among which mechanical loading plays a crucial role. Indeed, what is clear is that reduced bone mass induced by weightlessness is the result of an increase in bone resorption together with a decrease of osteoblast differentiation and function, leading to an impairment of bone formation.

So far, data on the interventions applicable to prevent bone loss in astronauts during space flights are lacking, and various experimental data provide evidence that osteoblast anabolic factors, such as the PTH, currently used on ground to treat osteoporosis, are likely not to be suitable in unloading conditions. Therefore, there is the need to develop this field further, by investigating more deeply the molecular mechanisms underlying the regulation of bone mass on ground and in space.

Abbreviations

ALP, Alkaline Phosphatase; BMD, Bone Mineral Density; Dlx-5, Distal-less homeobox-5; C/EBPα, CCAAT Enhancer Binding Protein α; CFU-AP, ALP-positive Colony Forming Units; CFU-O, Osteoblast Colony Forming Units; IGF-I, Insulin-like Growth Factor-I; IGFBP-3, Insulin-like Growth Factor

Binding Protein-3; MSC, Mesenchymal Stem Cell; OPG, Osteoprotegerin; Osx, Osterix; PGE2, Prostaglandin E2; PGHS-2, Prostaglandin G/H Synthase-2; PPARγ, Peroxisome Proliferator-Activated Receptorγ; PTH, ParaThyroid Hormone; PTHR1, ParaThyroid Hormone Receptor 1; RANK, Receptor Activator of Nuclear Kappa B; RANKL, Receptor Activator of Nuclear Kappa B Ligand; RCCS, Rotating Cell Culture System; RPM, Random Positioning Machine; Runx2, Runt-related transcription factor 2; RWV, Rotating Wall Vessel; Wnt10b, Wingless-int 10b.

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