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Research Paper

Effects of long time exposure to simulated micro- and hypergravity on skeletal architecture

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ABSTRACT

This manuscript reports the structural alterations occurring in mice skeleton as a consequence of the longest-term exposition (90 days) to simulated microgravity (hindlimb unloading) and hypergravity (2*g*) ever tested. Bone microstructural features were investigated by means of standard Cone Beam X-ray micro-CT, Synchrotron Radiation micro-CT and histology.

Morphometric analysis confirmed deleterious bone architectural changes in lack of mechanical loading with a decrease of bone volume and density, while bone structure alterations caused by hypergravity were less evident. In the femurs from hypergravity-exposed mice, the head/neck cortical thickness increment was the main finding. In addition, in these mice the rate of larger trabeculae (60–75 μm) was significantly increased. Interestingly, the metaphyseal plate presented a significant adaptation to gravity changes. Mineralization of cartilage and bone deposition was increased in the 2 g mice, whereas an enlargement of the growth plate cartilage was observed in the hindlimb unloaded group.

Indeed, the presented data confirm and reinforce the detrimental effects on bone observed in real space microgravity and reveal region-specific effects on long bones.

Finally these data could represent the starting point for further long-term experimentations that can deeply investigate the bone adaptation mechanisms to different mechanical force environments.

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1. Introduction

The skeletal system provides support to the body, protects internal organs and enables stability and movements. Skeletal elements are made of an inorganic mineralized matrix and of several cell populations responsible for the fine-tuned remodeling process that ensures the bone tissue correct homeostasis and functionality.

Bone is a non-homogeneous and anisotropic tissue. It is made of a cortical (lamellar) and a trabecular (spongious) compartment with a space inside containing bone marrow. All the three compartments interact each other in order to create a supportive dynamic tissue able to renew itself and to adapt in response to inner or external stimuli. A fundamental characteristic of weight-bearing bones, such as femurs is that the spongious compartment shows a particular spatial disposition of trabeculae. Indeed, trabecular orientation depends on the direction and intensity of the force vector that insists on each individual bone.

Long bones are characterized by an irregular shape since weight bearing bones are designed to support and distribute compressive, bending and torsional forces. Since the 19th century, thanks to Wolff's law, scientists know that loading can influence the architecture of the bone tissue (Frost 1994; Wolff, 2010). It is clear that mechanostimulation represents one of the most important factors in influencing bone strength and structure. The mechanosensor machinery is represented by the three-dimensional network built by osteocytes, which is responsible for sensing the loading/unloading condition and translate the environmental signal in biochemical signals. Thus, mechanotransduction can condition bone remodeling balance and, consequently, bone microarchitecture. The force vectors act on the proximal and distal edges of femur becoming parallel while they move away from the edges of the bone, to induce the formation of a thicker compact cortical tissue in the shaft.

Extensive research work performed on the loading/unloading effects on bone tissue homeostasis have led to the observation that a gravity force other than the Earth's is able to unbalance the bone turnover process. Indeed, microgravity as well as hypergravity exert a critical influence on bone microarchitecture (Carmeliet 1999; Kohles et al., 1996). We had the possibility to investigate and clarify some aspects related to effect of the loading/unloading condition on weight-bearing bones microarchitecture. This kind of studies plays an important role in the improvement of the knowledge on exercise-linked bone strengthening, aging and diseases leading to bone loss.

Spaceflights are considered the best environment to investigate the unloading effects on the skeleton. In this scenario the Mouse Drawer System (MDS) study (Cancedda et al., 2012) represents a milestone for the animal space experimentation. Indeed, this mission marked the longest duration animal experiment in space.

However, it is worth noting that in space gravity corresponds to about 90% of gravity force on Earth, but it is felt like near-zero gravity for its centrifugal force around the Earth. Thus it should be much more correct to refer to “unloading” rather than to “microgravity” when speaking about space-related studies. However “microgravity” is commonly used as a synonym for “unloading condition” and this paper followed this convention (Ruggiu and Cancedda 2014).

Since years, investigators take advantage of microgravity simulation techniques that can be performed on Earth; several studies have been carried out in vitro by means of cell bioreactors and tissue engineering approaches (Pardo et al., 2005; Ko et al., 2010). A microgravity environment can be simulated on Earth through several in vivo systems, such as in the case of bed-rest tests for human volunteers (Ruggiu and Cancedda 2014; LeBlanc et al., 1987; Noble 2003; Zerwekh et al., 1998; Ziambaras et al., 2005), or hind limb unloading (HLU) test for rodents (Saxena et al., 2011; Visigalli et al., 2010). Particularly, a limited number of publications revealed that short-term mice exposition to simulated microgravity (HLU) produced a bone volume density loss similar to that observed by our long-term experimentation. This change was attributed to the decrease of bone formation in association with normal or even increased bone resorption (Carmeliet, 1999). Another work reported how a short-term period (18–22 days) of exposure to microgravity affects skeletal tissue dynamics in rat aboard different space flights (Simmons 1981). These experiments revealed a reduced osteogenesis and new bone apposition in the weight-bearing bones (femurs and tibiae). Moreover, the rate of osteoid maturation and the degree of mineralization in these bones was diminished (Morey and Baylink, 1978; Wronski et al., 1980). Recently, mice aboard STS-131 space shuttle for 15-days showed decrement in bone volume fraction and bone thickness in pelvis for osteocytic osteolysis, and cell cycle arrest during osteogenesis (Blaber et al., 2013). The bone growth reduction could be associated with a failure of differentiation of osteoprogenitor cells and their conversion to osteoblasts (Roberts et al., 1981) while in long bones one should also consider the replacement of the osteogenic marrow with fat tissue (Wronski et al., 1980).

Conversely, hypergravity condition can be obtained using a centrifuge on Earth. Some studies reported the effects of hypergravity on the long bones of different species (Doden et al., 1978; Prodanov et al., 2013; Martinez et al., 2008), highlighting a cell mediated bone positive remodeling.

However, the number of studies on the response of the mature skeleton to gravity changes is limited and several aspects remain unsolved. Unfortunately, major technical and economical constraints do exist to the implementation of experiments to be performed. First of all, in particular for microgravity investigations, the limited number of flight opportunities and the practical difficulties in implementing scientific systems that fit to the space vessels and facilities surely hamper the possibility to perform a large-scale scientific research work.

In order to improve our knowledge of the effects of different mechanical cues linked to different loading conditions to interfere on bone deposition, we investigated the alterations of the bone microstructure occurred with the exposure of 2 months old C57BL/10 J mice, that were expected to complete the bone growth process during the experimental period, to simulated microgravity and hypergravity for 90 days.

To this purpose, we took advantage of HLU for microgravity and centrifugation for hypergravity as valid scientific