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Animal physiology across the gravity continuum

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ABSTRACT

Gravity has been an ever-existing force impacting various processes since the beginning of time. Biological properties of living organisms change when the gravitational force is altered, and not surprisingly, these changes are perceived from cellular to organismal levels. Variations in gravitational levels induce adaptive responses that influence dynamic physiological functions. In a microgravity environment where weightlessness is experienced, astronauts often suffer from space motion sickness, cardiovascular deconditioning, bone demineralization, muscle atrophy, as well as pooling and redistribution of fluids in the upper parts of the body. Additionally, indirect effects mediated by fluid shear stress and hydrostatic pressure strongly affect systems both *in vitro* and *in vivo*. In this review, we reiterate some interesting data that has been obtained from studies conducted in both microgravity and hypergravity and provide key mechanistic insights that could be responsible for the continuum of physiological changes observed in these conditions. We have mainly focused on long-duration space orbiting experiments rather than short-term parabolic flights and sounding rockets. Even after more than 500 missions, space is still not a place for either regular visits or habitation largely due to the challenges posed to normal growth and development of organisms, which is further complicated by the lack of successful and reliable countermeasures. Hence, we reinstate the use of artificial gravity simulations in tackling space-incurred physiological disturbances.

1. Introduction

Gravity has been continuously influencing various life forms on Earth both physically and biologically, thereby playing a momentous role in shaping evolution [1,2]. Gravity is a physical force consisting of both magnitude and direction and thus manipulation of these two components of the gravity vector could have serious implications. Microgravity is a condition where people or objects experience a state of weightlessness such as that attained in space, while hypergravity refers to a condition where the force of gravity exceeds that on the surface of the Earth [3]. In recent decades, there has been an exponential increase in the number of research studies carried out in altered gravity conditions. Gravity as a fundamental physical force regulating the survival of organisms had been identified during early experiments designed to observe the effects of microgravity on embryo development.

Gravity directs orientation in the chick embryo, which undergoes rotation in the uterus to determine its anterior-posterior axis in a direction perpendicular to gravity's longitudinal axis [4]. So, the instructional role of gravity on a radially symmetrical blastodisc to develop into a bilateral embryo is lost in microgravity allowing the embryo to still develop without a defined axis [5]. Similarly, gravity appears to influence dorsal-ventral orientation in the zebrafish embryo, which when allowed to rotate freely after fertilization redirects its animal-vegetal axis horizontally in a direction perpendicular to the gravitational field resulting in an upward facing embryonic shield indicative of the future dorsal side [6]. During the initial phase of development of an amphibian embryo, gravity acts to spatially orient the animal-vegetal axis so that the position of the future dorsal-ventral axis remains gravitationally neutral. Systemic events following sperm entry and cortical rotation can determine dorsal-ventral axis only if this gravitational neutrality is achieved earlier. Such intriguing gravity experiments performed on a wide range of animal models have been summarized in Table 1 [7].

Gravitational adaptation is seen in larger animals like snakes whose heart locations vary based on their habitat. For instance, hearts of aquatic snakes are significantly closer to the centre of the body than

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Table 1
Animal models studied in altered gravity conditions.

Physiological Systems	Animal Models	References
The Cardiovascular System	Murine (*Mus musculus, Rattus norvegicus*)	[16,22,155–157]
	Primates (*Homo sapiens*)	[17,158–162]
The Immune System	Arthropods (*Drosophila melanogaster*)	[52,163]
	Pisces (*Carassius auratus*)	[164]
	Amphibians (*Pleurodeles waltl*)	[39,165]
	Murine (*Rattus sp., Mus musculus*)	[34,42,43,50,51,166,167]
	Primates (*Macaca mulatta, Homo sapiens*)	[37,38,44,45,167]
The Musculoskeletal System	Nematodes (*Caenorhabditis elegans*)	[168]
	Pisces (*Danio rerio*)	[59,63]
	Amphibians (*Xenopus laevis*)	[169]
	Murine (*Mus musculus, Ratus ratus*)	[61,69,71,72,170–172]
	Canine (*Canis lupus familiaris*)	[53]
	Primates (*Saimiri sp., Papio sp., Nasalis larvatus, Homo sapiens*)	[173–175]
The Vestibular System	Molluscs (*Aplysia sp.*)	[176]
	Pisces (*Danio rerio, Oreochronis mossambicus*)	[73,75–77,80] [177–180]
	Amphibians (*Xenopus laevis, Pleurodeles waltl, Cynops sp.*)	[87,181,182] [84–86,183,184]
	Arthropods (*Gryllidae sp., Pleocyemata sp.*)	[185,186] [78,91,187–189]
	Murine (*Mus musculus, Rattus norvegicus*)	[190] [74,79]
	Avian (*Gallus gallus*)	
	Primates (*Macaca sp., Homeo sapiens*)	
The Nervous System	Nematodes (*Caenorhabditis elegans*)	[191] [100,101]
	Arthropods (*Drosophila melanogaster*)	[192] [93,95,98,99,102,104,105] [107,108]
	Amphibians (*Xenopus laevis*)	
	Murine (*Mus musculus, Rattus norvegicus*)	[96,97]
	Primates (*Macaca mulatta, Homo sapiens*)	
The Reproductive System	Rotifers (*Macrotrachela quadricornifera*)	[193] [193]
	Nematodes (*Panagrolaimus rigidus*)	[119,194–196] [110,111,114,116–118,120,122]
	Amphibians (*Rana pipiens, Xenopus laevis, Pleurodeles waltl*)	[197,198]
	Murine (*Rattus sp., Mus musculus*)	[112]
	Avian (*Coturnix japonica*)	
The Integumentary System	Murine (*Mus musculus*)	[129]
	Primates (Homo sapiens)	[124–126,128,130,131,199–201]
The Adipose Tissue	Murine (*Rattus norvegicus*)	[135,136,139,140,202]
	Primates (*Homo sapiens*)	[137,203,204]
The Ocular System	Pisces (*Danio rerio*)	[205]
	Amphibians (*Xenopus laevis*)	[206]
	Murine (*Mus musculus*)	[147]
	Primates (*Homo sapiens*)	[145,146,207–210]
The Digestive System	Murine (*Mus musculus*)	[141–144,211,212]
	Rodents (*Meriones unguiculatus*)	[213]

those of arboreal or terrestrial forms, albeit present at an intermediate location in semiaquatic and relatively non-climbing forms. A central location minimizes the heart's load to pump blood for the whole body and hence possibly an evolutionary adaptation among aquatic snakes whose underwater conditions mimic a microgravity environment. Whereas a heart located more anteriorly (or superiorly as in upright mammals, including humans) as in climbing or terrestrial snakes favours blood flow to the brain and the rest of the body [8]. Therefore, gravity has been so instructive in determining the various developmental processes and bodily organizations of all life forms on Earth. In this review, we summarize the effects of extreme gravity conditions, both microgravity and hypergravity on the physiology of animal models studied thus far.

2. The cardiovascular system

2.1. Cardiac system (heart and circulation)

The cardiovascular system (CVS) consists of the heart, blood and blood vessels. The heart pumps blood through the vessels that supply nutrients and oxygen, transport blood cells, remove metabolic wastes including carbon dioxide, and maintain homeostasis. Weightlessness experienced in a spaceflight causes cardiovascular deconditioning syndrome, a common manifestation of which is orthostatic intolerance, syncope, a decreased ability to exercise and an increased resting heart rate. Autonomic dysregulation with reduced cardiac-baroreflex sensitivity and shift in the sympathetic-parasympathetic balance has been a major contributor to this syndrome [9]. Under normal physiological conditions when an individual is in an upright position, excessive blood pooling in the legs reduces venous return causing decreased cardiac output and maintains arterial blood pressure [10]. Spaceflight causes loss of hydrostatic gradients resulting in a fluid shift from lower to upper parts of the body. This gives the characteristic 'puffy face' appearance and 'bird legs' to crew members [11]. These adaptations to weightlessness vary depending on the extent of time spent in space [12]. The cephalad fluid shift is considered an initiating event associated with cardiovascular adaptations that follow when an astronaut enters a weightless environment. The autonomic nervous system (ANS), adequate blood volume, intact skeletal and respiratory muscle pumps are all essential components for rapid cardiovascular adjustments to orthostasis [13].

A study of 12 astronauts from 6 space missions measuring cardiac parameters before, during and after spaceflight showed that heart rate, arterial pressure and frequency of cardiac dysrhythmias (irregular heartbeat rhythm) all decreased during the spaceflight [14]. Similarly, another mission consisting of 3 orbital astronauts (2 men and 1 woman) found decreased diurnal rhythms of heart rate and body activity during flight followed by normal values post-flight. This misaligned diurnal rhythm could be attributed to altered function of the ANS influenced by weightlessness [15]. Gravitational field is a major determinant of cardiovascular function that provides a hypotensive stimulus to the baroreceptors (located within the aortic arch and the carotid sinuses, they detect increases in blood pressure) every time an upright posture is assumed on Earth. The arterial and cardiopulmonary baroreceptors respond by initiating vagal reflex withdrawal and sympathetic excitation resulting in increased heart rate and peripheral vascular resistance. This stimulus to which the CVS must respond several times a day on Earth is therefore withdrawn when gravity is eliminated. This lack of repeated stimulation is what probably causes the decrease in variabilities often observed in microgravity [14].

Paraventricular nucleus (PVN) of the hypothalamus is an important centre of the brain that integrates autonomic functions with cardiovascular responses to maintain homeostasis [16]. Rats subjected to a head-down tilt to simulate microgravity for about 10 min showed increase in mean arterial pressure and decrease in heart rate. These responses associated with acute microgravity are due to PVN-mediated modulation of heart rate brought about by autonomic changes involving both the sympathetic and parasympathetic arms. An increase in vascular resistance in the cerebral vessels offsets the increase in mean arterial pressure. Ventricular repolarization dispersion is a condition associated with heterogeneities in action potential duration of the ventricular tissue. Comparison of differences in repolarization adaptation among 22 men subjected to 5 days of head-down bed rest found impairment in the process. Rapid flow of Ca^{2+} and K^{+} followed by a much slower intracellular Na^{+} movement usually helps action potential duration adapt to rapid changes in the heart rate. Deconditioning in the ANS modulates

the control over Ca^{2+} and K^{+} currents leading to decreased initial phase adaptation of action potential duration to abrupt changes in the heart rate [17].

Reduced heart rate might be suggestive of elevated vagal activity and a possible fall in sympathetic activity of the heart. The absence of normal head-to-foot translocation of blood volume during spaceflight might lead to increased cardiac output with vasoconstriction lower than that usually required to maintain arterial blood pressure. Reduction in total blood volume along with loss of cardiac muscle mass and chamber volume, and altered contractile properties could limit the heart's response to challenges such as, exercise and maintaining an upright posture upon returning to Earth [12]. This is consistent with abnormal orthostatic responses observed immediately following a space mission which may reflect a space-induced vagal baroreflex dysfunction (reduced baroreflex sensitivity). Studies conducted on 13 astronauts (11 men and 2 women) of STS-40 and STS-55 space missions before, during and after two 8-day space missions revealed that exposure to microgravity augments sympathetic while diminishing vagal cardiovascular influences. Spaceflight reduces R-R interval responses to baroreceptor stimulation which is indicative of diminished vagal-cardiac nerve activity [18]. R-R interval refers to the time elapsed between two successive R waves of the QRS signal on the electrocardiogram, examining which gives a measure of the heart's ventricular rate.

Red cell mass, plasma and blood volumes decrease within the first day of spaceflight and this subnormal level persists throughout the duration of stay in space (refer Fig. 1). Hemodynamic responses to weightlessness such as, reduced stroke volume, cardiac output and left ventricular diastolic volume could result from this hypovolemia. Microgravity exposure decreases the maximal rate of O_2 uptake (VO_{2max}) in individuals returning from space. This occurs mainly due to attenuation of the O_2 transport pathway with the magnitude of decrease dependent on the duration of exposure. This reduction in O_2 transport is an effect of multi-organ system deconditioning that includes reduced total blood volume and red cell mass. Moreover, decreased cardiac and vascular functions, and altered capillary hemodynamics are more pronounced on acquiring an upright posture back on Earth attributed to a post-flight stress response [19].

Contrary to numerous studies conducted in microgravity, only limited experiments have been carried out in hypergravity conditions (refer Fig. 1). In a recent study designed to investigate hypergravity-induced hypoxia in the heart and liver of mice, it was demonstrated that hypergravity followed by re-oxygenation significantly increased cardiac HIF-1α levels along with increased levels of cardiac eNOS to protect against hypoxia-induced effects [20].

2.2. Vasculature

The endothelium causes vasodilation, modulates platelet adhesion and limits vascular inflammation thereby regulating microvascular homeostasis and local blood flow. Endothelial functions are influenced by blood flow-induced shear stress. Weightlessness entails increased physical inactivity along with decreased hemodynamic activity and local vascular shear stress. Thus, it is likely that endothelial dysfunction would make a vital contribution to the deleterious effects of weightlessness at the microcirculatory level [9]. In a recent twin study conducted by NASA, one of the twin brothers spent almost a year aboard ISS while the other stayed on Earth as a ground-based control. As expected, stroke volume and cardiac output increased along with distention of arteries and veins in the upper parts of the body of the former. The study also revealed an increase in carotid intima-media thickening inflight soon after launch which is a manifestation of increased internal jugular diameter and vascular remodelling of the carotid artery. This is consistent with the observations of previous short-term missions (4–6 months) suggesting that the increase in carotid intima-media thickening and vascular stiffness might be related to increased insulin resistance, oxidative stress and inflammation. Also, a higher apo B/A1 ratio which

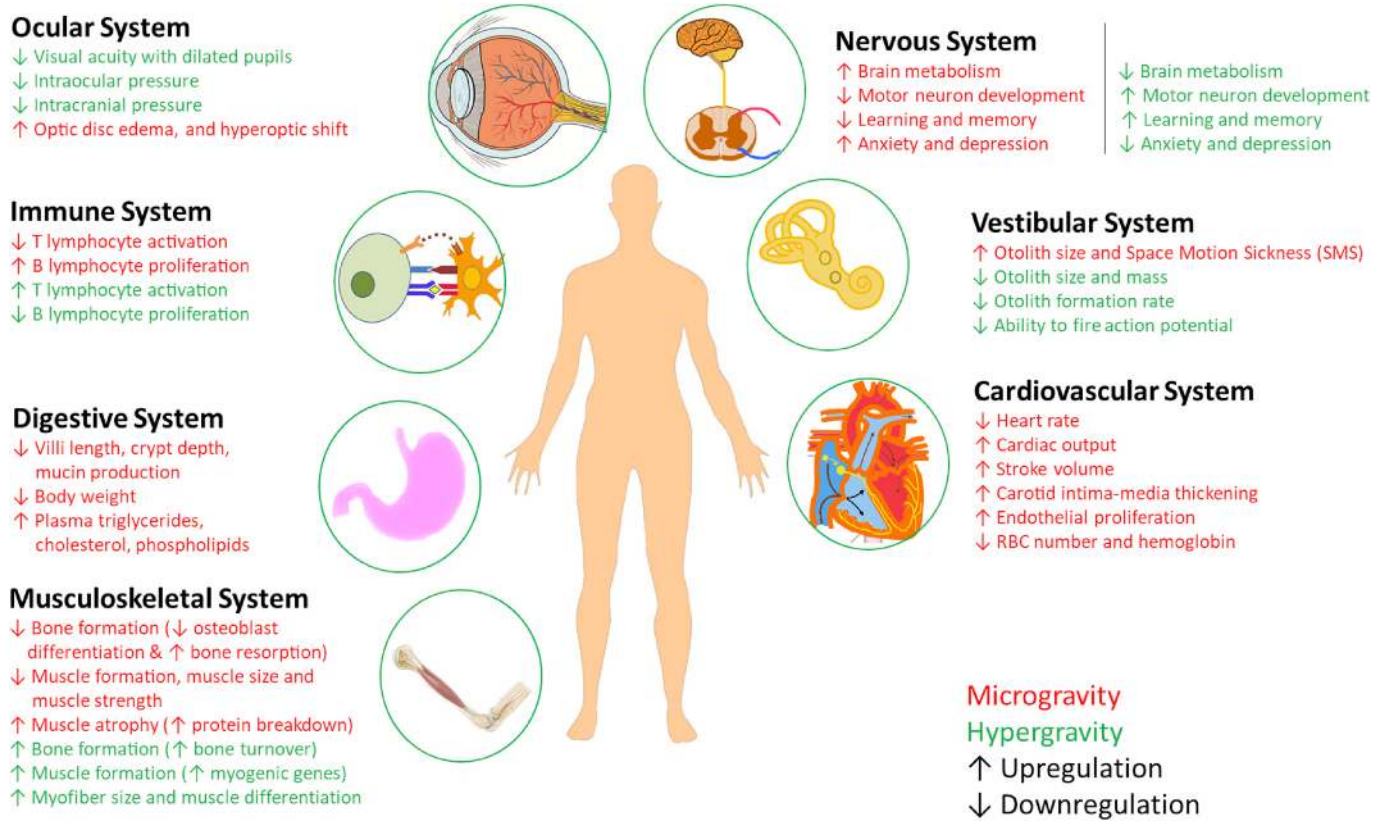

Fig. 1. Effects of altered gravity on physiological systems.

is indicative of increased cardiovascular disease risk has been observed in the latter part of the mission as compared to pre-flight values [11].

Endothelial properties were studied before and after 56 days of bed rest in a cohort consisting of 16 women, 8 each in control and exercise groups. The study revealed that prolonged bed rest is associated with impairment of endothelium-dependent vasodilation and increase in the number of circulating endothelial cells suggesting endothelial damage [9]. Endothelial cells are mechanosensitive in nature that undergo morphological and physiological changes, such as increased proliferation following exposure to a simulated microgravity condition [21]. Microgravity, therefore, has been considered as an angiogenesis-inducing force, although *anti*-thromobogenic ability of endothelial cells had been demonstrated to be lowered in simulated microgravity.

2.3. Red blood cells and platelets

Gravity modulates the characteristics of red blood cells (RBCs) which are major contributors to the microcirculation in addition to endothelial cells. Spaceflight causes decrease in plasma volume with concomitant reduction in red cell mass involving loss of erythrocytes and hemoglobin leading to a condition referred to as spaceflight anemia. Mouse erythrocytes exposed to microgravity showed modified rheology and underwent greater hemolysis. Also, spaceflight induced modifications in cell membrane composition and lipid peroxidation status along with increase in the levels of glutathione, superoxide dismutase and catalase, all of which still insufficient to prevent spaceflight-induced oxidative stress [22]. A recent study which involved exposing human erythrocytes to simulated microgravity in a 3D clinostat found them to be reacting to external stimuli by initially adapting their metabolic patterns and the rate of consumption of cellular resources. On a longer duration, however, the cells responded to even small differences in external mechanical environment by displaying structural and morphological changes as well as displaying signs of accelerated aging [23].

In contrast, 9 men subjected to hypergravity in a short-arm human centrifuge demonstrated that 30 min of continuous artificial gravity (2 g) augmented RBC aggregation without severely affecting their structure [24]. Further, erythroid differentiation is inhibited in microgravity by a mechanism involving suppression of GATA-1 mRNA expression [25]. Platelet activation is also inhibited under microgravity conditions [26]. Gravity-sensitive variations in platelet function possibly occur due to altered association of glycoprotein GPIbα with the cytoskeleton. Hypergravity causes activation of platelets which could lead to thrombotic events, especially in individuals with pre-existing medical conditions or cardiovascular system defects [27].

3. The immune system

3.1. T lymphocytes

The immune system is remarkably complex consisting of a multitude of distinct cell types each with a unique function. Innate immunity mounts a non-antigen-specific response, while adaptive immunity comprising of cell-mediated and humoral arms produces a delayed, antigen-specific response ensuring long-term memory of infection. Space travelers are often found to be infection-prone upon their return to Earth which could be ascribed to a reduced state of resistance to infections. Studies conducted on various animal models have shown that immunosuppression occurs in an organism following a space mission mainly attributed to reduction in the number of activated T lymphocytes [28]. The response of T lymphocytes to a mitogen usually decreases during spaceflight. Proliferation of lymphocytes from the lymph nodes in mice reduced even when stimulated with a T lymphocyte mitogen concanavalin A following a 4-day spaceflight [29]. A similar reduction was demonstrated in human lymphocyte cultures exposed to simulated microgravity in a clinostat [30]. Generally, a T lymphocyte is first stimulated by a primary signal when a T cell receptor (TCR) interacts with a processed antigen-bound major histocompatibility complex (MHC). This is followed by secondary signals involving T cell CD28 ligand binding to CD80/86 of the antigen presenting cells and IL-2 binding to its receptor on the T cell surface. All these signals are indispensable for T cell activation [31]. Under microgravity conditions, a decrease in IL-2 synthesis attenuates both activation and proliferation of T cells [32]. The decrease in the number of T cells is also linked to cytoskeletal integrity, with reduction in the amount of actin [33] having a negative impact on TCR-MHC clustering, formation of immune synapses and other T cell activation factors.

Gravity also affects cell migration ability and cytokine secretion. Alteration in the actin or tubulin assembly due to any kind of physical stress modulates IL-2 signaling directly [34]. Experiments conducted in a random positioning machine (RPM) that simulates a microgravity environment showed that changes to the cytoskeleton might be transduced as destructive signals in the Rho/Actin and Ras/MAPK signaling pathways which in turn could cause inactivation of serum response factor [35]. Further, the presence of serum response factor binding sites in majority of T cell genes inhibited in microgravity thus explains the decreased T cell activation [36]. Transcription factors such as, NF-κB and CREB that are essential for the activation of early T lymphocytes were also downregulated [37]. In the aforementioned twin study, a major proportion (91.3%) of genes that showed altered expression in CD4 and CD8 cells during spaceflight reverted to normal levels within 6 months of returning to Earth. Surprisingly, spaceflight did not appear to attenuate the recruitment of influenza-specific T lymphocytes as indicated by the response to mid-flight vaccination. This suggests that major immune functions, including chemotaxis, antigen presentation and distribution were all retained unlike previously thought [11].

3.2. B lymphocytes

Observation of spaceflight-induced effects on the immune system of 30 cosmonauts flown on both short-term and long-term missions to the ISS revealed that the levels of IgM and IgG serum antibodies in response to cytomegalovirus, herpes simplex virus, herpes virus type 6 and Epstein-Barr virus did not change significantly [38]. However, a brief, albeit reversible increase in the levels of IgG and IgA were observed in cosmonauts who spent more than two weeks in space. Also, IgM levels remained stable even after prolonged missions. Whether these changes are indicative of compromised resistance to infections during space missions remains unclear. Adult *Pleurodeles* that spent 5 months aboard Mir space station when immunized orally and sacrificed 10 days post-flight revealed the use of different VH families in IgM heavy-chain transcripts. This indicates a modified antibody response mounted against the antigens (present in food) during spaceflight [39]. In addition to V(D)J recombination that generates antibody diversity in vertebrates, naïve B cells undergo isotype class switching and somatic hypermutation to enhance binding and effector abilities of the expressed antibodies. In a similar experiment, *Pleurodeles* demonstrated a lower frequency of somatic hypermutation following hyperimmunization during their stay aboard Mir. Several factors including reduced T cell activation, cytoskeletal perturbation, and alterations in cytokine signaling cause this reduction in frequency of somatic hypermutation thereby decreasing antibody affinities [40]. This decrease in IgM heavy chains and lymphoid-determining factor, Ikaros further suggest a decrease in immunocompetent B cell lymphopoiesis [41].

Likewise, studies have shown impairment in mitogen-induced response of B and T lymphocytes in mice exposed to 2 g centrifugation for 21 days along with an increased IgG concentration [42]. Mice subjected to 1.6 g for 7–40 days demonstrated a reduction in the total number of white blood cells, specifically a reduced number of lymphocytes in the peripheral blood, spleen and thymus [43].

3.3. Natural killer cells

The cytotoxicity of natural killer cells was found to be reduced in cosmonauts following a 3-week spaceflight [38,44]. It has been shown that a decrease in the cytotoxicity of human natural killer cells correlates inversely with the time of exposure to simulated microgravity [45].

3.4. Macrophages

The release of reactive oxygen species by macrophages was found to be reduced in both real and simulated microgravity whilst being augmented in hypergravity conditions [46]. It was also shown that microgravity did not significantly affect the cytoskeletal framework of macrophages unlike that of lymphocytes as mentioned before [47]. Several studies have recorded the incidence of leukocytosis among astronauts irrespective of the duration of spaceflight [48,49]. Not surprisingly, ground-based microgravity studies using anti-orthostatic suspension mouse models have shown impairment in superoxide response to phagocytosed bacteria [50].

In contrast, rats exposed to simulated hypergravity by chronic centrifugation were unresponsive to antigenic stimulation, thereby maintaining a poised immune system raising a speculation of immune system staying homeostatic under hypergravity conditions [51]. However, hypergravity has been demonstrated to improve resistance to Toll-mediated fungal infection in *Drosophila* except in the *yuri gagarin gene* gravitaxis mutant [52]. Such discrepancies of results across studies may be attributed to disparity in age, gender, species and the method used to simulate microgravity in the respective experiment.

4. The musculoskeletal system

4.1. Bone

The skeletal system which primarily gives protection to vital organs consists of the bone and its associated dense connective tissues, including cartilage, tendon and ligament, all of which assist in movement and locomotion. Bones of the skeleton provide structural support to the body enabling upright position, maintain acid-base balance and mineral homeostasis, serve as reservoir of growth factors and assist hearing by allowing sound transmission into the inner ear. The associated connective tissues form a variety of joint structures allowing effective leverage for muscular activity. Spaceflight-induced microgravity leads to systemic bone loss and muscle atrophy in the trunk and legs of astronauts, making these structures with anti-gravity functions most susceptible. Low bone mass results in severely reduced bone mineral density increasing the risk of fractures which may also impact the subsequent healing process [53,54].

Bone strength is maintained by modifications in its cortical thickness, cross-sectional area, mineralization quality and microstructure. Mineralized matrix in the dense cortical and trabecular bones is sustained independently by the activities of osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells). Alterations in mechanical loading either upregulate or downregulate the activity of these cells [53]. Weight-bearing bones like, femur, tibia and vertebrae in male mice space-flown for a month suffered accelerated bone loss compared to the non-weight-bearing bones. A significant reduction in the trabecular fraction of these bones indicated a decreased osteoblast differentiation and an increased bone resorption. Interestingly, among the non-weight-bearing bones, ribs and sternum that are involved in breathing received higher mechanical load. Consequently, these recorded a significant decrease in bone area without much disruption in tissue area and cortical thickness as compared to ground-based controls implying reduced skeletal maturation-related cortical expansion [55]. Of note, in an experiment conducted to study spaceflight-induced effects on fracture healing, 7-week-old mice femur was operated and compared with that of space-flown counterparts. Here, a comparably identical loss in the trabecular fraction of tibia (24–28% reduction) suggested similar unloading effects due to both spaceflight and surgery [56]. This raises doubt about fracture healing in space, an environment which itself causes unloading-induced atrophy.

Pertinent, but often overlooked are the weight-bearing ankle bones distal to the femur that also suffer similar bone deterioration. The calcaneous, navicular, and talus bones in the ankle of mature mice space-flown for 1 month followed by an 8-day recovery phase post-flight experienced a loss in trabecular volume although less than femur and vertebrae described above. Cortical thinning and bone erosion occurred predominantly at sites of muscle insertions and hence could be attributed to muscular atrophy that is inherent in microgravity. Surprisingly, an 8-day ambulatory period post-flight further worsened the bone loss endured in spaceflight. Post-flight stress response and higher bone resorption during the initial phase of recovery might be strong candidates for this deterioration in bone microarchitecture [57]. Hence, space sojourns induce bone remodelling with site-specific loss according to the function of weight-bearing bones [58]. Zebrafish larvae exposed to simulated microgravity in a 2D clinostat from 5 to 10 days post fertilization (dpf) showed significantly reduced bone formation in key structures of the head skeleton [59]. Unlike the weight-bearing bones in humans and rodents that are usually affected as a result of microgravity-induced muscle strain, cranial bone elements in the fish are affected directly due to changes in gravitational unloading.

Studies using 2T3 preosteoblasts where alkaline phosphatase, *runx2* mRNA and parathyroid hormone receptor 1 levels (osteoblastic markers) were downregulated, while cathepsin K (osteoclastic marker) was upregulated in an RPM also indicated suppressed bone formation [60]. Fluid shear stress on the osteoblasts transduces mechanical signals into a biochemical response thereby triggering osteoblastic activity. However, decreased interstitial fluid pressure due to microgravity reduces this signal transmission to osteoblasts thus impairing their activity. Hence, resistive exercises that would draw fluid into these bones could help mitigate bone resorption to a certain level. On the other hand, location and attachment sites dictate the biochemical function of tendons and ligaments that assist musculoskeletal activity. Similar to bone physiology, immobilization, disuse and injury can cause a decrease in extracellular matrix of these connective tissues [53].

Mice exposed to 3 g hypergravity for a period of 1 month showed reduced osteoclastic activity in the tibia indicating suppression of bone resorption and further lead to an increase in bone mass [61]. Exposure of mice to hypergravity for 2 weeks significantly improved the mass of humerus, femur, and tibia along with an increase in the expression of osteoblastic genes like, *Osx* and *Bmp2,* again indicating high bone turnover [62]. Artificial 1 g reversed spaceflight-induced trabecular separation in humerus and tibia of mice by an increase in osteoblastic activity thus emphasizing the impact of mechanical loading on these cells [62]. Zebrafish larvae exposed to 3 g hypergravity from 5 to 9 dpf in a large diameter centrifuge displayed broadening of the head skeleton. This lead to an increase in the distance between the symmetrically arranged, paired bone elements showing higher ossification due to an increased bone formation. Hypergravity stimulated the expression of a gene regulatory network consisting of *fos, fosb, egr1, edn1, socs3a, gadd45b,* and *klf2a,* which affect most of the processes of the skeletal system and vestibular gravity sensing. On the other hand, hypergravity suppressed the expression of vitamin D receptor that is involved in calcium uptake. Absence of any major effects in hypergravity conditions indicated that larval cranial cartilage is less affected by mechanical constraints [63]. Thus, communication between osteoblasts and osteoclasts which drives a major mechanical sensory network is disturbed in altered gravity conditions [64,65].

Although key physiological systems are discussed separately, it should be noted that the body's integrative physiology is exposed to altered gravity as a whole, thereby affecting the various functions of systems interacting with it. For instance, interactions between the hematopoietic stem cells (HSCs) and their resident bone marrow niches

(endosteal and vascular niches) regulate the balance between quiescence, proliferation, and differentiation of HSCs mainly by IL-7 signaling from stromal cells and a network of major transcription factors. Changes in the bone microstructure in microgravity and hypergravity conditions thus affect the maturation and function of blood cells. In hind limb unloaded mice, a decrease in bone microstructure correlated with a reduction in B cell lymphopoiesis specifically targeting the committed pro-B to pre-B cell transition rather than multipotent HSCs by virtue of bone marrow compartmentalization [66]. Similarly, a reduction of B lymphocytes in the bone marrow (61%) and spleen (41%) of mice flown aboard the Bion-M1 biosatellite for 1 month followed by a 1-week recovery period is suggestive of an increased susceptibility to infections [67].

4.2. Muscles

Microgravity-induced physiological adaptations also include reduction in muscle mass, strength and function leading to decreased functional performance and metabolism upon returning to Earth's gravitational environment. Mechanical unloading primarily leads to reduced muscle protein synthesis causing muscle atrophy and subsequent decrease in muscle mass due to accelerated breakdown of muscle proteins [68]. Anti-gravity or postural muscles which include soleus, adductor longus and vastus intermedius consisting of predominantly slow-twitch fibres appear more vulnerable to mechanical unloading by shifting towards a faster myosin heavy chain composition [69].

Mice exposed to microgravity for 14 days in the Columbia space shuttle (STS-62) showed significant reduction in muscle mass (8–9%) and myofiber cross-sectional area (10–35%) of extensor digitorum longus and gastrocnemius. Such a reduction in these predominantly fast-twitch muscles indicated a continued atrophy caused by reduced skeletal muscle protein synthesis or elevated protein degradation. The fact that diaphragm was unaffected upon unloading suggested that the functional role of the muscle rather than myofiber composition directs muscular response to microgravity [69]. Factors contributing to this muscle loss include reductive remodelling, pre-flight physical fitness, and inability to maintain energy balance during spaceflight. Reduction in muscle size and strength is mainly attributed to decreased work load on the muscles [70]. Denervation experiments on specific muscles suggested that gravitational unloading of postural muscles probably altered the pattern of neuromuscular activation of these muscles, while unloading of non-postural muscles had negligible effects on the pattern of neuromuscular activation. Indeed, microgravity experienced in a spaceflight leads to preferential remodelling of the neuromuscular junctions [69].

Gravitational loading by 3 g hypergravity for 4 weeks in mice improved muscle mass around the tibia, increased myofiber size and promoted muscle differentiation in the soleus indicating hypertrophy. This is caused by the upregulation of myogenic genes like, *Myf6*, *Myod*, and *Myog,* thereby inducing muscle mass in a dose- and duration-dependent manner [61,71]. Chronic centrifugation at 2*g* again induced muscle hypertrophy in mice by specific downregulation of muscle ubiquitin ligases and suppression of autophagy-related genes indicating increased myogenesis and decreased muscle degradation [62]. However, vestibular lesions attenuated hypergravity-induced increase in muscle and bone mass, muscle differentiation and myofiber size of the soleus muscle implying that these changes are mediated by vestibular signals [61,72]. Adapting to reduce dependence on the musculoskeletal system for work performance and proper energy balance, if not achieved, would likely pose serious health threats in microgravity environment. Inflight exercise programmes would be more effective only if astronauts are in perfect energy balance which still remains a big challenge [70]. The opposing physiological effects observed in microgravity and hypergravity conditions are presented in Fig. 1.

5. The vestibular system

5.1. Otoliths and inner ear sensory cells

The vestibular system functions to maintain body equilibrium in the Earth's gravitational field by working as a sensory apparatus coordinating balance and movement. It carries information about acceleration of the head, including linear accelerations sensed by otoliths and angular accelerations relayed by semicircular canals. Otoliths are calcified structures that are responsible for internalization of gravitational cues in vertebrates. Any perturbation to gravitational sensing could result in orientation problems leading to space motion sickness (SMS) which is commonly experienced by astronauts [73]. When this occurs during head movement, semicircular canals relay normal information, but otoliths provide altered information due to a higher asymmetry resulting in disparity between semicircular canal and otolith signals (sensory mismatch theory) [74]. An initial period of such vestibular insult is usually followed by a rapid adaptation to the microgravity condition. The symptoms of SMS also include paleness, cold, sweat, nausea, dizziness, and vomiting which are experienced during the early phase of transition from 1 g to 0 g. Fishes are widely used model organisms by virtue of homology of their vestibular system to that of humans. Also, the effects due to gravity are less pronounced on their muscles, vascular tonus system, and supporting tissues, unlike their large otoliths which are highly susceptible to gravity changes. In fishes, sensory mismatch manifests as looping responses and spinning movements. Cichlid larvae exposed to clinorotation showed increased otolith size and growth rate [75].

Hypergravity delayed the rate of formation of otoliths by inhibiting calcium deposition which led to decreased otolith mass. Otoliths also grew slower in hypergravity conditions and their asymmetry diminished representing an adaptive response to allow afferent inputs reaching the brain remain within the range of neuronal vestibular compensation [76]. The inertia provided by the weight of otoliths facilitates deflection of ciliary bundles of the inner ear sensory cells which have a critical period for gravitational sensing (discussed later) [77]. Microtubules in the hair cells were also disorganized which resulted in the loss of their shape. Neonates of pregnant rats exposed to 2 g hypergravity from embryonic day 10 to postnatal day 8 in a four-armed animal centrifuge displayed a transient excitability phase of neonatal utricular hair cells in firing action potentials demonstrating that development is affected by an enhanced gravity condition [78].

5.2. Neuro-vestibular system and vestibulo-ocular reflexes

Otoliths sense gravito-inertial acceleration, which is a resultant vector from all other linear accelerations and are involved in a number of ocular compensatory mechanisms through the linear vestibulo-ocular reflex (VOR). Otolith-induced eye movements of two rhesus monkeys space-flown for about 2 weeks showed altered spatial orientation of the VOR [79]. An increase in the number of synaptic contacts within the vestibular nucleus of 5-day-old swordtail fishes space-flown for 16 days as part of the STS-90 Neurolab space mission possibly reflects a countermeasure that acts upon the efferent system to enhance otolith growth in microgravity conditions [80]. Four oyster toadfishes examined upon their return from a 5-day spaceflight displayed maximal excitement and directional selectivity along with sensory changes in their utricular afferents [81].

The VOR, which responds to both translational and rotational movements of the head utilizes information from the inner ear vestibular labyrinth to generate eye movements and stabilizes gaze during head movements [82]. Roll-induced VOR (rVOR) is a form of utriculo-ocular reflex that is specifically related to gravity. The rVOR gain and amplitude measured in 3 aquatic vertebrates space-flown for 9–12 days showed that the first appearance of rVOR has a gravity-sensitive critical period of development [83]. The major finding in *Xenopus* embryos was

a gravity-related critical period when an rVOR can be first induced in an organism. The rVOR gain and amplitude was unchanged in tadpoles space-launched close to the emergence of ear vesicles (stages 25–28) or after hind limb bud was formed (stages 47–50) along with normal body shape development. On the other hand, tadpoles space-flown shortly before first appearance of the rVOR (stages 33–36) revealed an rVOR depression, while those launched shortly after first appearance of rVOR (stage 45) experienced a reflex augmentation [84,85]. Similar age-related sensitivity to microgravity was also demonstrated in *Pleurodeles* 3 days following the 10-day Soyuz mission TM-7/TM-6. Flight and ground *Pleurodele* larvae (16–18 dpf) showed no rVOR, which however could be stimulated only in the ground larvae on the fourth day post-flight. Any such change in rVOR post-flight would mean that vestibular development had been impaired by altered gravity [86]. Likewise, studies in cichlid larvae revealed rVOR augmentation post-flight when launched before the appearance of first rVOR (stages 11–12). However, if launched after the first reflex (stages 14–16), rVOR development was unaffected thus demonstrating a sensitive critical period when development of the macular VOR depends on gravitational stimulus. Also, during this narrow period of early development microgravity exposure sensitizes the vestibular system [87,88]. Of note, the underlying neuronal pathways together with synaptic contacts between eye muscles and neurons are formed prior to the first emergence of rVOR. Similar results were also obtained with zebrafish larvae following exposure to simulated microgravity (refer Table 1).

Hypergravity (3 g) exposure for 9–12 days decreased both gain and amplitude of rVOR in *Xenopus, Pleurodeles*, and cichlid fish. Unlike microgravity conditions, a critical period was not observed in studies conducted in 3 g hypergravity [87,89]. A 10-day 3 g exposure of *Pleurodeles* had no effect on the developmental progress if exposure phase occurred prior to functional rVOR maturation indicating that hypergravity leads to a slowly developing vestibular sensitization [86]. Prolonged exposure of rats to hypergravity impaired the peripheral vestibular organ and the amplitude of otolith-spinal reflex due to decreased sensitivity [90]. These studies clearly demonstrate that the period of first appearance of rVOR is an important milestone for the development of the underlying neuronal network [91].

6. The nervous system

6.1. Central nervous system

The nervous system is a complex network in animals that coordinate sensory and motor information by transmitting signals throughout the body. It consists of the central and peripheral branches of the nervous system. The central nervous system (CNS) is composed of the brain and the spinal cord, while the latter consists mainly of nerves. Efferent or motor neurons transmit signals from the brain whereas afferent or sensory neurons transmit signals from the rest of the body. The body's response to any stimulus is directed by the CNS. Alterations in gravity levels affect the brain and other parts of the CNS thereby relaying modulatory effects on the aforementioned systems. Several lines of evidence point towards significant changes in the structure, function, and metabolism of the CNS. MRI images of the brain before and after space missions of long duration (164.8 days) involving 18 astronauts and short duration (13.6 days) involving 16 astronauts were compared. Both showed a similar upward shift of the brain, narrowing of the central sulcus and cerebrospinal fluid (CSF) spaces at the vertex, albeit more predominant after long-term exposure to microgravity. Spaceflight-induced intracranial hypertension that accompanies visual pathologies occur due to cephalad shift of body fluids along with disruptions in the flow of CSF and venous outflow [92]. Proteomic data from the brain of mice following a 13-day mission on STS-135 found variable changes in the brain for instance, white matter had elevated sympathetic activity, protein/organelle transportation, synaptic plasticity and catecholamine formation, while grey matter had increased formation of dendritic spines, vesicles and myelin sheath [93]. Energy consumption accelerated in the CNS to counter the stress endured in microgravity as evidenced by higher glycolytic rate, mitochondrial respiration as well as synthesis of fatty acids and complex lipids [94].

Consequently, the CNS is more prone to oxidative injury due its higher oxygen demand [95]. The pre-frontal cortex suffered oxygen deprivation as observed during hypergravity exposure of 12 healthy participants (6 men and 6 women) in a short-arm human centrifuge. This was accompanied with a decrease in oxyhemoglobin levels and a subsequent increase in deoxyhemoglobin levels, which were however rescued to a certain extent upon exposure to microgravity [96,97].

6.2. Development of neurons

An overall delay in development of the vestibular system was shown by delayed reflexes like righting (contact and air) in rat neonates which spent extended time periods in microgravity [98]. Delayed surface righting in spaceflight is indicative of partial development of motor neurons and the brain regions responsible for it, with the duration of spaceflight being a limiting factor [99]. Of note, negative geotaxis observed in insects flying under normal gravity conditions is affected by the Earth's geomagnetic field. Since this is reduced in space, i.e., in microgravity conditions, an indirect cause-effect relationship between altered gravity and geotaxis is plausible, while the effect of altered gravity on genes related to geotaxis such as, *cry, pyx* and *pdf* in *Drosophila* remains to be investigated [100]. The *pyx* gene has been shown to be associated with gravity sensing by Johnston's organ in *Drosophila,* which sends mechanosensory neuron bundles (gravity responsive) to the antennal mechanosensory and motor centre [101].

Pregnant rats subjected to 2 g hypergravity and pups born and raised in a similar environment showed a delay in the maturation of some brain areas and neuron bundles. Hypergravity (2g) delayed development of motor neurons thus affecting monoaminergic neuronal projections, reticulo- and vestibulo-spinal tracts in addition to other descending pathways from the CNS to spinal motor neurons [102].

6.3. Cognition, memory, and learning

Cognitive impairments leading to neurodegenerative diseases have also been implicated with gravity changes mediated by altered vascular functions. The reactivity of vascular smooth muscle cells influencing vasodilation and contraction thereby regulating cerebral blood flow was decreased in microgravity experiments. This was due to reduced depolarization of neurons that regulate Ca^{2+}-induced Ca^{2+} channels in these cells which may lead to Alzheimer's disease and Parkinson's disease that are often associated with impaired learning, memory and cognition. Moreover, hampering brain perfusion and Ca^{2+} signaling could potentially disrupt the blood-brain-barrier (BBB) which also has a causal effect on such medical conditions [103]. Not surprisingly, simulated microgravity has been reported to increase aquaporin 4 levels, a marker for decreased BBB integrity. Microgravity-induced impaired learning and memory in rats has been linked to reduced spine density, neuronal loss, connectivity changes and cytomorphometric changes (decreased area, perimeter, synaptic cleft, length of active zone, etc.) in hippocampal CA1 neurons. Furthermore, simulated microgravity downregulates SNARE proteins that mediate vesicle fusion thereby perturbing the release of neurotransmitters in the brain leading to behavioural changes [104]. Observations from 6 to 12 month-long missions indicated that prolonged microgravity may negatively impact cognitive performance post-flight which could be aggravated by astronauts participating in media events and research studies following their return to Earth [11].

6.4. Affect system (anxiety and depression)

Brain regions housing the affect system (anxiety and depression) are also sensitive to gravity changes [103] which could be responsible for

mood swings. Microgravity studies have shown that lateral hypothalamus auto-stimulation conjugated with sympatho-vagal imbalance was sensitive to anti-depressors leading to depression-like symptoms [105, 106]. The levels of GDNF and CDNF, both survival factors in the brain dopamine system were decreased in long-term space-flown mice. Hence, disregulation of these factors greatly contribute to space-induced impairment in the motivational component of reward-motivation behaviour [107].

Hypergravity (2 g) exposure of mice for 14 days showed down-regulated BDNF (involved in regulating brain development and neuroplasticity) in ventral hippocampus and hypothalamus along with 5-HT1BR (part of the 5-HT system of serotonin receptors whose dysfunction causes anxiety and mood disorders) downregulation in the cerebellum, which more or less reverted upon deceleration to normal gravity [108]. Studies have shown that hypergravity, both chronic (2 g–3 g for 3 weeks) and mild (2 g for 2 h) hypergravity resulted in depression-like symptoms such as, high anxiogenic profile and sex-dependent anxiety, respectively [103]. These studies support a molecular basis for the observed behavioural changes in altered gravity conditions.

7. The reproductive system

Perpetuation of species is inevitable to avoid extinction and ensure balance in the ecosystem. Studies have shown that microgravity and cosmic radiations modify the reproductive capacity in space [109]. Lesions and irreversible pathological injury to the testis, decreased androgen receptor expression [110] and testosterone levels [111,112], as well as reduction in the number of Leydig cells were observed in animals (mice and quail) exposed to simulated microgravity conditions [113]. Moreover, increased levels of caspase 8, caspase 3, NF-κB, Fas ligands, and Fas or death receptors leading to apoptosis revealed the severity of testicular cell damage in mice exposed to simulated microgravity by suspension techniques [111]. Microgravity simulated in a rotary cell culture system (RCCS) also promoted meiotic progression of mouse spermatogonia spontaneously [114]. Mouse oocytes cultured in a rotatory wall vessel (RWV) to simulate microgravity showed excessive peripheral vacuolization, a common cytoplasmic abnormality which additionally caused autophagy in these cells [115]. Moreover, microgravity in an RCCS induced disorganization of spindle fibres causing cytoplasmic blebbing in cultured mouse oocytes thus inhibiting their maturation [116]. In a recent experiment, male mice individually caged in microgravity and artificial gravity (1 g by centrifugation aboard space station) over a span of 35 days showed decrease in the weights of accessory glands such as, prostate, seminal vesicles and coagulating glands. This is consistent with decrease in total blood and plasma volume during spaceflight. Hence, these observations suggested that the weights of organs which store secretions were more susceptible to gravity changes while that of testes and epididymis remained unchanged [117].

In hypergravity conditions, pregnant rats either suffered miscarriage or gave birth to offspring which did not survive for long. Few pups died *in utero* and few others undelivered, albeit complete development pointing to the critical time when these animals were subjected to centrifugation-an earlier exposure resulting in prenatal death [118]. On the other hand, there is less doubt about the success rate of fertilization in space. The presence of both female and male pronuclei in the egg cytoplasm affirmed that fertilization does occur at altered gravity conditions. Microgravity reduced microvilli elongation, while hypergravity enhanced the same in *Pleurodeles* eggs subjected to space and centrifugation conditions, respectively [119].

7.1. Post-partum development

Fertilization is not the end of a success story in reproduction, at least in mammals where it involves complex processes such as, implantation, pregnancy, parturition and early development of offspring all of which are essential for successful propagation of a species. A critical issue in mammalian reproduction is the *in utero* developmental process and species-specific sensory, motor and neural systems. These process continue to develop throughout postnatal life which helps mammals adapt to various conditions, including those imposed by the Earth's gravitational field. Also, their maternal care-giving behaviour and postnatal infant development are quite unique where the mother plays a major role during the neonatal period. This is especially true for species like rats that produce highly immature offspring with underdeveloped sensory and motor abilities. Physical stimulation through licking and handling of pups are among important behaviours for initiating a broad range of neonatal functions. Thus, factors like space environment that perturb behavioural and/or physiological processes in the mothers or their offspring are likely to affect the bi-directional maternal-offspring system. These changes include altered retrieving behaviour (pups repeatedly floating way from the nest), diminished milk intake, warmth and tactile stimulation from the mother. Pregnant mice flown from gestational day 9–11 as part of the NIH.R1 and NIH.R2 space missions respectively, displayed intact circadian cycles upon re-adapting to 1 g on gestational day 20, while the frequency of grooming and rearing behaviour was significantly lowered [120]. In another 9-day NIH.R3 space mission involving pups at 5, 8 and 14 days of postnatal age, only 14-day neonates survived and gained comparable weight, while the much younger neonates suffered from malnourishment, hypothermia and dehydration during spaceflight resulting in a few deaths [121].

The most dramatic changes from microgravity exposure occurred in the later part of pregnancy, which included space-flown dams experiencing two times more labour contractions relative to 1 g controls. In sharp contrast, pregnant mice exposed to either 1.5 g or 2 g hypergravity from gestational day 11 through parturition had significantly reduced labour contractions relative to 1 g controls. In both conditions, the duration and timing of parturition remained unchanged and the number of offspring born was comparable. This suggests a selective effect of altered gravity on the mechanism controlling the frequency of labour contractions, possibly through the emergence of late-term gap junctions in the uterine myometrium. At the onset of labour, the myometrium transitions from a relatively quiescent to a highly excitable state. Gap junctions with their connexin subunits increase substantially in late-term which allows adjacent myometrial smooth muscle cells to form a functional syncytium facilitating coordinated contractions during labour. Consistently, a major determinant of postnatal survival in hypergravity appears to be maternal reproductive experience and maternal post-partum behaviour [122]. Such complications in the maternal-offspring system demanded watching the animals in space conditions which revealed mice displaying route-tracing, somersaulting, repetitive unvarying and functionless locomotion, and unique circling behaviour which are usually less common. The circling behaviour resembles running wheels in terrestrial studies, which could have emerged as a stress response to spacecraft experience [123].

8. The integumentary system

Skin is the outermost covering of the body and represents the first immune barrier to foreign substances and pathogens. Also, skin plays an important role in moisture control and thermoregulation. It interacts directly with the environment and responds immediately to any external stimulus. Twenty individuals (17 men and 3 women) when subjected to head-down bed rest for 14 days demonstrated a decline in cutaneous vasodilation and sweat rate [124]. Skin sensory inputs from the soles of feet are coupled with the vestibular system to orient the body in a gravitational environment. Foot sole skin hypersensitivity of astronauts post-flight could be attributed to increased sensitivity of fast-adapting-velocity receptors and decreased sensitivity of slow-adapting-pressure receptors [125]. Skin thinning and loss of elasticity was also observed in astronauts after spaceflight. Thinning of epidermis would allow low wavelength ultraviolet and cosmic

radiations to penetrate the deeper layers of the skin thereby increasing the risk of developing skin cancer [126,127]. Studies on human dermal fibroblasts have shown that there is a 143% increase in collagen synthesis in simulated microgravity [128]. Transcriptome analysis of mice exposed to microgravity for 3 months aboard ISS revealed that dermal atrophy took place even after increased procollagen synthesis, which could be due to early degradation of newly formed defective procollagen [129]. The diversity of normal lipophilic fungal microbiota also decreased in cheek and chest skin samples of 10 astronauts obtained 5–6 months after their return from space. The percentage constitution of uncommon microbes such as, *Malassezia* (a genus of fungi) and *Cyberlindnera jadinii* (torula, a species of yeast) increased which could have colonized the astronauts before their spaceflight, but still retained the ability to grow under microgravity conditions [130]. Upregulation of *FGF18*, *ANGPTL7*, *COMP* and *CDK1* genes, which are normally downregulated in growing hair follicles, were observed in 10 astronauts following a 6-month-long space mission indicating inhibition of hair growth cycle in microgravity conditions [131]. Skin cells cultured under microgravity conditions showed increased epidermal contact, proliferation, nuclear and cellular hypertrophy. Therefore, microgravity-coupled bioreactors could be used to enhance proliferation of engineered skin tissues for the treatment of burn injuries since shear stress in these bioreactors would be nullified by microgravity [132].

9. Other systems

9.1. The adipose tissue

Triglycerides which are a substitute metabolic energy source during diminished glucose reserves are stored in large amounts by the adipose tissue [133]. Animals subjected to hind limb suspension unloading and astronauts in space follow a similar trend in that they go through a state of negative energy balance. In such a state, body fat stores are mobilized to compensate for the deficit in nutrient intake and a progressive protein loss characterized by loss of body weight, fat and protein content takes place. Spaceflight induces a shift from glucose metabolism to fatty acid metabolism in humans leading to increased utilization of triglycerides [134,135]. Low sympathetic activity state achieved in 8 healthy men subjected to a 5-day head-down bed rest demonstrated sustained sympathoinhibition leading to increased lipolytic activity in the adipose tissue as well as increased thermogenic activity in their mitochondria. Ectopic adiposity was observed with accumulation of visceral and intermuscular adipose tissue which could be due to fibro-adipogenic progenitors shifting more towards an adipocyte lineage [136–138]. Weightlessness reduces norepinephrine release, which influences β-adrenergic receptors to become hypersensitized to limited amounts of norepinephrine thus transitioning towards higher triglyceride metabolism. In contrast, pregnant rats exposed to 2 g hypergravity from mid-gestation through lactation showed decrease in glucose metabolism and lipolysis rate influenced by both g level and gestation period, which could entail limited supply of triglycerides for milk fat synthesis in a hypergravity environment [139].

9.2. The digestive system

In rats and humans, calorie intake and carbohydrate value remained unchanged in space as compared to ground control values. However, loss in body weight along with elevated plasma triglycerides, cholesterol and phospholipids was observed [140]. In rats, decrease in villi length, crypt depth, and mucin production of intestinal epithelial cells was observed when they were space-flown for 12 days [141–143]. There were changes observed in the microflora of the intestine and other mucosa during short-term or long-term spaceflights [144]. The gut microbiome responds dynamically to changes in diet and so changes in gut microbial composition and function were also seen during flight periods. The microbial ecosystem of the gut rebounds within weeks after landing thereby bringing it back to pre-flight status. The diversity of the microbiome that was preserved in the NASA twin study signifies minimal health risk associated with changes incurred due to spaceflight [11].

9.3. The ocular system

Astronauts frequently experience one or more ocular issues such as, optic disc edema, choroidal folds, cotton wool spots, nerve fiber thickening, and globe flattening. The symptoms of these ocular conditions collectively referred to as spaceflight-associated neuro-ocular syndrome (SANS) include cephalad fluid shift and its associated adaptations during spaceflight. Distension and increased pressure in the internal jugular vein may contribute to congestion in the blood vessel supplying the retina [11,145]. Recent research findings suggest that astronauts bound for a long-duration space mission could have intraocular implants before the start of their journey to protect vision and redress neuro-ocular issues [146]. In contrast, mice exposed to 10 g hypergravity in a centrifuge had decreased intraocular pressure which caused hypoxic damage to the retina followed by an increase in the expression of VEGF ligand and receptor [147]. The visual acuity of humans also decreased along with dilated pupils and thickened cornea when they were exposed to a hypergravity environment [148].

10. Conclusion

Research in the area of space science has largely focused on mitigating detrimental effects of the microgravity environment given the fact that exposure to it is inevitable. Although it is clear from our comprehensive review that most of the effects of space missions are only transient declining after a short period of time, the suffering endured while adapting back to 1g is something that needs to be addressed still. It should be noted that the effects of microgravity and hypergravity are not always opposing which raises an important question whether g levels really constitute a continuum. We have discussed contrasting effects between these gravity extremes in the preceding sections of this review. However, absence of these observations in the physiology of animal models (including humans) as a whole simply indicates that adaptations to hypergravity and microgravity occur through different mechanisms. An understanding of such mechanisms revealed by studies conducted both in spaceflight (real) and simulated (artificial) gravity conditions has helped design a range of countermeasures to resolve this issue. Aerobic exercises for cardiovascular deconditioning and resistive exercises for the musculoskeletal system have been the rule of thumb, nevertheless insufficient. The physiological challenge induced by artificial gravity simultaneously stimulates all the body systems by approximating the Earth's gravitational environment. This inflicts a stress on the cardiovascular and musculoskeletal systems, activates antigravity muscles, and stimulates the vestibular system thus simulating an Earth-like environment [149]. However, such equipment is a thing of distant future, especially earmarked for deep space exploration missions.

In a study involving 15 men who underwent a 6° head-down bed rest for 21 days it was demonstrated that a further exposure of these individuals to artificially induced gravity (1 g at heart level) for 1 h daily prevented cardiovascular deconditioning that is commonly associated with microgravity [150]. The effect was, however, not directly cardiac-mediated but through an improved sympathetic activity that had earlier decreased during the head-down bed rest. Previous studies using bed rest and water immersion to simulate microgravity have also shown that artificial gravity regimens along with exercise are capable of preventing orthostatic intolerance associated with weightlessness. This is merely an extension of the idea proposed using a short-arm human centrifuge for space adaptation syndrome by subjecting astronauts to repeated, long-term, low-intensity acceleration to prevent spaceflight-induced physiological deconditioning [151].

The next major concern is the immune system dysfunction endured

by astronauts in space for which specific and personalized countermeasures have been discussed recently. A strict pre-mission screening for aerobic and immune fitness followed by in-mission stress-relieving breathing exercise and visualization meditation accompanied by proper immune surveillance are recommended. Immune boosting medicines aboard, like polyclonal immunoglobulin and IL-2 are prescribed to rectify immune decrements. Finally, a rich diet including a combination of probiotics and vitamin D supplementation could help sustain proper immune health. For instance, probiotics including *Lactobacillus acidophilus, Bifidobacterium lactis, and Lactobacillus casei* could help alleviate spaceflight-related sicknesses, like respiratory infections, antibiotic-associated diarrhea, and dermatitis [152,153].

Intriguingly, a multitude of similarities between spaceflight-induced and aging-related physiological deconditioning has been recently explored. Aging is associated with deterioration of cardiovascular and musculoskeletal systems predisposing older individuals to orthostatic intolerance or dizziness upon assuming an upright position. The deconditioning caused by long-duration confinement in bed among older/bed-ridden individuals thus parallels spaceflight-induced deconditioning among astronauts. An integrated approach to deconditioning could lead to a comprehensive solution for both astronauts as well as long-term bed-ridden individuals [154]. Finally, a personalized artificial gravity training regimen would be more beneficial in preserving the integrity of various physiological systems to help astronauts quickly adapt to Earth conditions when they return home.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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