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Review Article

Hypergravity and its effects on bones and the musculoskeletal system: a narrative review

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Abstract

The entrance of mankind into the *Space Age*, accomplished by the second half of the 20th century, and the advances in modern physics have completely transformed the way we perceive the role of gravity. The musculoskeletal system is long known to be heavily affected by the gravitational forces, resulting in marked loss in bone mineral density in the setting of microgravity. The meticulous study of the underlying pathophysiologic mechanisms is pivotal in order to identify possible therapeutic targets for the management of the microgravity-induced changes in bone physiology during spaceflight missions, as well as the osteopenia induced changes in the setting of osteoporosis affecting a vast majority of elder individuals. In the present bibliographic narrative review, the importance of mechanisms employed by cells in order to perceive and respond to altered gravity are discussed. Current *in vitro* and *in vivo* studies focusing on the effect of hypergravity on the musculoskeletal system are also presented. Overall, the variability in study design of the available published data makes the deduction of safe conclusions rather challenging and uncertain. It is important that future studies address the matter by employing similar research methodology and study design in order to increase the comparability of their results.

Keywords: Hypergravity, Microgravity, Space, Bone cells, Musculoskeletal

Introduction

Gravity holds a pivotal role in shaping the Universe the way we perceive it nowadays. Planets, stars and whole galaxies are constantly interacting with each other through the gravitational attraction between them. Gravity is responsible for the revolution of the Earth around the Sun and the Moon around the Earth, thus having tremendous impact on the existence of life on Earth.

The need of human beings to understand the natural world that they live in has led, from the early ages of human history, in many theories and explanations. As early as the 4th century B.C. *Aristotle*, the classical ancient Greek philosopher, formulated the theory of the four elements, according to which the world is made up of four primary elements ("*earth*", "*air*", "*fire*" and "*water*") and the properties of each entity is dictated by the combination of the elements they consist of[1]. Aristotle believed that "*earth*" and "*water*" have the inherent tendency to move towards the center of the Earth, while "*air*" and "*fire*" the tendency to move away from it. *Aristotle's* theory was one of the first attempts to explain the force of gravity and prevailed for many centuries, until *Isaac Newton* gave the notion of attracting forces its current gravitational meaning. Isaac Newton formulated the universal law of gravitation in 1687, which states that every particle attracts every other particle with a force that is directly proportional of their masses and inversely related to the square of the distance between them[2]. Two more centuries went by until *Albert Einstein* completely transformed our perception about gravity. In

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1915 he proposed the theory of general relativity, according to which gravity is not like the other forces encountered in nature. Einstein suggested the revolutionary idea that gravity is an aftereffect of the fact that the space-time continuum is curved by the presence of mass and energy[3,4]. As a result, matter is not moving in circular trajectories compelled by any force, but is rather moving in "straight" lines of curved space-time, known as geodesic curvatures.

Despite the immense advances of physics in the effort to explain the natural world we live in, the nature of gravity remains a debatable issue and no consensus exists yet among the prevailing theories. The two most well-established theories of our era, that of general relativity and that of quantum mechanics, strongly disagree on the nature of gravity. Therefore, new theories, like string theory, have emerged in order to fill in the gap of the existing ones[3]. Nowadays, the rapid evolution of technology has enabled scientists to observe and measure phenomena in the universe dominated by gravity, like black holes[5]. In these extreme conditions we could gather important information about gravity that can put the existing theories to test and also allow new theories to emerge.

More importantly, by reproducing these conditions, we could infer valuable deductions regarding the role of gravity on shaping life on Earth and employ them in order to deepen our understanding in the pathophysiology of disease processes and beyond that, to design new treatment options.

Literature search method

The MEDLINE/PubMed database was searched using "*Hypergravity*", "*Centrifugation*", "*Bone Metabolism*" and "*Cell Proliferation*" as keywords. All references from the identified articles were searched for relevant information. The end date of the literature search was set to October 2018. Our search was focused on the latest published information regarding the effect of hypergravity on the musculoskeletal system.

How cells perceive gravity

Cytoskeleton is an essential component of the support system of eukaryotic cells, which is comprised of an extended network of microtubules, microfilaments and intermediate filaments. Gravity has been shown to hugely affect cell cytoskeleton[6]. This disruption of the normal cell architecture could affect a plethora of procedures ranging from cell signaling to cell proliferation and apoptosis[7]. This view was further amplified by the study of substances that are directly related with normal cytoskeleton and intercellular communication. Examples include the under-expression of protein ICAM-1 in the setting of microgravity and the fundamental role of the family of Rho GTPases, which act as mechanosensitive molecular switches that drive the reorganization of cytoskeleton[8,9].

The exact mechanism the cells employ in order to perceive the gravitational changes in not yet fully understood. There is evidence indicating that the model of tensegrity is involved, as proposed by *Ingber* in 1999[10]. According to *Ingber*, cells are interconnected by wiring of various points of their cytoskeleton and the surrounding extracellular matrix (ECM) mainly through integrins. Therefore, they are under constant tension, just like connective tissue keeps bones aligned and stable, conveying mechanical forces without the existence of direct contact between them. The balance of the forces applied by the ECM in the connection points with the cytoskeleton is highly important and thus any disruption can lead to changes in cell shape, profoundly affecting intracellular signaling and subsequently gene expression[10,11]. When cells perceive the gravitational change microtubules are rapidly re-orientated and actin filaments increase their density in order to provide optimal mechanical support. The simultaneous opening of calcium channels also assists this process by altering the cell membrane potential, as TRP (transient receptor potential) cationic channels work in mastigophora Euglena[11].

Centrifugation as a method to create hypergravity conditions

In order to overcome the changes on the physiology of the human body during spaceflights created by microgravity, it has long been investigated the issue of creating artificial gravity. Centrifugation has been proven successful in creating the angular momentum needed to give a similar perception with that of actual gravity. The rotation around a constant axis creates centripetal force. Nevertheless, unlike the real gravitational field, artificial gravity created by centrifugation behaves differently to some extent. For example, the gravitational force created is related to the radius of the centrifugation. When subjecting human beings in centrifugation, the *Coriolis* forces created by the rotational movement can influence their vestibular system, causing nausea and motion sickness[12]. In order to achieve the gravity that is normally perceived by individuals on Earth, large radius, around 15 meters, and slow rpm (rotations per minute) are required, around 10 rpm[12]. The same principles apply when the issue of creating hypergravity conditions is addressed. However, in the setting of experimental conditions used with cells cultures and laboratory subjects, the gravitational forces achieved and the duration of centrifugation is rather variable among the existing literature. Laboratory animals are subjected to less extreme g forces of about 2-10 g, while the g forces applied to cell cultures can reaches very high values (20, 50 or even 150 g)[13-15].

Studies on the effects of hypergravity on bones and musculoskeletal system

As already mentioned, the cell cytoskeleton is one of the most important structures heavily influenced from gravitational alterations. Its ability to sense mechanical forces and convey them intracellularly makes it work as a "gatekeeper" responsible to protect and adapt cell shape and morphology by any means. Therefore, many studies focused on the effects of hypergravity on cytoskeleton. Grute et al. (1995) subjected human dermal fibroblasts in gravity 1-20 g for 8 days[16]. They noticed rearrangement of their cytoskeleton into a "star"-shape formation instead of the normal round morphology when exposed to gravity above 15 g. Migration of tubulin intracellularly was also altered in order to better support the nucleus under stress conditions. The extracellular meshwork was thickened by increasing the density of collagen fibers without, though, affecting the proliferation rate of the cells. Similar results were reached by Kacena et al. in osteoblasts submitted in 1-4 g gravitational field[17]. The authors noted a marked increase in the number and thickness of actin filaments, fibronectin and vinculin but no change in the proliferation rate of osteoblasts. Other, more recent, studies also indicate the increase in actin fiber density, but not number, even in low g forces (about 5 g)[18-20]. These results are in line with a recent study performed on human tendon cells cultured in 15-20 g for 16 hours[21]. While many studies suggest that the cell proliferation rate remains unaffected by increases in gravity in an effort to direct their resources in better managing the increased stress they are under, the issue is still debatable as the results of various studies are contradicting. Gebken et al. (1999) reported a slight reduction (about 20%) in cell number after incubation in 13 g for a day[22]. On the other hand, a study performed by Miwa et al. in 5 g hypergravity indicated an increased osteoblast proliferation rate without change in osteoblast differentiation, possibly mediated by autocrine and paracrine signaling of PGE_2, as supported by other studies in osteoblasts and endothelial cells[23,24].

Hypergravity has also been shown to significantly affect collagen synthesis. Gebken et al. (1999) found a significant increase in mRNA levels of collagen Ia2 in 13 g driven by marked increase in phosphorylation of the p44/42 MAP-kinases (ERK1/2)[22]. Moreover, the transcription activity of lysine hydroxylase and lysine oxidase, enzymes pivotal in collagen synthesis and crosslinking, has been shown to increase in 20 g conditions[25]. Van Loon et al. in 1995 on his thesis regarding the effects of a wide range of accelerations from microgravity to hypergravity noted that increased stress resulted in increased mineralization and calcium release in *in vitro* cultures of the long bones of fetal mice[16]. As an inherent component of the musculoskeletal system, myoblasts are also affected by hypergravity. A study performed in mice myoblasts concluded that there is increased myosin expression and subsequently increased myoblast differentiation rate in 20 g[26]. Neural cells also exhibit alterations in cell metabolism directly related to increases in gravitational forces (from 1 to 150 g) and stimulation of transcriptional activity of genes involved in neural cell maturation[15].

When the musculoskeletal system is studied, *Wolff's* law should always be bear in mind. As early as in 1892, *Wolff* stated that changes in the form and function of bone are followed by changes in both bone's inner architecture and its external appearance and vice versa[27]. In other words, this means that the bones (as well as muscles and tendons) have the inherent ability to adapt to the load under which they are placed[28]. The importance of Wolff's law is highlighted in many *in vivo* studies. The majority of *in vivo* studies, predominantly performed in mice, have been proven very useful in elucidating the effects of hypergravity on bones. An experiment by Vico et al. in 1999 resulted in increased bone mass, especially cancellous bone in tibial metaphysis, of rats after centrifugation in 2 g for 4 days[29]. These results were in accord with those of studies performed more than 20 years previously, as those by Jaekel et al. in 1977 and Keil et al. in 1969[30,31]. More recently, Ikawa et al. (2011) also showed increased bone mineral density in rat trabecular bone under hypergravity of about 3 g, via reduction in both bone resorption and formation, as indicated by biochemical and histomorphometric analysis[32]. Data from the study of Canciani et al. in 2015 also suggest that long-term (of about 3 months) exposure to 2 g result in increased bone formation and mineralization. Nevertheless, a safe range between 2 g and 3 g was suggested by Gnyubkin et al. (2015), as cortical thinning, decreased bone formation and increased osteoclast function was observed after long exposure (of about 20 days) under 3 g environment[13]. Finally, a human study performed in flight pilots indicated that gravitational forces between 2 and 6 g can also increase BMD in a region-specific manner[14].

Discussion

The entrance of mankind into the Space Age that was accomplished by the second half of the 20th century has completely transformed the way we perceive gravity. Given the current increase in the number of manned space missions, it has been easier than ever before to study the effects of altered gravitational field on the human physiology. However, the effects of microgravity, in which astronauts are exposed during space flights, are favored over those of hypergravity. Even in the setting of hypergravity mainly low values of gravity are usually studied (1-10 g), as these are more relevant to the return of astronauts on Earth. Nevertheless, the exposure of human cells in conditions of extreme gravity could shed more light in the way cells behave in such conditions, but also the mechanism of adaptation employed by cells and organisms. The elucidation of the effects of hypergravity could also suggest possible therapeutic methods for the management of the microgravity-induced changes in bone physiology during spaceflight missions, as well as the changes induced in bone mineral density in the setting of osteoporosis or those induced by prolonged bed rest.

The data available in current literature are rather variable. The design of each study, in terms of the gravitational force achieved and the duration of the exposure of either the cell cultures or the test subjects, is practically unique for each study, making the comparison between them and the deduction of safe conclusion very challenging. In the present literature review, an effort was made to include the latest data published regarding the effect of hypergravity on the musculoskeletal system.

Conclusion

Given the importance of gravity in the shape and function of the musculoskeletal system and bone growth, the study of hypergravity is invaluable and promising. New treatment options can arise addressing both the microgravity-induced osteopenia and the osteopenia induced in the setting of osteoporosis in the elderly or that induced in bedridden individuals. Towards this direction, the design of studies with similar methodology is important in order to deduct safe and homogenous conclusions.

Authors' contributions

***CA**: collected literature, drafted the manuscript, **GIL**: proof-read the manuscript, drafted the manuscript, provided critical review, gave final permission for submission.*

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