Bioeffects of Microgravity and Hypergravity on Animals*

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Abstract: Gravity alterations in space cause significant adaptive effects on the human body, including changes to the muscular, skeletal, and vestibular systems. However, multiple factors besides gravity exist in space; therefore, it is difficult to distinguish gravity-related bioeffects from those of the other factors, including radiation. Although everything on the Earth surface is subject to gravity, gravity-induced effects are not explicitly clear. Here, different research methods that have been used in gravity alterations, including parabolic flight, diamagnetic levitation, and centrifuge, are reviewed and compared. The bioeffects that are reported to be associated with altered gravity in animals are summarized, and the potential risks of hypergravity and microgravity are discussed, with a focus on microgravity, which has been studied more extensively. It should be noted that although various microgravity and hypergravity research methods have their limitations, such as the inevitable magnetic field effects in diamagnetic levitation and short duration of parabolic flight, it is evident that ground-based clinical, animal, and cellular experiments that simulate gravity alterations have served as important and necessary complements to space research. These researches not only provide critical and fundamental biological information on the effects of gravity from biomechanics and the biophysical perspectives, but also help in developing future countermeasures for astronauts.

Keywords: Gravity, microgravity, hypergravity, bioeffects, static magnetic field (SMF)

1 Introduction

Since the launch of human spaceflight in 1961, the bioeffects of altered gravity have been reported. The microgravity in space is a main risk factor that threatens the health of astronauts, acting synergistically with other space environmental hazards [1-2]. The multi-omics analysis highlights pathways and mechanisms of vulnerability in human spaceflight; however, effects caused by altered gravity are difficult to isolate via space biology studies alone. During the launch phase of accelerated ascent, the astronauts experience hypergravity. In space, the astronauts are in a state of microgravity [3]. Over 20 years ago, it was reported that microgravity and hypergravity could result in a variety of bioeffects in physiological, cellular, and molecular levels, and in particular, there are obvious changes in physiological systems, such as the musculoskeletal, neuro-ventricular, and cardiovascular systems [4]. In the early stages of spaceflight, body fluids shift from the lower limbs to the head owing to the lack of gravity and reach a stable state within a few days [5]. After a longer period of flight, the pressure removal-induced muscle and bone reduction became more apparent [6-7]. Certain crew also experienced a neuro-ocular syndrome characterized by optic disk edema, retina folds, and flattening of the sclera after long-term missions [8].

It is obvious that physiological systems are forced to adapt at varying degrees in microgravity space to counteract the environment [9]. Regarding the time span experienced by the astronauts, microgravity

Manuscript received February 3, 2023; revised February 13, 2023; accepted February 22, 2023. Date of publication March 31, 2023; date of current version March 3, 2023.
* Corresponding Author, E-mail: xinzhang@hmfl.ac.cn
* Supported by the National Natural Science Foundation of China (U21A20148) and Collaborative Innovation Program of Hefei Science Center CAS (2022HSC-CIP002).
Digital Object Identifier: 10.23919/CJEE.2023.000011

effects act much longer than hypergravity, even for short-term missions. Therefore, research is more focused on microgravity, and only a few studies are focused on hypergravity [10]. Therefore, it is necessary to get a more comprehensive understanding about the physiological changes caused by altered gravity from space-based and ground-based experiments, including both microgravity and hypergravity, which will provide critical information to facilitate the development of related countermeasures for astronauts.

2 Experimental conditions used in medicine or physiology studies

Definitely, research results in space provide important basis for ground experiments. However, owing to the very limited flight opportunities, various devices have been developed to simulate the space environment. This part specifically focuses on methods and facilities for medical and physiological experiments, but not other methods that are more frequently employed to study physical and chemical phenomenon, such as the drop tower.

2.1 Microgravity generation facilities and analogs

2.1.1 Parabolic flight

Aircraft flight along a parabola can provide repeated microgravity and hypergravity cycles. The microgravity time depends on the speed of the vehicle and angle of entry into the maneuvering trajectory [11]. The aircraft ascends at an angle after a period of steady flight, then the engine reduces but still maintains a certain power to compensate for air resistance, thus entering a microgravity state (Fig. 1). The cycle interval of the parabolic flight is determined by the operator, either as a continuous roller coaster-like flight, or as a transition via a cruise flight interval. This simulation method provides the opportunity to truly fly-off the ground at a relatively low cost, and its controlled microgravity meets the experimental requirements. However, the processes of hypergravity states are interspersed with microgravity, which makes it difficult to separate the individual conditions exactly. Moreover, the entire microgravity phase lasts for a fairly short period of time; hence, this method is not very suitable for long-term follow-up studies [12].

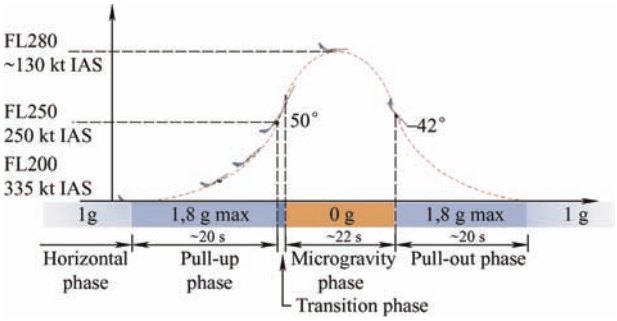

Fig. 1 Maneuvering trajectory during a parabolic flight (The figure illustrates the pull-up, microgravity, and pull-out phases of a parabolic flight. FL 280, FL250, and FL200 represent the flight altitudes, and 130 kt IAS, 100 kt IAS, and 335 kt IAS represent the indicated airspeed to match the altitude. Reproduced with permission from Ref. [11], Copyright of ©2016 Reach)

2.1.2 Diamagnetic levitation

Although a parabolic flight can achieve both microgravity and hypergravity, the duration is very short. An alternative means to simulate both hypergravity and microgravity is via gradient static magnetic field (SMF). Owing to the bore size limitation, this method is mainly employed to study cell models or small-sized model organisms. For example, Guevorkian et al. [13] used SMFs provided by a resistive magnet to investigate the locomotor behavior of paramecium (Fig. 2). SMFs provided by superconducting magnets were also used for multiple gravitational biology studies (Fig. 3) [14-16].

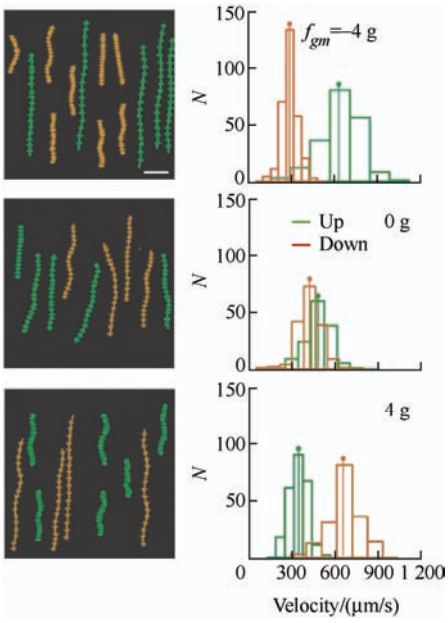

Fig. 2 Trajectory and velocity distributions of upward swimming and downward swimming paramecium under different gravities (Reproduced with permission from Ref. [13], Copyright of ©2006 PNAS)

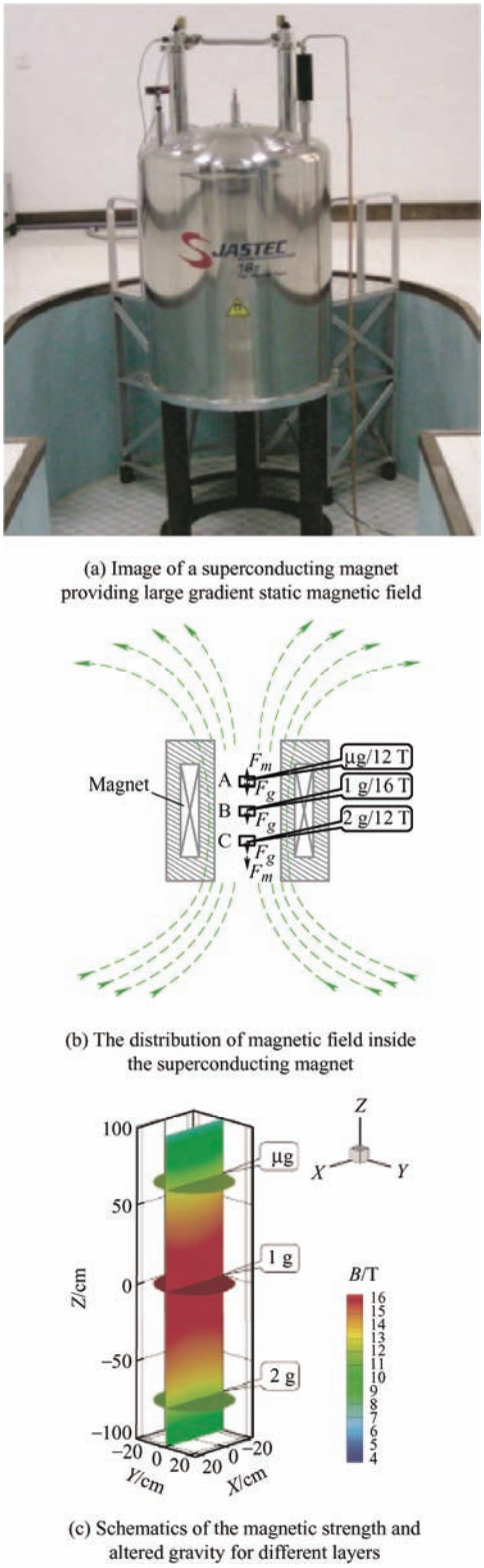

Fig. 3 Schematic of superconducting magnet (Reproduced with permission from Ref. [16], Copyright of ©2018 Bioelectromagnetics)

2.1.3 Hindlimb unloading

Hindlimb unloading (HU) is routinely applied to simulate the displacement of body fluids that occurs in astronauts during spaceflight and is a widely employed ground-based simulation method for rodents. The HU model utilizes a tail ring to immobilize the tail of rodents and raises the hindquarters of the rats or mice by adjusting the height, during which the head is in a relatively low position (Fig. 4). This results in fluid shift from the head, and lack of ground support from the hind limbs [17]. Different from the other microgravity generating devices, the gravity remains unchanged during HU, and only the pressure exerted on the hind limbs is shifted by the postural alteration. At the same time, the suspended rodent has a much reduced body movement. Specifically, HU provides an analog of microgravity condition, which can be used for long-term simulated microgravity studies, especially for effects from the cardiovascular, musculoskeletal, and central nervous systems.

Fig. 4 Rodent hindlimb unloading suspension model (Reproduced with permission from Ref. [17], Copyright of ©2020 Reach)

2.1.4 Bed rest

Bed rest is frequently used as a promising alternative to simulate the gravity unloading for humans, which can be performed for up to 70 days. This helps to understand the adaptive changes of physiological systems and tissues to microgravity during spaceflight. Initially, bed rest employed the horizontal supine position, which can eliminate the Gz vector. However, six degrees of head-down tilt was eventually introduced because it is more consistent with the body fluids displacement toward the head in actual space conditions [18]. For example, Laurie et al. [19] have documented changes in optic disc edema after 30 days of strict 6° head-down-tilt bed rest (HDBR) in

subjects, which provides a reference for clinical countermeasures.

2.1.5 Clinostat

To date, clinostats are highly established as a paradigm for simulating microgravity, especially for “in vitro” experiments. For example, the random positioning machine (RPM) and rotating wall vessel (RWV) developed by NASA are extensively employed for microgravity investigation. The RWV reactor rotates around a horizontal axis with a rotation frequency that matches the cell settling speed, thereby keeping the cells in complete suspension. The interior is a low-shear, low-turbulence culture environment filled with culture medium. Similarly, RPM operates with its direction and velocity altered stochastically based on the two-axis feature (Fig. 5) [20]. However, it should be noted that it is impossible to completely remove the role of gravity from either type of rotator. In contrast, the rotation of the vessel cancels the directionality of gravity to a modest extent.

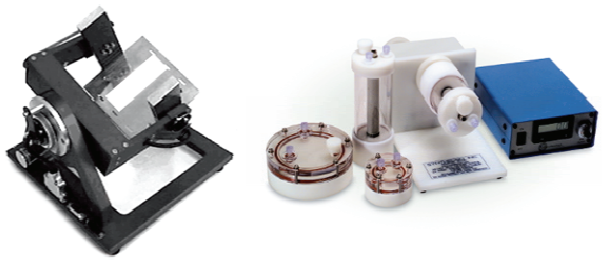

Fig. 5 RPM (left) and RWV bioreactor (right) (Reproduced with permission from Ref. [20], Copyright of ©2013 Nature)

2.1.6 Others

In addition to the experimental conditions aforementioned, there are also a few other methods that can be employed to simulate microgravity or hypergravity. For example, drop towers are also frequently utilized in microgravity research, which can provide short time “weightless” conditions for basic physics, materials science, and other studies [21-23]. The sustained period of microgravity depends on the height of the tower. Sounding rockets and other non-orbiting launch facilities are capable of moving above the atmosphere without orbiting around the Earth. Recovered by a parachute after a stable free-fall, sounding rockets have been utilized in several disciplines, especially to study gravity-sensing mechanisms in plants [24]. In addition to the International Space Station (ISS), CubeSats can also be used for experiments that can be miniaturized in orbital microgravity. Such modular microsatellites are also utilized to test spacecraft technology or conduct experiments with equipment serving large satellites [25-26].

Ground-based microgravity analogs are widely employs as a promising alternative to the space environment. For example, the analogs derived from the use of buoyancy to balance gravity are commonly used in training astronauts or testing procedures [27]. Furthermore, there is dry immersion, which can accurately reproduce the short-term physiological effects of space. Test subjects wrapped in waterproof, high-elasticity fabric are immersed in water to the neck, replicating the unloading in space [28]. The unilateral lower limb suspension (ULLS) method specifically employed to study the functionality of muscles in the spatial environment has similarities to tail suspension in rodents. ULLS involves crutches and a platform shoe to ensure that the other foot always drops naturally and avoids contact with the ground. This method is inexpensive but lacks standards, thus increasing the variability of the data [29-30].

2.2 Hypergravity generation facilities and analogs

To the best of our knowledge, there are several methods that can be used to study hypergravity, including centrifuge, parabolic flight, and magnetic field.

Hypergravity centrifuges are most commonly utilized to simulate hypergravity conditions, which can be used for cellular and animal research. The utilization of rotors to place organisms in different hypergravity environments can be cross-referenced with studies in microgravity conditions to better understand the effects of altered gravity on organisms. The European Space Agency (ESA) has constructed a sophisticated gravity experimental platform specifically designed for animal models. This platform allows for prolonged studies of aquatic animals and

rodents that have been exposed to hypergravity, with the added capability of adjusting gravity up to 4g using the rodent rotor [31]. Cesari et al. [32] utilized the large diameter centrifuge (LDC) facility to treat human microvascular endothelial cells at high g values and obtained improvements in cell motility and function.

In addition to the centrifuge, hypergravity also occurs during parabolic flight and in magnetic fields. For example, Acharya et al. [33] exposed human cardiomyocytes (hCMs) to this gravity condition to study functional changes. However, the acute hypergravity and microgravity during this period last for a relatively short period. The gradient SMFs of high intensities not only enable the levitation of small mammals, but can also provide a hypergravity environment (Fig. 3b). Inevitably, animals are exposed to different gravities along with high-intensity magnetic fields, which is the major factor that currently limits the result interpretation.

To help people get a clear understanding of the methods that are frequently used to study microgravity and hypergravity, we summarized these methods in Tab. 1 to compare their advantages and disadvantages (Tab. 1). In addition, there are a few very comprehensive reviews about the advantages and limitations of various microgravity platforms, which will provide more detailed information about the microgravity-related research [20, 27].

Tab. 1 Comparison of various commonly employed methods in microgravity and hypergravity studies

Advantages and disadvantages	Parabolic flight	Diamagnetic levitation	HU	Bed rest	RPM	RWV	Centrifuge	Space station
Microgravity	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Hypergravity	Yes	Yes	No	No	No	No	Yes	No
Longest duration	~20 s	Up to hours or weeks	Up to weeks or months	Up to weeks or months	Up to hours or weeks	Up to hours or weeks	Up to hours or weeks	Up to months or years
Biological systems	Humans and cells	Animals and cells	Animals	Humans	Cells	Cells	Animals, cells	Human, cells
Limitations	Short time	Inevitable magnetic field effects	Stress behaviors; localized necrosis	long adaptation period	Size limited; fluid shear	Size limited; fluid shear	More demanding on the rotors	Costly; rare opportunities

3 The bioeffects of altered gravity

This section mainly summarizes reported gravity-related physiological effects, including the immune, skeletal, muscular, cardiovascular, visual, and neurological systems.

3.1 Physiological effects of microgravity

3.1.1 Immune dysregulation

Spaceflight serves as a unique stress model, where the human immune system is exposed to continuous or intermittent stimulation by multiple extreme factors that threaten the safety of astronauts and performance of missions. Gravity alters the distribution of body fluids, which may affect established immune pathways at 1 g. During and after a spaceflight, various immune parameters are changed, but the amplitude and mode of these changes vary widely from mission to mission, even among crew members on the same mission [34-35]. Alterations in the immune system induced by spaceflight or analogs occur primarily in cell-mediated immune responses, including leukocyte proliferation, cytokine production, and the distribution of leukocyte subsets [36]. Studies conducted on T cells during spaceflight, or using ground-based culture systems, have exhibited reduced cytokine production, proliferation, and effector functions [37]. Analysis of blood samples from space astronauts experiencing short-duration spaceflight revealed that cytotoxicity, central memory, and senescence of $CD8^+$ T cell subsets were altered during spaceflight. At the same time, virus specific T cell function was depressed both

during and after the flight. Cytokine production profiles following mitogenic stimulation were significantly altered during and after the spaceflight [38]. During long-term spaceflight, alterations in $CD8^+$ T cell subsets, reductions in T-cell function, and decreased production of cytokine stimulated by mitogen were observed. Crucian et al. [39] attributed the decrease in cytokine secretion during flight to a reduction in bulk production capacity rather than the discharge of cytokine-secreting T cells from the peripheral circulation. However, B-lymphocytes were not significantly affected after the spaceflight. Spielmann et al. [40] determined that B cell homeostasis was maintained during a prolonged spaceflight. Spaceflight had no effect on the number and ratio of different B-cell subpopulations, and there was no difference in the kappa free light chain (FLC) between pre-flight samples and in-flight or recovery samples. Here, IgG and IgM remained unchanged during and after the spaceflight compared to the pre-flight values. The maintenance of B-cell homeostasis will contribute to the development of viable countermeasures against altered immune function in space.

Downregulation of immune functions may account for viral reactivation events and opportunistic infections associated with multi-mission astronauts [37]. The decline in cellular immune function is usually accompanied by the reactivation and shedding of the potential infectious virus. Human cytomegalovirus (CMV) causes immune diseases in individuals with immature or impaired immunity. Urine samples from the astronauts indicated a significant increase in CMV shedding frequency before flight and an increase in CMV antibody titers in certain crew. It is certain that CMV reactivation happened before the spaceflight, and CMV may cause further reactivation in spaceflight [41].

Published data on aerospace and immunization can be categorized as the time or space (ground or universe) in which the data were collected (Tab. 2). There are a few points worth mentioning. The first is that pre-flight, in-flight, and post-flight data need to be differentiated because the stress response of immune system constantly changes before and after flights, and recovery may occur upon return. The second is that some aspects of flight may be simulated from ground analogs, but none of them can replicate all flight aspects [42]. Here, we focus on studies that simulate gravity rather than human stress in extreme environments, such as extreme cold. More importantly, in ground-based simulated microgravity experiments, especially at the cellular level, gravity will act as the dominant inducer to affect the cells.

Parabolic flight is considered as a viable model for studying the short-term effects of gravity changes, and can be used to assess the effects of gravity changes on human physiology. By analyzing the human blood, Stervbo et al. [43] determined that parabolic flight maneuvers prompted changes in the number of various leukocyte subsets. Memory T-cell and B-cell subsets were reduced, and the number of basophils and eosinophils was reduced as well. Only circulating neutrophils increased during parabolic flight. The use of long-duration HDBR has also been investigated to understand the complexly orchestrated regulations. Five days of HDBR reduced the expression of CD62L on resting granulocytes [44].

Blunting of the immune response in the microgravity environment is due to insufficient occurrence at the immune cell level. The study by Bradley et al. [37] illustrated that long-term culture of T cells in simulated microgravity (SMG) resulted in increased expression of the inhibitory receptor, cytotoxic T lymphocyte-associated antigen-4 (CTLA-4). Boonyaratanakornkit et al. [45] discovered that impaired induction of early genes was regulated primarily by transcription factors NF-κB, cAMP-response element binding protein (CRE-B), ETS-like transcription factor (ELK), activator protein-1 (AP-1), and after crosslinking with the T-cell receptor the signal transducer and activator of transcription (STAT) contributes to T-cell dysfunction in altered gravity environments.

Tab. 2 Microgravity-induced changes in immune system

Species	Categories	Subjects	Exposure time	Overall effects	Indications	Refs.
Human	In-flight	Urine and blood	4 d	Cytomegalovirus shedding frequency increased Cytomegalovirus antibody titer was further increased	Latent viral reactivation	[41]
		Blood	N/A	CD8+ T cell subsets were altered T cell function was depressed Cytokine production profiles were altered	T cell and cytokine	[39]
	Post-flight	Peripheral blood	L-10, R+0, R+3	Significant increases in Epstein-Barr virus capsid antigen antibodies Anti-Epstein-Barr virus nuclear antigen antibodies were significantly decreased	Latent viral reactivation	[46]
		Thymopoiesis	184 d	Significant suppression of thymopoiesis upon return from spaceflight	Reduced thymopoiesis	[47]
		Blood	5-11 d	Monocyte capacity to engulf *E. coli*, oxidative burst, and degranulation were all reduced	Altered monocyte function	[48]
	Ground-analog (Parabolic flight)	Blood	1/8 d	Changes in numbers of different leukocyte subsets Naïve and memory T and B cell subsets decreased under gravitational stress	Affected the human immune system	[43]
	Ground-analog (Bed rest)	Blood	5 d	Lower numbers of basophils and eosinophils Decreased CD62L on lymphocytes and elevated soluble CD62	Induced immunological responses	[44]
	Ground-analog (RPM)	Peripheral blood leukocytes	1/12 d	Alterations in the expression of ten key genes during simulated microgravity	Altered immune function	[45]
	Ground-analog (RPM, FFM)	Peripheral blood	N/A	Mitogenic T cell activation decreased	Specific immune system alterations	[49]
Mice	In-flight	Femur bone and marrow	30 d	Reduction of B cells in the spleen	Cytokine dysregulation	[50]
		Splenocytes	15 d	Reductions in the expression of key early T-cell activation genes in mouse splenocytes during spaceflight	Altered T cell activation	[51]
	Post-flight	Spleen	13 d	Decreased T cell CD25 expression Decrease in CD11c+MHC I+, CD11c+MHC II+, and CD11c+CD86+ cells	T cell function	[52]
	Ground-analog (CUMS)	Thymuses, blood	14 d	Stressors affect a fraction (25%) of the TCR-β repertoire and can increase self-reactivity	T cell function	[53]

L-10: launch minus 10 days; R+0: return/landing day; R+3: 3 days after landing; CUMS: chronic unpredictable mild stressors; RCCS: Rotary cell culture system; FFM: Free fall machine; RPM: Random positioning machine; N/A: Not applicable.

3.1.2 Spaceflight-induced changes to bone

Reduced mechanical use is considered as the main contributor to bone loss in space. In microgravity, the skeletal system no longer acts on the pull of gravity; hence, there is no need to keep the bones strong enough to support the weight of the body, which is defined as skeletal unloading.

Bone remodeling in the gravitational environment on the ground is tightly coupled; however, it is disrupted in the microgravity environment. After five days of exposure to microgravity, the bone resorption pits increased significantly, while the cellular morphology of osteoblasts indicated an expanded shape [54]. Similarly, microgravity stimulated osteoclast formation and activity indirectly by regulating key regulators secreted by osteoblasts [55]. Simulated microgravity studies also demonstrated a marked suppression of hMSC differentiation into osteoblasts [56]. The rotary cell culture system also exhibited inhibition in the formation of osteoblasts [57]. Osteocytes are the most abundant but least understood cells in bones and are considered responsible for sensing stresses and strains in bone. Mechanical unloading regulates intrinsic osteocyte responses in concert with hormonal and cytokine inputs [58].

Microgravity-induced bone loss is similar to that of the disuse osteoporosis on Earth. Astronauts on the ISS experienced substantial losses of the trabecular and cortical bones in the hip and less in the spine after long missions [59]. Bone loss that occurs during spaceflight is the result of increased bone resorption and decreased intestinal calcium absorption. Bone resorption markers were also significantly increased in studies conducted during flight compared to pre-flight. Changes in these markers are valid intermediate endpoints for fracture risk reduction and can provide valuable additional data for treatment success, with a significantly lower true intestinal calcium absorption rate during flight compared to pre-flight values [60-63].

Recovery of lost bone after return to Earth may be influenced by a variety of factors, such as age, nutritional intake, and post-flight activity. The average

bone mineral loss at all sites after long-duration spaceflight ranged from 2% to 9%, and the recovery model by Sibonga et al. [64] predicted a 50% recovery of bone loss at all sites within 9 months. Experimental reports based on space flight and ground simulations are presented in Tab. 3.

Tab. 3 Skeletal changes caused by microgravity

Categories	Subjects	Exposure time	Overall effects	Refs.
In-flight	Blood and urine	4-6 months	Bone resorption was increased Both bone-specific alkaline phosphatase and osteocalcin were significantly increased	[62]
	Blood and urine	4-6 months	True intestinal calcium absorption was significantly lower Risk of renal stone formation increased	[63]
	Hip and spine	4-6 months	Cortical bone mineral loss in the hip occurred Integral, cortical, and trabecular vBMD were lost in the hip at high rates	[59]
	Lumbar spine, hip, and calcaneus	4-6 months	Averaged losses of bone mineral after long-duration spaceflight ranged between 2% and 9% across all sites	[64]
	Hip and spine	6 months	iBMD in the lumbar spine and proximal femur of all subjects was decreased	[65]
	Osteoblast	9 days	The cytoskeleton had a reduced number of stress fibers and unique abnormal morphology The flight nuclei were 30% smaller	[66]
		5 days	Osteoblast had shorter and wavier microtubules, smaller and fewer focal adhesions, and thinner cortical actin and stress fibers Space-flown osteoblasts presented extended cell shapes and often fragmented or condensed nuclei	[54]
Ground-based	Osteoblast	7 days	Modeled microgravity inhibited the osteoblastic differentiation of hMSC	[56]
		1, 4, 58 days	Simulated microgravity induced an impairment of osteogenesis Simulated microgravity triggered the dedifferentiation impulse of human primary osteoblasts	[67]
	Osteoclast	7 days	Inhibition of osteogenic markers Enhanced expression of adipogenic markers	[57]
		7 days	Microgravity increased osteoclastogenesis Bone resorption was promoted	[55]
	BMSC	4, 10 days	Induced the expression of RUNX2 and OSX in BMSC Mesenchymal stem cells upregulated HSP60, HSP70, cyclooxygenase 2 and superoxyde dismutase 2	[68]
	hMSCs	7 days	F-actin stress fibers were disrupted in hMSCs	[69]
	Preosteoblast	1 day	ALP activity was dramatically decreased The expression of ALP gene was downregulated. Expression of markers and regulators for osteoblasts differentiation were downregulated	[70]
		3 days	Inhibited alkaline phosphatase activity Decreased ALP activity and inhibited RUNX2, BMP4, PTHR1 and osteomodulin gene expression	[71]
	Osteocytes	3 days	Induced an autonomous up-regulation of SOST/sclerostin and RANKL/OPG	[58]

iBMD: Integral bone mineral density; vBMD: Volumetric bone mineral density; hMSC: Human bone marrow mesenchymal stem cell; ALP: Alkaline phosphatase; BMSC: Bone mesenchymal stem cells; RUNX2: Runt-related transcription factor 2; OSX: Osterix; HSP: Heat shock protein; BMP: Bone morphogenetic proteins; PHTR1: Parathyroid hormone type 1 receptor; SOST: Sclerostin; RANKL: Receptor activator for nuclear factor-κB ligand; OPG: Osteoprotegerin.

3.1.3 Cardiovascular adaptations

Prolonged exposure to the extremes of microgravity produces adaptive changes in the human body that is termed ‘‘deconditioning’’ in research, particularly in the cardiovascular system. Despite the exposure of the cardiovascular system to both radiation and microgravity in space, it exhibits distinct gravity dependence. The altered vascular function in the center of space results in post-flight physiological challenges.

The cardiovascular system undergoes certain well-known alterations during spaceflight. Adaptation of autonomic nervous system to the microgravity environment leads to reduced plasma volume and left ventricular mass. During spaceflight, the transfer of blood volume to the central vein triggers a regulatory response that reduces the total blood volume [72]. In addition, blood pressure regulation is impaired in response to a decrease in the overall circulating blood volume of fluid. According to clinical data, the crew experiences a decrease in arterial diastolic pressure, which may be influenced by mechanical stimulation, neurological and hormonal activity, and changes in radiation balance during spaceflight [73]. Cardiac output is elevated early in short-duration spaceflight owing to the larger stroke volume with only small reductions in the heart rate [74]. The central venous pressure of astronauts in space tends to be close to or lower than on the ground because of the elimination of the effects of gravity on the internal organs of the body, which normally exert external pressure on the veins, and the lower central venous pressure is often accompanied by a larger central blood volume.

Rapid hemodynamic changes and fluid transfer during the launch of a crew by rocket or into outer space may induce paroxysmal atrial fibrillation. Changes in autonomic nerve balance and atrial pressure may also affect atrial electrophysiological properties, which may be associated with atrial fibrillation in astronauts [75].

The reduction in metabolic demand and oxygen uptake owing to the resting nature of microgravity in actual or simulated microgravity environments resulted in cardiac atrophy, i.e., a physiological adaptation of the body to reduced myocardial load and work, exhibiting myocardial plasticity under different loading conditions. The left ventricular mass in subjects at bed rest decreased after six weeks, and mean ventricular wall thickness decreased with decreasing left ventricular mass, with additional atrophy occurring after 12 weeks, suggesting physiological remodeling under altered load [76].

The vascular endothelium is an important regulatory organ responsible for the release of vasoactive factors, preventing platelet aggregation and leukocyte adhesion, and regulating vascular smooth muscle cell proliferation. During the six-month-long mission of the astronauts on the ISS, the destruction of red blood cells, also known as hemolysis, occurred. This is a primary effect of microgravity in spaceflight. Upon landing, erythrocyte mass, plasma volume, hematocrit, and reticulocyte count all exhibited decreases. Changes in the vascular composition of the astronauts were observed on landing, such as a decrease in red blood cell mass and plasma volume, and hemoglobin, hematocrit, and reticulocyte count did not return to pre-flight levels within two weeks of landing. In addition, one year after landing, effects on erythrocytes persisted, including hemolysis and increases in reticulocyte and hemoglobin levels [77]. Concisely, the space microgravity environment is associated with persistent increases in hemoglobin degradation products, carbon monoxide in alveolar air, and iron levels in serum. The understanding of space anemia by Trudel et al. [78] is that the reduction in red blood cells is an acute adaptive event in response to major hemodynamic events such as headspace fluid transfer, hemoconcentration, and low erythropoietin levels upon entry into space.

3.1.4 Muscle atrophy

Muscle remodeling and atrophy in space is caused by skeletal muscle adapting to reduced loading. Accordingly, the motor performance and connective tissue integrity of skeletal muscles are disrupted, and strength and fatigue resistances are reduced [79].

The results of removing the effects of gravity on humans include a decrease in the calf muscle mass and performance and a slow to fast transition of the fiber type in the gastrocnemius and flounder muscles [80]. After a 17-day spaceflight, the cross-sectional areas of the knee extensors and hip muscles of the astronauts decreased by 8%, with changes consistent with the

results of earlier ground-based studies of similar durations [81]. Decreased muscle size, strength, and endurance may also be associated with gene expression in skeletal muscle cells. Sandona et al. [82] reported that gene expression of atrophy-associated ubiquitin ligases was upregulated in both flounder and extensor muscles in spaceflight, while autophagy genes were under control. Effects of long-term spaceflight on the structure and function of slow and fast fibers in human skeletal muscles as evidenced by a substantial fiber mass loss in gastrocnemius and flounder muscle biopsies [83]. The calf muscle is considered one of the skeletal muscles most affected by microgravity and plays a role in supporting the torso and maintaining an upright morphological posture in the ground environment. Interestingly, Trappe et al. [84] discovered no significant changes in functional characteristics of the calf muscle after exposure to 17 days of spaceflight and bed rest. The study concluded that these results were likely influenced by the exercise paradigms.

Alterations in the muscle morphology of skeletal muscles were also reported after spaceflight, with a higher percentage of slow flounder muscle fibers acquiring short filaments comparable to gastrocnemius fiber types. The short filament content of gastrocnemius type I fibers was also higher after flight. This suggests that slow fibers adapted to spaceflight acquired more short filaments of fast muscle characteristics. During the 17-day mission, significant reductions occurred in all muscle regions except the hamstrings and there was a significant decrease in muscle volume in all muscle groups except the neck, with the landing being reversed 30-60 days after landing [85].

During long-duration spaceflight on the ISS, astronauts mitigate the risk of skeletal muscle loss via exercise countermeasures; however, lower extremity muscle volume and strength still decline, and this are related to factors such as intensity, duration, frequency, and individual differences in exercise [86].

Methods to simulate the microgravity of skeletal muscles on the ground include complete horizontal or head-down bed rest, dry immersion, unilateral upper and lower extremity unloading. Comparisons of muscle strength and size changes between these models suggest that each may be useful for investigating some aspects of skeletal muscle in the context of the limited results of spaceflight [87]. Studies of bone loss in healthy older adults have indicated that bed rest leads to significant skeletal muscle loss, especially in the lower extremities [88]. Testing counter strategies for skeletal muscle loss reveal that aerobic and resistance exercise protocols effectively prevent loss of thigh muscle volume, while nutritional supplementation does not effectively offset the loss of lower extremity muscle mass or strength and actually promotes loss of thigh muscle volume [89].

3.1.5 Altered neural/ocular function

Altered gravity leading to changes in sensorimotor/vestibular functions have been reported, exhibiting motion sickness, spatial disorientation, reduced postural control and movement, and deficits in manual and fine motor control. After entering space, the effects of gravity are substantially diminished, leading to a mismatch between the expectations of the sensory consequences of motion and the actual experience, which in turn affects behavioral performance during the first few days of exposure to microgravity [90]. Moreover, experimental results suggest that loss of otolith loading leads to increased sensitivity of the vestibular pathway and adaptation over time [91-92]. Accordingly, neural mechanisms of upright posture appear to be disrupted after short- and long-term spaceflight. Further, associated with perception in space are type II hair cells that communicate primarily with branches of primary vestibular afferent endings. Ross et al. [93] determined that synapses in type II hair cells are uniquely affected by altered gravity, thereby speculating that type II hair cells may be chiefly gravitational sensors.

Clinical symptoms of spaceflight associated neuro-ocular syndrome (SANS) include unilateral and bilateral optic disc edema, globe flattening, choroidal and retinal folds, hyperopic refractive error shifts, and focal areas of ischemic retina. The exact etiology of SANS-related symptoms is unknown, but may be involved with elevated intracranial pressure (ICP) and the zonation of cerebrospinal fluid (CSF) in the orbital optic nerve sheath [94].

3.1.6 Cellular and molecular effects

Cells and molecules exposed to microgravity gradually

adapt to the spatial environment, resulting in changes to the genome, epigenome, and proteome, and these changes create risks for a range of pathologies [95].

Early experiments demonstrated that the self-assembly of microtubules is associated with gravity, which triggers the self-organization process. The gravitational orientation breaks the symmetry of the initially homogeneous state and leads to the emergence of forms and patterns. This provides an explanation for the fact that microtubules in samples prepared under microgravity conditions exhibit little self-organization and are locally disordered [96]. After 24 hours of simulated microgravity, the major cytoskeletal components are reduced, and actin filaments and microtubules tend to be disorganized making them more rounded morphologically. The stiffness and viscosity of the cells are significantly reduced. The stiffness of the cortex experience a relatively direct and significant decrease compared to the stiffness of the entire cell body. Similarly, Janmaleki et al. [97] determined that the mechanical behavior of endothelial cells altered significantly induced by microgravity. However, the effects of simulated microgravity on cell proliferation are controversial. Cultured in clinostat, the population growth of rBMSCs was inhibited, and cells were suppressed in the G0/G1 phase of the cell cycle. At the same time, there was a differentiation of rBMSCs toward osteoblasts, which means osteogenic capacity was decreased under simulated microgravity [98]. In addition, the RPM can alter the gene expression profile of 2T3 preosteoblasts and inhibit the differentiation of preosteoblasts to osteoblasts. In terms of the process of reduced bone formation, it is unknown whether the two events, differentiation and changes in gene expression, occur sequentially or simultaneously [99].

3.2 Hypergravity and simulated hypergravity-induced effects

As aforementioned, there are fewer studies on hypergravity because it is experienced for a much shorter period than microgravity. Functional immune dysregulation and stress responses to hypergravity depend on the level of gravity [100].

Gravity, as an “in vitro” signal, also affects the function of endothelial cells. Hypergravity induces ATP release and actin reorganization, which activates cell proliferation and migration of bovine aortic endothelial cells (BAECs) via RhoA activation and focal adhesion kinase (FAK) phosphorylation [101]. A similar report proved that the distribution of integrins is altered and the cytoskeletal network is reorganized after the cells are exposed to hypergravity. In parallel, the expression of genes controlling vasoconstriction and inflammation and pro-apoptotic signaling are both downregulated [102]. Under high gravity, human lymphocytes are exposed to mitogenic cutin, and cause divided and significantly increased cell proliferation rates. In contrast, under microgravity conditions, lymphocytes exhibit a significantly inhibited proliferation rate [103]. Moreover, increased gravity is determined to alter cell viscosity. Mouse osteoblasts and human endothelial cells exhibit increased viscosity with increased hypergravity [104]. Hypergravity also induces positive effects on C2C12 myogenic cells at the morphological and functional levels, including promotion of cell proliferation, differentiation, and protein synthesis [105].

A variety of cells have increased proliferation rates when exposed to high gravitational fields, including HeLa cells, chicken embryo fibroblasts, sarcoma Galliera cells, friend leukemia virus transformed cells and human lymphocytes [106]. Gravity also has a significant effect on the differentiation of bone MSCs. Hypergravity induces differentiation of rBMSCs into force-sensitive cells (cardiomyocytes and osteoblasts), whereas SMG induces force-insensitive cells (adipocytes) [107].

Microgravity-induced muscle atrophy and bone loss have been demonstrated previously. In contrary, hypergravity (2 g) causes a significant increase in both bone mass, elevated expression of genes related to bone formation, and an increase in muscle volume [108]. Behavioral studies have indicated that hypergravity caused developmental delays in motor aspects that are largely dependent on the vestibular system [109]. Correspondingly, hypergravity was indicated to affect muscle and improve bone strength via vestibular signaling [110]. Here, we categorize the effects induced by various hypergravity conditions in different species (Tab. 4).

Tab. 4 Bioeffects caused by hypergravity

Species	Cell types	Categories	Overall effects	Refs.
Human	Peripheral blood lymphocytes	Spaceflight	93% inhibition of activation by ConA formation of cell aggregates	[103]
	Umbilical vein endothelial cell	Large diameter centrifuge	A delayed response occurs between gravitational loading The cellular viscosity increases	[104]
Bovine	Aortic endothelial cells	Low speed centrifuge	Induces ATP release and actin reorganization Activates cell proliferation and migration in BAECs	[101]
	Aortic endothelial cells	Thermostated centrifuge	Cytoskeletal network reorganized Reduction in expression of genes controlling vasoconstriction and inflammation Proapoptotic signals are downregulated	[102]
Rats	N/A	Centrifuge	Leads to a retarded development of motor aspects	[105]
	N/A	The animal centrifuge	Induces impairment of learning and memory Causes neuronal apoptosis in the cortex and hippocampus in rats	[107]
	PC12 cells	Bench centrifuge	Induces a faster and higher neuronal differentiation	[111]
Mice	Osteoblastic (MC3T3-E1)	Special vest filled with extra loads	Full cellular relaxation after exposure to hypergravity	[112]
	N/A	Large radius centrifuge	2 g centrifugation impairs mitogen-induced response of B and T cells 3 g centrifugation induces a durable HPA axis activation and anxiety associated	[100]
	N/A	Gondola-type centrifugal device	The upregulation of myogenic genes Down-regulation of muscle-specific ubiquitin ligases and autophagy-related genes Increases expression of osteogenic genes in mice	[106]
	C2C12 mouse myoblasts	Large diameter centrifuge system	Positively affects both proliferation and protein synthesis A positive effect on differentiation	[108]
	N/A	Custom-made gondola-type rotating box	Increases muscle mass, myofiber size, and muscle differentiation in the soleus	[110]
	N/A	Rotational device	Influences NGF levels in the central nervous system of adult mice Leaving BDNF values unchanged	[113]
	N/A	Large-radius centrifuge	12.1% of the taxa are significantly impacted in 3 g microbiota Most of them (78%) being enriched	[114]
	Hl-1 cardiomyocyte cell	Hypergravity centrifuge	Spontaneous calcium oscillations and cytosolic calcium concentration are both increased	[115]

BAECs: Bovine aorta endothelial cells; ConA: Mitogen concanavalin A; NGF: Nerve growth factor; BDNF: Brain derived neurotrophic factor; HPA: Hypothalamic-pituitary-adrenal; N/A: Not applicable.

4 Pros and cons of magnetic field-simulated gravity alterations

It is well known that magnetic levitation provided by gradient SMFs has been widely employed by researchers in the field of physical, chemical, material, and life sciences. It should also be noted that there are both pros and cons for using SMFs to simulate gravity alterations when investigating gravity-related bioeffects.

SMF-based gravity investigations have multiple advantages. First, SMF is the only ground-based method that can be used to simulate zero gravity, hypergravity, and microgravity in multiple folds. In fact, the multiple gravitational environments generated by the SMFs, using either superconducting or resistive magnets, are capable of accommodating small-sized model organisms. Second, SMFs provide a static physical tool that allows the 3D growth with complex cell structures without agitation. Third, the imposed magnetic force works non-invasively on the cell population, avoiding the fluid shear stress generated in the RPM and RWV. Fourth, with the development of superconducting and resistive magnet technology,

long-term investigations are accessible.

In the meantime, SMF-based gravity investigations also have obvious disadvantages. In fact, the major limitation is that it is hard to decouple completely the effects of magnetic field and the effects of altered gravity. Moreover, although Liu et al. [116] did not find adverse effects of short-term exposure of mice suspended in superconducting magnets at 17 T, we have also previously demonstrated that 1-2 h of up to 33 T high SMF exposure does not generate obvious harmful effects on healthy mice [117-120]. Our recent study revealed that >10 T/m high SMF can induce harmful effects on diabetic mice, especially on severe type 1 diabetic mice [121]. Therefore, more researches are still required to clarify specific pathological conditions and SMF parameters that limit SMF-based gravity investigations.

Nevertheless, we believe that by setting up proper controls, such as gradient vs. uniform SMFs and side-by-side comparisons with other ground-based and in-flight methods, abundant information on the differential effects of zero gravity, hypergravity, and microgravity can be obtained. This will help develop effective protective methods for astronauts to perform long-term space duties in the future.

5 Conclusions and future perspectives

Current studies on spaceflight, complemented by on-ground experiments have shown that gravity alterations can induce multiple physiology perturbations, including dysregulation of the immune function, musculoskeletal loss, cardiovascular adaptation responses, and altered neurological and ocular functions. Moreover, besides the hypergravity during launch, as the Mars, Moon, and space all have different gravities, more comprehensive and systematic future studies will be required to unravel the bioeffects of different gravitational forces, specifically those of different types of microgravity and hypergravity. However, although there are various simulation methods to the study the bioeffects of hypergravity and microgravity, they have their own advantages and limitations. Evidently, none of the microgravity analogs can produce all the biological effects observed in an actual space environment. Therefore, along with the necessary parallel on-ground and in-space experiments to understand the gravity-related bioeffects completely, we should develop more ground-based techniques that can be implemented in spaceflight.

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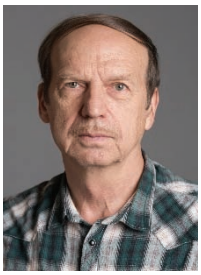

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