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Spaceflight osteoporosis: current state and future perspective

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Osteoporosis is one of the established major consequences of long-duration spaceflights in astronauts seriously undermining their health after their returning on Earth. Indeed, astronauts typically lose more bone mass during one month than postmenopausal women on Earth lose in one year. To date, countermeasures mainly consist in exercise and supplementation while pharmacological treatment as those used in postmenopausal women are not routine. However, it is evident that exercise and supplementation alone are not enough to maintain bone homeostasis. In this paper we describe the current countermeasures for bone loss during long-term spaceflight, review the modern treatment which are successfully employed to prevent osteoporosis on Earth and that could be quickly used also for astronauts and finally focus on the recent cellular and molecular understanding of bone homeostasis which might provide the basis for the development of future targeted therapies.

Key words: osteoporosis, vitamin D, spaceflights, bone metabolism

It is well known that the exposure to microgravity induces significant changes in multiple biological systems, mainly in muscle-skeletal, because of removing gravitational loading (Hawkey 2003). Since the first long-duration space missions, a significant loss of calcium and bone was reported in astronauts for whom this still represent a major health danger in particular for increased risk of fracture and premature osteoporosis in later life (Droppert 1990; Cavanagh et al. 2005; Hughes-Fulford 2011). Indeed, the human skeleton develops on Earth under the gravity force that leads to the maintenance of balance in the calcium metabolism and the normal mechanical loading environment of bone (Keller et al. 1993). Therefore, the exposition to a new environment with low gravity during long-term spaceflight disrupts bone homeostasis in the skeleton causing a remodeling of bone structure and a release of calcium at a rate that is almost 10 times greater than that in a postmenopausal woman (Iki et al. 1996; Sirola et al. 2003). This adaptation to microgravity leads to skeleton weakness, with higher risk of fracture and potential long-term health risks for astronauts on their return on Earth (Droppert 1990; Cavanagh et al. 2005; Hughes-Fulford 2011). For these reasons, the physiologic changes in bone in astronauts during spaceflight and the effect of exercise, the supplementation of calcium and vitamin D, and the efficacy of hormone and antiresorptive therapies on bone metabolism and bone mass have been extensively studied (Parfitt 1981; Heer 2002; Cavanagh et al. 2005; Baecker et al. 2010). However, recent insights into the genes involved in osteoporosis and into the molecular regulation of bone mass in human provide the basis for the development of new preventional/therapeutical strategies for astronauts (Lei et al. 2011; Oganov et al. 2011; Sibonga 2013).

In this article, we review the evidence for loss of bone mass during long-term spaceflight, the mechanisms of microgravity-related osteoporosis, the current countermeasures (*i.e.* exercise, supplementation, drug therapies), and the future perspectives.

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Loss of bone mass in the space

Since Apollo and Skylab missions, spaceflights were used to study the effect of microgravity on human body and physiology. These missions led to the first evidences of significant osteoporosis in spaceflight (Vogel 1975) Indeed, during exposure to microgravity; calcium and bone mass are continuously and progressively lost (Hughes-Fulford 2011). Despite the short duration (only 9 to 12 days) of the Apollo missions XIV-XVI, the reported loss of bone was almost 2% (Vogel 1975). During longer (1 to 3 months) spaceflights in the Skylab era, the loss of bone appeared considerable (Vogel 1975). Peripheral quantitative computed tomography (CT) analysis of distal tibia in astronauts after six months of spaceflights showed up to 24% of trabecular bone lost and after six months from the return on Earth the recovery of bone mineral density (BMD) was not complete (Vico et al. 2000). Indeed, the recovery of skeletal density after long-duration space missions was estimated to exceed one year (Sibonga et al. 2007). These data have suggested that i) mechanical stress, as that induced by Earth's gravity, was a crucial underlying factor for normal bone homeostasis; ii) exposure to weightlessness was a causal factor of osteoporosis; iii) the loss of bone mass was more quick in astronauts than postmenopausal women; and iv) the severity of osteoporosis was proportional to the length of exposure to microgravity (Vogel 1975; Hughes-Fulford 2011). Moreover, it has been demonstrated that microgravity environment is able to induce a plethora of detrimental effects to the osteoblasts/osteoclasts equilibrium culminating in a more intense activity of osteoclast (Nabavi et al. 2011).

The application of dual X-ray absorptiometry (DXA) techniques to detect bone changes during spaceflight provided new information about the localization of the osteoporosis (LeBlanc et al. 1998; 2000). Of interest, this method highlighted regional losses during spaceflight of 1.06-1.56% per month in the spine, pelvis, and proximal femur. No significant changes were found in the upper extremities (LeBlanc et al. 1998; 2000). This was the first evidence of a distribution pattern of bone mass loss, which was higher in the lower than the upper extremity (LeBlanc et al. 1998; 2000).

Bone physiology

The homeostasis of bone formation and function is regulated by both, osteoblasts, which synthesize type I collagen and other proteins that combine to form osteoid, and osteoclasts that excavate the calcified matrix (Canalis et al. 1988). When bone is formed, only few osteocytes die and their viability is maintained by mechanical stimulation, which if lacking causes, on the one hand osteocytes apoptosis and on the other osteoclasts recruitment (Canalis et al. 1988). Bone belongs to most complex organs and tissues and its remodeling process is regulated by systemic influences (hormones), stress action on trabecular and cortical systems (physical activity/weight bearing), growth factors and cytokines produced by the bone cells which act locally and on the other bone cell types (Baron et al. 1984). There are many causes that may upset this delicate balance: some are physiological like aging that without prevention, induces osteoporosis which the most dramatic consequence are fractures, other causes can be identified in genetic, others in feeding (malnutrition or deficiency in micro- and macronutrients) and more in case of bed-rest and the absence of gravity (Riggs et al. 1969; Yamaguchi et al. 1987; 1998; Yamaguchi 2006; Nabavi et al. 2011). All agree that no matter what the cause is, the most effective forms of prevention and treatment must to identify to ensure optimal bone functionality.

Exercise

The first countermeasure adopted after the discovery of bone loss during spaceflights was exercise. Astronauts were (and to this day are) required to perform exercise up to three hours per day on machine which should reproduce Earth's gravity effect (Nicogossian et al. 1995). Such device consisted in passive treadmill, cycle ergometer, and bicycle ergometer complemented with bungees tethering the subjects in place and acting as resistance (Nicogossian et al. 1995; Schneider et al. 2003). However, none of these machines presented a force measurement capability and recent studies demonstrated that this practice during long-term spaceflights is not an effective countermeasure for bone loss (LeBlanc et al. 2000; Lang et al. 2004; Ohshima et al. 2012).

There are several reasons why exercise alone is inadequate to prevent osteoporosis in astronauts. Indeed, it is not yet clear if i) the load applied to the body of the subjects by the machines in space is enough to achieve a force similar to Earth's gravity or higher; ii) the duration of exercise programmed is sufficient to maintain bone homeostasis; and iii) the single dose per day of exercise is the optimal management to substitute the effect of the "all day dose" of gravity on Earth. Until these issues will not be deeply and carefully examined, the real weight of exercise as a countermeasure to

weightlessness-related osteoporosis cannot be determined. However, it seems already likely that also in the future exercise alone will not be sufficient to stop bone loss during spaceflights and that a pharmacological treatment is needed to prevent osteoporosis.

Supplementation

The inadequate sunlight exposure during space flights, justify calcium and vitamin D supplementation to contrast the lowering of their serum level and the subsequent development of osteoporosis. However, this preventive measure seems to be not sufficient to prevent osteoporosis because cannot counteract the increase in bone resorption and the decrease in bone formation (Holick 1998; Caillot-Augusseau et al. 2000; Zittermann et al. 2000). A solution regarding this latter aspect could be provided by an active form of vitamin D analog, the eldecalcitol. In fact, this molecule showed an improvement of bone biomechanical properties by normalizing bone turnover and in some countries in which it has just been approved for osteoporosis treatment (Smith et al. 2013). Moreover, even the vitamin K is well known to have a role in calcium balance and bone metabolism and its supplementation seems to be useful to counteract the reduction on bone formation (Vermeer et al. 1998; Shiraki et al. 2000). Several studies have demonstrated the basic role of zinc for the bone growth and the content of a large proportion of body zinc in skeleton (Ronaghy et al. 1974; Herzberg et al. 1990). On the other hand, zinc deficiency is a common finding in case of bone growth retardation and during fetal and postnatal period of skeletal abnormalities (Hurley et al. 1972; Masters et al. 1986). In short, the recommendation of zinc supplementation stems from its several functions: nutrition during bone growth, stimulation osteoblastic bone formation, inhibition osteoclastic bone resorption, and promotion of fracture healing (Yamaguchi et al. 1992; Yamaguchi et al. 1997; Igarashi et al. 2001). Another factor, which plays a role very similar to zinc, is genistein (Yamaguchi et al. 1997; 1998). Furthermore, zinc and genistein have been demonstrated to have a synergistic effect though the mechanism has not been completely understood (Yamaguchi et al. 1998).

Drug therapies

To date, in literature are available well-written guidelines about pharmacological treatment of osteoporosis, which consider efficacy, safety, cost, convenience, and other benefits. However, if on the one hand they identify the range of patients to be treated, on the other hand there are no specific recommendations that what type of drug use in different cases (Kanis et al. 2008). Assuming that therapy should be prescribed individualized for each patient in order to increase adherence and optimize the results, we summarize briefly below the main characteristics of the osteoporosis drugs available today.

Various bisphosphonate formulations, oral and intravenous, have been demonstrated to reduce significantly vertebral, non-vertebral, and hip fracture (Chesnut et al. 2004; Wells et al. 2008). In contrast, their use have been associated with acute phase reaction as fever, myalgia, arthralgia and acute renal failure, and long term consequences as atypical fracture and osteonecrosis of the jaw (Strampel et al. 2007, Schneider 2009). Moreover, oral bisphosphonates may produce gastrointestinal tolerability and esophageal ulcerations (MacLean et al. 2008).

As bisphosphonates, hormone therapy (estrogen or combined estrogen/progestogen) seems to reduce vertebral, non-vertebral, and hip fracture and it is designed primarily for women in menopause. However, it may represent a cardiac, thromboembolic, and breast cancer risk (Rossouw et al. 2002; Beral et al. 2003; Cauley et al. 2003). In this regard, a novel selective estrogen receptor modulator (bazedoxifene) is going to be developed. The preliminary experimental studies indicate that they seem to be well tolerated with no evidence of utherotrophic activity and no adverse effects on plasma lipids (Smith et al. 2015).

The use of calcitonin, has minor side effects and it has been demonstrated an ability to reduce vertebral fracture risk (Chesnut et al. 2000). Parathyroid hormone (teriparatide) has been shown to reduce vertebral and non-vertebral fractures and apart from minor side effects such as headache, nausea, and dizziness, osteosarcoma has been observed only in rats (Vahle et al. 2002; Hodsman et al. 2005). Strontium ranelate has a dual effect of reducing bone resorption and increasing bone formation and thus is effective in reducing of vertebral and non vertebral fracture risk, but on the other side, its use is associated with nausea and diarrhea, especially during first three months and vascular and neurological side effects (Meunier et al. 2004; Reginster et al. 2005). Selective estrogen receptor modulators (SERMs) may reduce vertebral fracture risk, but they have side effects such as hot flushes and thromboembolism (Ettinger et al. 1999; Barrett-Connor et al. 2006). SERMs, with tissue selective estrogen complex and Denosumab represent

the emerging osteoporosis therapies (Lindsay et al. 2009; Waugh et al. 2011; Bolognese et al. 2013). In particular, the last one is a monoclonal antibody approved for osteoporosis treatment in both the Europe Union and the United States (Waugh et al. 2011; Bolognese et al. 2013). Denosumab has been demonstrated effective in reducing vertebral, non-vertebral, and hip fracture risk, but they observed a high incidence of eczema, dermatitis, rash, and cellulitis (Cummings et al. 2009; Waugh et al. 2011; Bolognese et al. 2013). It is evident in the light of all the treatments available, how it is important to know all the effects, desired, and side of drugs, but even more the characteristics of the patient requiring the treatment. Moreover, as recently demonstrated, improved knowledge and patient engagement is necessary not only from an ethic point of view, but it can also increase treatment adherence and compliance (LeBlanc et al. 2015).

Future perspective

It is very interesting that osteoporosis occurs in subjects exposed to microgravity despite daily exercise. This suggests the presence of underlying cellular and molecular mechanisms responsible for bone loss which understanding might lead to identify new therapeutic targets. Several authors have reported that gravity is necessary for cytoskeleton integrity of osteoblast-like cells and many other cell types (Hughes-Fulford et al. 1996; Acuto et al. 2000; Lewis et al. 2001; Schatten et al. 2001; Vassy et al. 2001; Hughes-Fulford et al. 2003). Moreover, it has been demonstrated that osteoblasts exposed to microgravity present reduced gene expression of transforming growth factor-β (TGF-β), fibroblast growth factor-2 (FGF-2), osteocalcin (OC), proliferating cell nuclear antigen (PCNA), c-*myc*, and cyclooxygenase-2 (Cox-2) when compared to normal gravity (Hughes-Fulford et al. 2006). Of interest, the gene of low-density lipoprotein receptor-related protein 5 (LRP5) plays a pivotal role in bone mass accrual while Wnt-mediated signaling via LRP5 affects bone accrual during growth and peak bone mass (Gong et al. 2001). Moreover, the mutation of *LRP5* gene has been associated with diseases in which there was high bone mass (Boyden et al. 2002; Little et al. 2002). Due to its inhibitory action on the Wnt signaling pathway, the protein Dkk-1 has been suggested as a potential therapeutic target for modulating bone mass.

Cells perceive their microenvironment not only through soluble signals but also through physical and mechanical stimuli, such as gravity, extracellular matrix stiffness, and confined adhesiveness. Indeed, by mechanotransduction systems, cells translate these cues into biochemical signals controlling multiple aspects of cell behavior and function, including growth and differentiation (Dupont et al. 2011). There are evidences that mechanical loading is responsible of the activation of the Wnt signaling pathway and that could be involved in the mechanotransduction (Johnson et al. 2004). Another pathway involved could be the Hippo pathway through the overexpression of the nuclear factors Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ, also known as WWTR1) (Dupont et al. 2011).

MicroRNAs (or miRNAs) are a class of short non-coding RNAs that regulate gene expression in several physiological and pathological cellular processes (Fassina et al. 2012). Recently, *miR-2861* has been identified as a promoter of osteoblast differentiation in primary mouse osteoblasts by repressing histone deacetylase 5 (HDAC5) expressions (Li et al. 2009). Notably, *miR-2861* is transcribed in stromal cells during bone morphogenetic protein 2-induced (BMP2-induced) osteogenesis and its overexpression enhanced BMP2-induced osteoblastogenesis (Li et al. 2009). Contrariwise, the inhibition of the expression of this miRNA reduced such osteoblastogenesis (Li et al. 2009). Of interest, *miR-2861* was found to be conserved in humans and a blocking mutation in this gene has been reported to cause primary osteoporosis (Li et al. 2009). Another miRNA (*i.e.* miR-34) has been demonstrated to be induced by BMP2 during osteoblast differentiation and to be critical during osteoblastogenesis by regulating Notch signaling pathway in bone homeostasis (Bae et al. 2012). This is very fascinating because preclinical models consistently underlined the feasibility and efficacy of miRNA-based therapies and recently a so-called "locked nucleic acid (LNA) anti-miR" construct has been successfully used to knock down a specific miRNA expression in a phase II clinical trial (Garzon et al. 2010; Lanford et al. 2010).

Conclusion

The data here reviewed indicate that our knowledge about bone mass loss due to long-duration spaceflights is limited. To date, countermeasures mainly consist in exercise and supplementation, which are inadequate. Additional and accurate data are needed to determine the real weight of exercise as countermeasure of osteoporosis. However, it seems likely to be insufficient to

prevent bone loss. The considerable experience with pharmacological treatment of osteoporosis on Earth, despite the differences of the environment, should be quickly transferred to astronauts during spaceflights and the effects controlled. Moreover, the recent understanding of cellular and molecular regulation of bone homeostasis can provide a consistent scientific basis for the development of new-targeted therapies.

References

Acuto O, Cantrell D: T cell activation and the cytoskeleton. Annu Rev Immunol 18, 165-184, 2000. http://dx.doi.org/10.1146/annurev.immunol.18.1.165

Bae Y, Yang T, Zeng HC, Campeau PM, Chen Y, Bertin T, Dawson BC, Munivez E, Tao J, Lee BH: miRNA-34c regulates Notch signaling during bone development. Hum Mol Genet 21, 2991-3000, 2012. http://dx.doi.org/10.1093/hmg/dds129

Baecker N, Frings-Meuthen P, Smith SM, Heer M: Short-term high dietary calcium intake during bedrest has no effect on markers of bone turnover in healthy men. Nutrition 26, 522-527, 2010. http://dx.doi.org/10.1016/j.nut.2009.06.006

Baron R, Vignery A, Horowitz M: Lymphocytes, macrophages and the regulation of bone remodeling. Bone Mineral Research 2, 175-243, 1984.

Barrett-Connor E, Mosca L, Collins P, Geiger MJ, Grady D, Kornitzer M, McNabb MA, Wenger NK; Raloxifene Use for The Heart (RUTH) Trial Investigators: Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. N Engl J Med 355, 125-137, 2006. http://dx.doi.org/10.1056/NEJMoa062462

Beral V: Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet 362, 419-427, 2003. http://dx.doi.org/10.1016/S0140-6736(03)14596-5

Bolognese MA, Teglbjærg CS, Zanchetta JR, Lippuner K, McClung MR, Brandi ML, Hoiseth A, Lakatos P, Moffett AH, Lorenc RS, Wang A, Libanati C: Denosumab significantly increases DXA BMD at both trabecular and cortical sites: results from the FREEDOM study. J Clin Densitom 16, 147-153, 2013. http://dx.doi.org/10.1016/j.jocd.2012.02.006

Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP: High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med 346, 1513-1521, 2002. http://dx.doi.org/10.1056/NEJMoa013444

Caillot-Augusseau A, Vico L, Heer M, Voroviev D, Souberbielle JC, Zitterman A, Alexandre C, Lafage-Proust MH: Space flight is associated with rapid decreases of undercarboxylated osteocalcin and increases of markers of bone resorption without changes in their circadian variation: observations in two cosmonauts. Clin Chem 46, 1136-1143, 2000.

Canalis E, McCarthy T, Centrella M: Growth factors and the regulation of bone remodeling. J Clin Invest 81, 277-281, 1988. http://dx.doi.org/10.1172/JCI113318

Cauley JA, Robbins J, Chen Z, Cummings SR, Jackson RD, LaCroix AZ, LeBoff M, Lewis CE, McGowan J, Neuner J, Pettinger M, Stefanick ML, Wactawski-Wende J, Watts NB: Women's Health Initiative Investigators. Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. JAMA 290, 1729-1738, 2003. http://dx.doi.org/10.1001/jama.290.13.1729

Cavanagh PR, Licata AA, Rice AJ: Exercise and pharmacological countermeasures for bone loss during long-duration space flight. Gravitational and Space Biology Bulletin 18, 39-58, 2005.

Chesnut CH 3rd, Silverman S, Andriano K, Genant H, Gimona A, Harris S, Kiel D, LeBoff M, Maricic M, Miller P, Moniz C, Peacock M, Richardson P, Watts N, Baylink D: A randomized trial of nasal spray salmon calcitonin in postmenopausal women with established osteoporosis: the prevent recurrence of osteoporotic fractures study. PROOF Study Group. Am J Med 109, 267-276, 2000. http://dx.doi.org/10.1016/S0002-9343(00)00490-3

Chesnut III CH, Skag A, Christiansen C, Recker R, Stakkestad JA, Hoiseth A, Felsenberg D, Huss H, Gilbride J, Schimmer RC, Delmas PD; Oral Ibandronate Osteoporosis Vertebral Fracture Trial in North America and Europe (BONE): Effects of oral ibandronate administered daily or intermittently on fracture risk in postmenopausal osteoporosis. J Bone Miner Res 19, 1241-1249, 2004. http://dx.doi.org/10.1359/JBMR.040325

Cummings SR, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, Delmas P, Zoog HB, Austin M, Wang A, Kutilek S, Adami S, Zanchetta J, Libanati C, Siddhanti S, Christiansen C; FREEDOM Trial: Denosumab for preven-

tion of fractures in postmenopausal women with osteoporosis. N Engl J Med 361, 756-765, 2009. http://dx.doi.org/10.1056/NEJMoa0809493

Droppert PM: The effects of microgravity on the skeletal system--a review. J Br Interplanet Soc 43, 19-24, 1990.

Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Bicciato S, Elvassore N, Piccolo S: Role of YAP/TAZ in mechanotransduction. Nature 474, 179-183, 2011. http://dx.doi.org/10.1038/nature10137

Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, Christiansen C, Delmas PD, Zanchetta JR, Stakkestad J, Gluer CC, Krueger K, Cohen FJ, Eckert S, Ensrud KE, Avioli LV, Lips P, Cummings SR: Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. JAMA 282, 637-645, 1999. http://dx.doi.org/10.1001/jama.282.7.637

Fassina A, Cappellesso R, Guzzardo V, Dalla Via L, Piccolo S, Ventura L, Fassan M: Epithelial-mesenchymal transition in malignant mesothelioma. Mod Pathol 25, 86-99, 2012. http://dx.doi.org/10.1038/modpathol.2011.144

Garzon R, Marcucci G, Croce CM: Targeting microRNAs in cancer: rationale, strategies and challenges. Nat Rev Drug Discov 9, 775-789, 2010. http://dx.doi.org/10.1038/nrd3179

Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML; Osteoporosis-Pseudoglioma Syndrome Collaborative Group: LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 107, 513-523, 2001. http://dx.doi.org/10.1016/S0092-8674(01)00571-2

Hawkey A: The physical price of a ticket into space. J Br Interplanet Soc 56, 152-159, 2003.

Heer M: Nutritional interventions related to bone turnover in European space missions and simulation models. Nutrition 18, 853-856, 2002. http://dx.doi.org/10.1016/S0899-9007(02)00905-X

Herzberg M, Foldes J, Steinberg R, Menczel J: Zinc excretion in osteoporotic women. J Bone Miner Res 5, 251-257, 1990. http://dx.doi.org/10.1002/jbmr.5650050308

Hodsman AB, Bauer DC, Dempster DW, Dian L, Hanley DA, Harris ST, Kendler DL, McClung MR, Miller PD, Olszynski WP, Orwoll E, Yuen CK: Parathyroid hormone and teriparatide for the treatment of osteoporosis: a review of the evidence and suggested guidelines for its use. Endocr Rev 26, 688-703, 2005. http://dx.doi.org/10.1210/er.2004-0006

Holick MF: Perspective on the impact of weightlessness on calcium and bone metabolism. Bone 22, 105S-111S, 1998. http://dx.doi.org/10.1016/S8756-3282(98)00014-3

Hughes-Fulford M, Lewis ML: Effects of microgravity on osteoblast growth activation. Exp Cell Res 224, 103-109, 1996. http://dx.doi.org/10.1006/excr.1996.0116

Hughes-Fulford M: Function of the cytoskeleton in gravisensing during spaceflight. Adv Space Res 32, 1585-1593, 2003. http://dx.doi.org/10.1016/S0273-1177(03)90399-1

Hughes-Fulford M, Rodenacker K, Jutting U: Reduction of anabolic signals and alteration of osteoblast nuclear morphology in microgravity. J Cell Biochem 99, 435-449, 2006. http://dx.doi.org/10.1002/jcb.20883

Hughes-Fulford M: To infinity and beyond! Human spaceflight and life science. The FASEB Journal 25, 2858-2864, 2011. http://dx.doi.org/10.1096/fj.11-0902ufm

Hurley LS, Tao SH: Alleviation of teratogenic effects of zinc deficiency by simultaneous lack of calcium. Am J Physiol 222, 322-325, 1972.

Igarashi A, Yamaguchi M: Increase in bone growth factors with healing rat fractures: the enhancing effect of zinc. Int J Mol Med 8, 433-438, 2001. http://dx.doi.org/10.3892/ijmm.8.4.433

Iki M, Kajita E, Dohi Y, Nishino H, Kusaka Y, Tsuchida C, Yamamoto K, Ishii Y: Age, menopause, bone turnover markers and lumbar bone loss in healthy Japanese women. Maturitas 25, 59-67, 1996. http://dx.doi.org/10.1016/0378-5122(96)01042-0

Johnson ML, Harnish K, Nusse R, Van Hul W: LRP5 and Wnt signaling: a union made for bone. J Bone Miner Res 19, 1749-1757, 2004. http://dx.doi.org/10.1359/JBMR.040816

Kanis JA, Burlet N, Cooper C, Delmas PD, Reginster JY, Borgstrom F, Rizzoli R; European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO): European guidance for the diagnosis and manage-

ment of osteoporosis in postmenopausal women. Osteoporos Int 19, 399-428, 2008. http://dx.doi.org/10.1007/s00198-008-0560-z

Keller TS, Strauss AM: Predicting skeletal adaptation in altered gravity environments. J Br Interplanet Soc 46, 87-96, 1993.

Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, Kauppinen S, Orum H: Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 327, 198-201, 2010. http://dx.doi.org/10.1126/science.1178178

Lang T, LeBlanc A, Evans H, Lu Y, Genant H, Yu A: Cortical and trabecular bone mineral loss from the spine and hip in long-duration spaceflight. J Bone Miner Res 19, 1006-1012, 2004. http://dx.doi.org/10.1359/JBMR.040307

LeBlanc A, Shackelford L, Schneider V: Future human bone research in space. Bone 22, 113S-116S, 1998. http://dx.doi.org/10.1016/S8756-3282(98)00013-1

LeBlanc A, Schneider V, Shackelford L, West S, Oganov V, Bakulin A, Voronin L: Bone mineral and lean tissue loss after long duration space flight. J Musculoskelet Neuronal Interact 1, 157-160, 2000.

LeBlanc A, Wang AT, Wyatt K, Branda ME, Shah ND, Van Houten H, Pencille L, Wermers R, Montori VM: Encounter Decision Aid vs. Clinical Decision Support or Usual Care to Support Patient-Centered Treatment Decisions in Osteoporosis: The Osteoporosis Choice Randomized Trial II. PLoS One 10, e0128063, 2015. http://dx.doi.org/10.1371/journal.pone.0128063

Lei SF, Papasian CJ, Deng HW: Polymorphisms in predicted miRNA binding sites and osteoporosis. J Bone Miner Res 26, 72-78, 2011. http://dx.doi.org/10.1002/jbmr.186

Lewis ML, Cubano LA, Zhao B, Dinh HK, Pabalan JG, Piepmeier EH, Bowman PD: cDNA microarray reveals altered cytoskeletal gene expression in space-flown leukemic T lymphocytes (Jurkat). FASEB J 15, 1783-1785, 2001. http://dx.doi.org/10.1096/fj.00-0820fje

Li H, Xie H, Liu W, Hu R, Huang B, Tan YF, Xu K, Sheng ZF, Zhou HD, Wu XP, Luo XH: A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. J Clin Invest 119, 3666-3677, 2009. http://dx.doi.org/10.1172/JCI39832

Lindsay R, Gallagher JC, Kagan R, Pickar JH, Constantine G: Efficacy of tissue-selective estrogen complex of bazedoxifene/conjugated estrogens for osteoporosis prevention in at-risk postmenopausal women. Fert Steril 92, 1045-1052, 2009. http://dx.doi.org/10.1016/j.fertnstert.2009.02.093

Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, Manning SP, Swain PM, Zhao SC, Eustace B, Lappe MM, Spitzer L, Zweier S, Braunschweiger K, Benchekroun Y, Hu X, Adair R, Chee L, FitzGerald MG, Tulig C, Caruso A, Tzellas N, Bawa A, Franklin B, McGuire S, Nogues X, Gong G, Allen KM, Anisowicz A, Morales AJ, Lomedico PT, Recker SM, Van Eerdewegh P, Recker RR, Johnson ML: A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. Am J Hum Genet 70, 11-19, 2002. http://dx.doi.org/10.1086/338450

MacLean C, Newberry S, Maglione M, McMahon M, Ranganath V, Suttorp M, Mojica W, Timmer M, Alexander A, McNamara M, Desai SB, Zhou A, Chen S, Carter J, Tringale C, Valentine D, Johnsen B, Grossman J: Systematic review: comparative effectiveness of treatments to prevent fractures in men and women with low bone density or osteoporosis. Ann Intern Med 148, 197-213, 2008. http://dx.doi.org/10.7326/0003-4819-148-3-200802050-00198

Masters DG, Keen CL, Lonnerdal B, Hurley LS: Release of zinc from maternal tissues during zinc deficiency or simultaneous zinc and calcium deficiency in the pregnant rat. J Nutr 116, 2148-2154, 1986.

Meunier PJ, Roux C, Seeman E, Ortolani S, Badurski JE, Spector TD, Cannata J, Balogh A, Lemmel EM, Pors-Nielsen S, Rizzoli R, Genant HK, Reginster JY: The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. N Engl J Med 29, 459-468, 2004. http://dx.doi.org/10.1056/NEJMoa022436

Nabavi N, Khandani A, Camirand A, Harrison RE: Effects of microgravity on osteoclast bone resorption and osteoblast cytoskeletal organization and adhesion. Bone 49, 965-974, 2011. http://dx.doi.org/10.1016/j.bone.2011.07.036

Nicogossian A, Pool S, Sawin C: Status and efficacy of countermeasures to physiological deconditioning from space flight. Acta Astronautica 36, 393-398, 1995. http://dx.doi.org/10.1016/0094-5765(95)00123-9

Oganov VS, Skripnikova IA, Novikov VE, Bakulin AV, Kabitskaia OE, Murashko LM: Characteristics of local human skeleton reactions to microgravity and drug treatment of osteoporosis in clinic. Aviakosm Ekolog Med 45, 16-21, 2011.

Ohshima H, Matsumoto T: Space flight/bedrest immobilization and bone. Bone metabolism in space flight and long-duration bed rest. Clinical Calcium 22, 1803-12, 2012.

Parfitt AM: Bone effects of space flight: analysis by quantum concept of bone remodelling. Acta Astronautica 8, 1083-1090, 1981. http://dx.doi.org/10.1016/0094-5765(81)90082-5

Reginster JY, Seeman E, De Vernejoul MC, Adami S, Compston J, Phenekos C, Devogelaer JP, Curiel MD, Sawicki A, Goemaere S, Sorensen OH, Felsenberg D, Meunier PJ: Strontium ranelate reduces the risk of nonvertebral fractures in postmenopausal women with osteoporosis: Treatment of Peripheral Osteoporosis (TROPOS) study. J Clin Endocrinol Metab 90, 2816-2822, 2005. http://dx.doi.org/10.1210/jc.2004-1774

Riggs BL, Jowsey J, Kelly PJ, Jones JD, Maher FT: Effect of sex hormones on bone in primary osteoporosis. J Clin Invest 48, 1065-1072, 1969. http://dx.doi.org/10.1172/JCI106062

Ronaghy HA, Reinhold JG, Mahloudji M, Ghavami P, Fox MR, Halsted JA: Zinc supplementation of malnourished schoolboys in Iran: increased growth and other effects. Am J Clin Nutr 27, 112-121, 1974.

Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J; Writing Group for the Women's Health Initiative Investigators: Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 17, 321-333, 2002.

Schatten H, Lewis ML, Chakrabarti A: Spaceflight and clinorotation cause cytoskeleton and mitochondria changes and increases in apoptosis in cultured cells. Acta Astronautica 49, 399-418, 2001. http://dx.doi.org/10.1016/S0094-5765(01)00116-3

Schneider SM, Amonette WE, Blazine K, Bentley J, Lee SM, Loehr JA, Moore AD Jr, Rapley M, Mulder ER, Smith SM: Training with the International Space Station interim resistive exercise device. Med Sci Sports Exerc 35, 1935-1945, 2003. http://dx.doi.org/10.1249/01.MSS.0000093611.88198.08

Schneider JP: Bisphosphonates and low-impact femoral fractures: current evidence on alendronate-fracture risk. Geriatrics 64, 18-23, 2009.

Shiraki M, Shiraki Y, Aoki C, Miura M: Vitamin K2 (menatetrenone) effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. J Bone Miner Res 15, 515-521, 2000. http://dx.doi.org/10.1359/jbmr.2000.15.3.515

Sibonga JD, Evans HJ, Sung HG, Spector ER, Lang TF, Oganov VS, Bakulin AV, Shackelford LC, LeBlanc AD: Recovery of spaceflight-induced bone loss: bone mineral density after long-duration missions as fitted with an exponential function. Bone 41, 973-978, 2007. http://dx.doi.org/10.1016/j.bone.2007.08.022

Sibonga JD: Spaceflight-induced bone loss: is there an osteoporosis risk? Curr Osteopor Rep 11, 92-98, 2013. http://dx.doi.org/10.1007/s11914-013-0136-5

Sirola J, Kroger H, Honkanen R, Jurvelin JS, Sandini L, Tuppurainen MT, Saarikoski S; OSTPRE Study Group: Factors affecting bone loss around menopause in women without HRT: a prospective study. Maturitas 45, 159-167, 2003. http://dx.doi.org/10.1016/S0378-5122(03)00150-6

Smith SY, Doyle N, Boyer M, Chouinard L, Saito H: Eldecalcitol, a vitamin D analog, reduces bone turnover and increases trabecular and cortical bone mass, density, and strength in ovariectomized cynomolgus monkeys. Bone 57, 116-122, 2013. http://dx.doi.org/10.1016/j.bone.2013.06.005

Smith SY, Jolette J, Chouinard L, Komm BS. The effects of bazedoxifene in the ovariectomized aged cynomolgus monkey. J Bone Miner Metab 33, 161-172, 2015. http://dx.doi.org/10.1007/s00774-014-0580-z

Strampel W, Emkey R, Civitelli R: Safety considerations with bisphosphonates for the treatment of osteoporosis. Drug Safety 30, 755-763, 2007. http://dx.doi.org/10.2165/00002018-200730090-00003

Vahle JL, Sato M, Long GG, Young JK, Francis PC, Engelhardt JA, Westmore MS, Linda Y, Nold JB: Skeletal changes in rats given daily subcutaneous injections of recombinant human parathyroid hormone (1-34) for 2 years and relevance to human safety. Toxicol Pathol 30, 312-321, 2002. http://dx.doi.org/10.1080/01926230252929882

Vassy J, Portet S, Beil M, Millot G, Fauvel-Lafeve F, Karniguian A, Gasset G, Irinopoulou T, Calvo F, Rigaut JP, Schoevaert D: The effect of weightlessness on cytoskeleton architecture and proliferation of human breast cancer cell line MCF-7. FASEB J 15, 1104-1106, 2001. http://dx.doi.org/10.1096/fj.00-0527fje

Vermeer C, Wolf J, Craciun AM, Knapen MH: Bone markers during a 6-month space flight: effects of vitamin K supplementation. J Gravit Phys 5, 65-69, 1998.

Vico L, Collet P, Guignandon A, Lafage-Proust MH, Thomas T, Rehaillia M, Alexandre C: Effects of long-term microgravity exposure on cancellous and cortical weight-bearing bones of cosmonauts. Lancet 355, 1607-1611, 2000. http://dx.doi.org/10.1016/S0140-6736(00)02217-0

Vogel JM: Bone mineral measurement: Skylab experiment M-078. Acta Astronaut 2, 129-139, 1975. http://dx.doi.org/10.1016/0094-5765(75)90049-1

Waugh N, Royle P, Scotland G, Henderson R, Hollick R, McNamee P: Denosumab for the prevention of osteoporotic fractures in postmenopausal women. Health Technol Assess 1, 51-59, 2011. http://dx.doi.org/10.3310/hta15suppl1/06

Wells GA, Cranney A, Peterson J, Boucher M, Shea B, Robinson V, Coyle D, Tugwell P: Alendronate for the primary and secondary prevention of osteoporotic fractures in postmenopausal women. Cochrane Database Syst Rev 1, CD001155, 2008. http://dx.doi.org/10.1002/14651858.cd001155.pub2

Yamaguchi M, Oishi H, Suketa Y: Stimulatory effect of zinc on bone formation in tissue culture. Biochem Pharmacol 36, 4007-4012, 1987. http://dx.doi.org/10.1016/0006-2952(87)90471-0

Yamaguchi M, Segawa Y, Shimokawa N, Tsuzuike N, Tagashira E: Inhibitory effect of beta-alanyl-L-histidinato zinc on bone resorption in tissue culture. Pharmacology 45, 292-300, 1992. http://dx.doi.org/10.1159/000139013

Yamaguchi M, Gao YH: Anabolic effect of genistein on bone metabolism in the femoral-metaphyseal tissues of elderly rats is inhibited by the anti-estrogen tamoxifen. Res Exp Med (Berl) 197, 101-107, 1997. http://dx.doi.org/10.1007/s004330050059

Yamaguchi M, Gao YH: Anabolic effect of genistein and genistin on bone metabolism in the femoral-metaphyseal tissues of elderly rats: the genistein effect is enhanced by zinc. Mol Cell Biochem 178, 377-382, 1998. http://dx.doi.org/10.1023/A:1006809031836

Yamaguchi M: Regulatory mechanism of food factors in bone metabolism and prevention of osteoporosis. Yakugaku Zasshi 126, 1117-1137, 2006. http://dx.doi.org/10.1248/yakushi.126.1117

Zittermann A, Heer M, Caillot-Augusso A, Rettberg P, Scheld K, Drummer C, Alexandre C, Horneck G, Vorobiev D, Stehle P: Microgravity inhibits intestinal calcium absorption as shown by a stable strontium test. Eur J Clin Invest 30, 1036-1043, 2000. http://dx.doi.org/10.1046/j.1365-2362.2000.00682.x