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Chapter

Effects of Microgravity on Human Physiology

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Abstract

The effects of microgravity conditions on neurovestibular, cardiovascular, musculoskeletal, bone metabolic, and hemato-immunological systems are described. We discuss "space motion sickness," sensorimotor coordination disorders, cardiovascular deconditioning, muscular atrophy, bone loss, and anemia/immunodeficiency, including their causes and mechanisms. In addition to the previously described deconditioning, new problems related to microgravity, spaceflight-associated neuro-ocular syndrome (SANS), and structural changes of the brain by magnetic resonance imaging (MRI) are also explained. Our proposed countermeasure, artificial gravity produced by a short-arm centrifuge with ergometric exercise, is also described in detail, and we confirmed this system to be effective in preventing the abovementioned deconditioning caused by microgravity exposure.

Keywords: microgravity, neurovestibular, neural plasticity, cardiovascular, musculoskeletal, bone metabolism, hematology and immunology

1. Introduction

Outer space offers several abnormal and/or unique environmental conditions, including microgravity, vacuum/hypovaria, acceleration, extreme temperature, space debris, space radiation, and confinement/isolation. As the latter four conditions may be mitigated by spacecraft engineering (i.e., pressurization and the bulkhead), we focused on microgravity and its effects on human physiology [1–8].

In spaceflight, astronauts face three periods of physiological adaptation induced by changing gravity: (1) changes upon entry to microgravity (initial adaptation), (2) changes after prolonged exposure to microgravity, and (3) readaptation to 1 G gravity on Earth after returning from space. Body systems influenced by microgravity are the neurovestibular, cardiovascular, musculoskeletal, bone metabolic, and immuno-hematological systems. The changes associated with these systems occur during the adaptation phases outlined above. We will briefly discuss each of these body systems.

2. Neurovestibular system

2.1 Space motion sickness

How do we humans sense our relative positions in three-dimensional space? There are three sensory systems in the human body that help us define position: the visual, somatosensory, and vestibular systems. Most of the information from the outside world is processed by the visual system, but the combination of somatosensory and vestibular systems from the inner body helps define the positional status.

The vestibular organs include the otolith organs and semicircular canals. The otolith organs, saccules (sagittal direction) and utriculi (horizontal direction), sense linear acceleration. The semicircular canals, anterior, posterior, and horizontal, detect angular velocity of the head. The vestibular organs in the inner ear detect and measure linear and angular acceleration. These responses—already a complex set of signals—are further integrated with visual and proprioceptive inputs.

Exposure to microgravity alters some of these input signals, leading to misinterpretation and inadequate responses by the brain. This may cause vertigo, nausea, vomiting, appetite loss, headache, pallor, etc. As the symptoms are like those of motion sickness, this set of symptoms is termed "space motion sickness," but unlike conventional motion sickness, antiemetic drugs cannot suppress the symptoms of space motion sickness. Approximately 60–80% of astronauts develop the symptoms within 2 or 3 days after launch. Space motion sickness is considered important because of its potential impact on the astronauts' operational performance.

Although sensory misinterpretation may play a role in space motion sickness, its exact mechanism remains unknown. There are, however, several hypotheses: (1) sensory conflict, (2) fluid shift, (3) otolith asymmetry, and (4) orientation adaptation [9, 10].

The sensory conflict hypothesis suggests that loss of tilt-related otolith upon entry into microgravity causes a conflict between actual and anticipated signals from sense organs subserving spatial orientation. Such sensory conflicts are thought to induce motion sickness in other environments.

The fluid shift hypothesis suggests that space motion sickness results from the caudad fluid shifts, which in turn result from the hydrostatic pressure gradients in the lower body to the thoracic cavity or to the cranial cavity when entering microgravity. The cephalad fluid shift leads to visible puffiness in the face and is thought to increase the intracranial pressure, the cerebrospinal fluid pressure, or the inner ear fluid pressure, altering the response properties of the vestibular receptors and inducing space motion sickness.

The otolith asymmetry hypothesis is based on the theory that a mass difference in otolith between the left and right ear is the origin of space sickness and that there is interindividual susceptibility.

The otolith adaptation theory, or otolith tilt-translation reinterpretation theory, is the theory that space motion sickness is caused during the process of the brain learning to reinterpret novel otolith quasi-static signals to represent linear acceleration in space rather than the usual interpretation of tilt relative to the vertical direction on Earth.

Until now, it was unclear which of these theories (if any) was most likely. However, evidence from Space Shuttle missions suggests that the otolith asymmetry and otolith adaptation theories are unlikely.

2.2 Countermeasures for space motion sickness

In the Shuttle program and in the case of the International Space Station (ISS), the most commonly used countermeasure for space motion sickness is

pharmacotherapy. Dornhoffer [11] reported the effects of four drug countermeasures (lorazepam, meclizine, promethazine, and scopolamine) for alleviating motion sickness induced by vestibular stimulation with a rotary chair and found that scopolamine was the only countermeasure to significantly change the mean duration of rotation compared with the placebo ($p < 0.008$), with a > 40% increase in rotation time.

In the Shuttle study, administration of promethazine at 20–50 mg was recommended by intramuscular injection or suppository. In the ISS study, meclizine and dimenhydrinate with cinnarizine were hypothesized to affect the medial vestibular nucleus. Promethazine is a vestibular suppressor, but a more recent report [12] demonstrated that *d*-amphetamine counters this suppression and inhibits the effects of fatigue on the saccadic reaction time.

We propose that artificial gravity is also effective in preventing space motion sickness because constant gravity on the otolith is effective against all four etiologies of this maladaptation.

3. Cardiovascular system

3.1 Effects of microgravity in the cardiovascular system

The changes in the cardiovascular system begin solely with the fluid shift associated with microgravity, followed by the decreased circulatory blood volume, cardiac size, and aerobic capacity, and the most prominent symptom, postflight orthostatic intolerance. These symptoms are generically known as "cardiovascular deconditioning" [13–17].

When the spacecraft reaches low Earth orbit (LEO), body fluids move from the lower body to the thorax, which is associated with the increase in the intraocular pressure and morphological alterations in the central nervous system, demonstrated by changes in the magnetic resonance imaging (MRI).

As a result of fluid shift, the leg volume decreases and the face becomes puffy. The leg volume decreases by 1 L, whereas subcutaneous tissue at the forehead thickens by as much as 7% compared with in the preflight supine position. The pulmonary capillary blood volume increases by approximately 25%, and intraocular pressure can nearly double. Fluid shift increases the cardiac volume and stroke volume at the beginning of the spaceflight (first 24 to 48 hours), but over time, the heart rate, stroke volume, and cardiac output stabilize to the preflight sitting level. The arterial blood pressure slightly decreases compared with the preflight level. Compared with "space motion sickness," cardiovascular and fluid balance adaptation is gradual. The symptoms appear in 3–5 days and disappear after 1–2 weeks, causing facial edema, nasal stiffness, heavy headedness, papilledema, or jugular vein dilatation. These symptoms upon exposure to microgravity disappear at most 2 weeks after the reduction in circulatory plasma volume [13–17].

The cardiovascular changes in actual spaceflight differ from those in stimulations such as head-down bedrest or dry immersion. First, the volume of fluid shift is much larger than the orthostatic change from the supine to upright positions. The fluid volume loss during simulated microgravity (e.g., head-down bedrest or dry immersion) is less than 50% of that observed in actual spaceflight. Second, the central venous pressure measured during spaceflight does not increase as much as in head-down bedrest. Third, the diuresis caused during simulated microgravity is to a lower degree.

Then what is the cause of reduced blood volume after adaptation to microgravity? Diedrich et al. [18] explained the reduced blood volume in space as (1)

a negative balance 2010 of decreased fluid intake and smaller reduction of urine output; (2) fast fluid shifts from the intravascular to interstitial spaces as a result of lower transmural pressure after reduced compression of all tissues by gravitational forces, especially of the thorax cage; and (3) fluid shifts from intravascular to muscle interstitial spaces because of lower muscle tone required to maintain body posture, and the attenuated diuresis during space flight is due to increased retention after stress-mediated sympathetic activation during the initial phase of space flight.

3.2 Decrease in the circulatory blood volume

The centralization of body fluid induces dehydration to adapt to the microgravity environment. The cephalad fluid shift causes an increase in venous return and marked increase in the stroke volume, inducing the alterations in the autonomic and endocrine systems to control the cardiovascular system.

On the first day of microgravity exposure, urine volume does not increase, but the circulatory blood volume suddenly decreases by 17%, probably due to the shift of water from the intravascular to the interstitial spaces and finally to the intracellular space. This induces an increase in the hematocrit level, which suppresses erythropoietin production and reduces the erythrocyte volume. Reductions in the circulatory plasma volume and erythrocyte volume equal an 11% reduction in the total blood volume, and this stabilizes the central blood volume to a new equilibrium, which nearly equals the central blood volume in the standing position at 1 G on Earth.

Upon the above alterations, the autonomic nervous system stabilizes the blood pressure by suppressing the sympathetic functions and activating the vagal functions by reducing the heart rate and suppressing muscle sympathetic nerve activity. Alterations include suppression of vasopressin by the Henry-Gauer reflex, facilitation of α-natriuretic peptide secretion, and suppression of the renin-angiotensin-aldosterone system, all of which facilitate urination. Thus, centralized body fluid is excreted, accounting for 10–15% of the circulatory blood volume, increased hematocrit level, and adaptation of the cardiovascular system 5–7 days after microgravity exposure. This ameliorates the facial edema and jugular distension. This adaptation causes cardiovascular deconditioning, including orthostatic intolerance, after returning to 1 G on Earth.

3.3 Reduced heart size

Once exposed to microgravity, the volume and pressure stimuli disappear. Constant postural change of lying down from upright standing on Earth loads intermittent volume on the heart, which ceases during microgravity. In addition, microgravity reduces the overall pressure load on the heart depending on the content of the countermeasure program. The mean arterial pressure slightly decreases. During spaceflight, the myocardial volume decreases by 8–10%.

3.4 Cardiovascular system after stabilization

After adaptation to microgravity, the cardiovascular system stabilizes, and the blood pressure is either unchanged or slightly lower [19]. Ambulatory blood pressure recording for 24 hours in eight astronauts revealed that the systolic, diastolic, and mean arterial pressures (mean ± se) in space were reduced by 8 ± 2 mmHg (p = 0.01; ANOVA), 9 ± 2 mmHg (P < 0.001), and 10 ± 3 mmHg (p = 0.006), respectively, with a maintained nocturnal dip of 8 ± 3 mmHg (p = 0.015). The cardiac stroke volume and output increased by 35 ± 10% and 41 ± 9% (p < 0.001),

respectively, the heart rate and catecholamine concentrations were unchanged, and systemic vascular resistance was reduced by 39 ± 4% ($p < 0.001$).

3.5 Alteration of aerobic exercise capacity

Microgravity exposure reduces the circulatory blood volume; however, the maximal oxygen uptake is maintained after a short duration of spaceflight. During long-term spaceflight, the aerobic capacity decreases without countermeasures, but aerobic exercise training can maintain it, although standard exercise only markedly reduces the postflight maximal oxygen uptake. After a short duration of spaceflight (9–14 days), the maximal oxygen uptake decreased by 22%, probably due to decreases in the maximal stroke volume and maximal cardiac output without alterations in the maximal heart rate, blood pressure, or whole-body arteriovenous oxygen. This decrease in maximal oxygen uptake is believed to be due to the decreases in intravascular blood volume, stroke volume, and cardiac output.

As crew members are expected to work on the Moon/Martian surface, and they are exposed to extensive heat stress in the extravehicular suits, this aerobic capacity is considered to be significant after landing on the Moon/Mars.

3.6 Alterations in sympathetic neural traffic under microgravity

Sympathetic neural traffic indirectly measured by the plasma noradrenaline level has been reported to increase during spaceflight from the preflight control level [14, 20], and vagal activity estimated by power spectral analysis of heart rate variability was reduced after long-term spaceflight [21, 22].

Microneurographically recorded neural traffic in humans is known to be muscle and skin sympathetic nerve activity (MSNA and SSNA), and MSNA controls the vasomotor function of the muscular bed, responding to blood pressure changes against gravitational stress [23–25]. MSNA was suppressed during exposure to short-term microgravity induced by parabolic flight [26], mild lower body positive pressure (10–20-mmHg LBPP) [15], and thermoneutral head-out water immersion [27] responding to the loading or unloading of cardiopulmonary receptor-stimulated cephalad fluid shift. On the other hand, MSNA was increased after exposure to long-term microgravity in spaceflight and its simulation induced by dry immersion [28] or 6° head-down tilt bedrest [17] due to different mechanisms, including plasma volume loss, changes in baroreflex, and vascular compliance.

3.7 Postflight orthostatic intolerance

Orthostatic intolerance is usually observed after returning to 1 G on Earth. The definition of orthostatic intolerance usually includes simple syncope, lightheadedness, or >20-mmHg reduction in systolic blood pressure.

Astronauts usually notice orthostatic intolerance during prolonged upright standing rather than while standing up. Just before fainting, they sometimes have tachycardia, suggesting that they have postural orthostatic tachycardia syndrome (POTS). This phenomenon is due to a state in which fluid shift easily triggers tachycardia, which also easily triggers Bezold-Jarisch reflex, and the vagal response suppresses the systolic blood pressure. Although all astronauts stood upright for 10 min, 63% were unable to finish the stand test in 10 min.

Less important factors for postflight orthostatic intolerance are reduced compliance of the lower legs, reduced baroreflex sensitivity, and increased basal sympathetic tone.

Reduction of the circulatory blood volume is the most important factor for postflight orthostatic intolerance. The decrease in stroke volume after spaceflight reflects this circulatory blood volume loss. Although this is the main cause, the recovery of circulatory blood volume to the normal state is not complete. The crew members are recommended to take 8 g of salt and 1 L of water, which ameliorates the orthostatic tolerance, albeit not completely.

Another factor is the limitation by vasoconstriction. The postflight blood pressure of non-finishers cannot be increased by the total peripheral resistance compared with the preflight state. During the postflight upright standing 70° tilt test, the total peripheral resistance cannot increase despite activation of muscle sympathetic nerve activity, probably due to the alterations in venoarterial reflex and smooth muscle atrophy of the resistant vessels. Overall, circulatory blood volume reduction and attenuated vasoconstriction are the main factors for orthostatic intolerance.

3.8 Cardiovascular deconditioning

What is the cause of this cardiovascular deconditioning? NASA's criteria of orthostatic intolerance are (1) presyncopal symptoms (pallor, cold sweat, nausea, blackout, and fainting), (2) gradual systolic blood pressure decrease <80 mmHg, (3) sudden systolic blood pressure decrease >15 mmHg, or (4) sudden heart rate decrease >15 bpm while on the 70° tilt bed for 15 min. A recent report stated that 65% of astronauts satisfied these criteria. Previously, this cardiovascular deconditioning was considered to be solely due to circulatory fluid loss, but other causes have also been explored.

In addition to the decrease in circulatory blood volume, other causes, i.e., altered arterial baroreflex gain, altered leg venous volume, easy fluid pooling in the space of atrophied skeletal muscles, attenuated muscle pump effects due to skeletal muscle atrophy, hypersensitivity of β-adrenergic receptors, and altered influence of vestibular (especially otolith) input, have been considered. Moreover, increased venous permeability of lower leg vessels and attenuated cardiopulmonary volume receptor reflex after −6° head-down tilt for 14 days were observed in our bedrest experiment. These changes are not the only cause of cardiovascular deconditioning, and multiple factors act in concert.

3.9 Spaceflight-associated neuro-ocular syndrome (SANS)

Several physiological and pathological neuro-ocular findings in astronauts/cosmonauts during and after long-term spaceflight, including hyperoptic shifts up to +1.75 diopters, optic disc edema (swelling), globe (eyeball) flattening, choroidal folds, and "cotton wool" spots in the fundus oculi, have been reported [29]. These findings have been documented as spaceflight-associated neuro-ocular syndrome. NASA has investigated the clinical, ultrasound, optical coherence tomography imaging, and fundus oculi findings of the above symptoms. In 2016, out of 47 or 64 astronauts examined, approximately 10 developed SANS (disc edema in 10/64, cotton wool spot in 7/64, choroidal folds in 11/47, globe flattening in 12/47, and refractive error in 9/47). It is unlikely that the duration of spaceflight is unrelated [30].

The exact cause of SANS has not been clarified, but its development is likely related to the increase in intracranial pressure due to the cephalad fluid shift. The increase in intracranial pressure is not necessarily due to microgravity exposure, but some percentage of astronauts had intracranial pressure change and developed SANS [29, 30].

Several countermeasures, e.g., lower body negative pressure, thigh cuffs, an impedance threshold device (ITD), vitamin B group administration, and artificial gravity, have been considered and are under trial. NASA and collaborating researchers continue to investigate SANS in preparation for future manned missions to space, including continued trips to the ISS, deep space gateway missions, a return to the Moon or Moon base, or a Martian expedition.

3.10 Brain structural plasticity during spaceflight

In 2016, structural changes in the brain during spaceflight were reported. Koppelmans et al. [31] evaluated retrospective longitudinal T2-weighted MRI scans and balance data from 27 astronauts (13, ~2-week Shuttle crew members, and 14, ~6-month ISS crew members) to assess spaceflight effects on brain structure. They observed extensive volumetric gray matter decreases, including large areas covering the temporal and frontal poles and around the orbits, and the effects were larger in ISS members than in Shuttle crew members. There were also bilateral focal gray matter increases within the medial primary somatosensory and motor cortex.

In 2017, a review on these MRI changes associated with spaceflight (actual or simulated) was reported. Van Ombergen et al. [32] discussed neuroplastic changes in the central nervous system and concluded that the cerebellum, cortical motor areas, and vestibular-related pathways are highly involved, demonstrating that these brain regions are indeed affected by actual and simulated spaceflight. Structural studies are now in progress, and functional relationships are under investigation. Long-term studies will be necessary to clarify the mechanism.

3.11 Effects of artificial gravity

We tested an intermittent short-arm centrifuge of 1.4 G with 60-W ergometric exercise with a step-up increase of 0.2 G and 15 W, respectively, for 30 min every day for 21 days during −6° head-down bedrest [33]. The circulatory blood volume was reduced by 20% in the control subjects, but no reduction was observed in the countermeasure subjects. Cardiac output and stroke volume were not changed in the countermeasure subjects, but they decreased in the control subjects. The baseline level of muscle sympathetic nerve activity (MSNA) was not changed in the countermeasure subjects, but it increased in the control subjects.

Therefore, everyday ergometric exercise under artificial gravity maintains the preflight cardiovascular state without adapting to microgravity.

4. Musculoskeletal system

4.1 Mechanism of muscle loss under microgravity

The first muscular measurements were performed in Skylab and Space Shuttle missions by the United States and in Salyut and Mir by the Soviet Union [34–37]. The most prominent muscle loss was observed in the calf muscle (the soleus and gastrocnemius) after a few weeks in space. The muscle loss exhibited interindividual variation, but the maximum loss reached as high as 10%. This volume loss in the lower extremities accounts for most of the muscle atrophy and the blood and interstitial fluid shift. Although fluid shift away from the legs influences the size of these muscles, this phenomenon alone cannot explain the changes in leg volume on MRI. Muscle atrophy appears rapidly, usually between 8 and 11 days of flight,

but can appear as early as the fifth day, as observed in one astronaut. Moreover, the effects of microgravity differ among muscles, with volume decreasing by 3.9% in the calf (the soleus and gastrocnemius) and 6% in the quadriceps femoris.

In addition to the morphological changes, functional alterations are associated with structural variations, and the muscular force is known to be reduced after spaceflight. In the Skylab 3 mission, it decreased by 20% after 53 days of microgravity exposure. Muscular electrical activity measured by electromyogram (EMG) had a lower EMG amplitude in addition to easy fatigability with a lower resistance.

The main cause of muscular loss is the disappearance of mechanical constraints and the subsequent decrease in muscular activity. The reduced muscular activity under microgravity is also associated with hypokinesia due to limited movement inside the spacecraft, which also can be observed in bedrest studies and animal experiments using tail suspension. The structural changes in skeletal muscle are also observed by microscopic examination under microgravity, which revealed that the proportion of type I red fibers decreased and they were replaced by type II white fibers. In 1995, pre- and postflight human muscle biopsies were performed on three and five astronauts during 5- and 11-day missions, respectively. In this study, the muscle fiber diameter decreased by 15 to 30%, and the number of capillaries around the muscle fibers decreased. The proportion of type I fibers changed from 43 (preflight) to 37% (postflight) after 5-day missions, and that of type II fibers changed from 57 to 67%. After 11 days of spaceflight, the proportion of type I fibers decreased from 45 to 39%, and that of type II fibers decreased from 55 to 61%, consistent with the animal experiments that demonstrated that gravity can influence genes regulating the protein synthesis of muscle protein degradation enzymes.

These changes were confirmed to be due to both an increase in protein breakdown and decrease in synthesis. Human biochemical examination also revealed a higher level of muscle protein degradation, increased level of urinary amino acids, and higher level of creatinine. The diet of astronauts is protein-rich, but the degradation process is such that nitrogen losses overcome the gains and the nitrogen balance becomes negative.

Recent studies have suggested the mechanism of disuse atrophy of the skeletal muscle, especially oxidative stress, to be an important regulator of pathways leading to muscle atrophy during periods of disuse. Redox disturbances, such as those in skeletal muscle myotubes, increase the expression of key components of the proteasome proteolytic system, which is a prominent factor in protein degradation in disused muscles.

Another hypothesized mechanism is the degradation of muscle proteins resulting from their ubiquitination. These molecular mechanisms underlie protein degradation during disuse.

4.2 Countermeasures for muscle loss

In order to prevent muscle loss, several countermeasures were available during microgravity exposure in Shuttle missions and continue to be available on the ISS. These include aerobic exercise, stretching, strength training, and electrical stimulation. Artificial gravity with exercise has been proposed as a potential measure for muscle loss because ground-based studies confirmed it to be effective, but a short-arm centrifuge has not been mounted. In addition to physical stimulation, medications, such as antioxidants, growth hormone, growth factors, ubiquitin, clenbuterol, anabolic steroids, and amino acids, are candidates against muscle loss.

Aerobic exercise is the most effective countermeasure for maintaining the fast twitch red fibers. On the ISS, the combination of an ergometer, treadmill, and the Advanced Resistive Exercise Device (ARED) is ideal to maintain muscle power and

the morphology of antigravity muscles (empowerment of both fast and slow twitch fiber muscles).

Stretching can minimize atrophy by maintaining the muscle as much as possible in the stretched condition. In the Russian space program, the penguin suit—a snug fitting, full-length, long sleeved jumpsuit made with elastic inserts at the collar, waist, wrists, and ankles and along the vertical sides of the suit—loads the body along the long axis with an adjustable force of 15–40 kg, while the other elastic elements make it possible to adjust the position of the limbs. The angle of the major joints, such as the knee and ankle, can be set, allowing the foot to be dorsiflexed, which will stretch the soleus. The effects of stretching are not well understood, but it is a valid countermeasure considering human physiology.

Strength training by resistance exercise is also employed as a countermeasure for muscle loss. Weight training studies recommended a good mix of exercise types to be 15% (eccentric), 10% (isometric), and 75% (concentric weight-bearing). The most recommended exercise for resistance training is squatting.

Electric stimulation is also expected to increase protein synthesis and prevent the decrease in oxidative enzymes inducing disuse atrophy.

4.3 Artificial gravity with exercise

We previously confirmed the effectiveness of artificial gravity in bedrest studies [33]. Our results demonstrated that artificial gravity of 1.4 G with ergometric exercise maintains the muscle strength and cross-sectional area of the quadriceps femoris measured by MRI. Another study revealed that artificial gravity with squatting exercise also maintains the function and morphology of the soleus and gastrocnemius [38].

5. Bone metabolism system

The main problem of the skeletal system is bone calcium (Ca^{2+}) loss during microgravity. The bone becomes fragile during microgravity exposure, which can harm an astronaut or cosmonaut even after returning to Earth. Moreover, the risk of renal stones is high during long-term missions due to hypercalcemia [39].

Ca^{2+} plays an essential role in bone structure, contraction of skeletal and cardiac muscles, neural transmission, blood coagulation, cell permeability, and hormonal signaling. The serum Ca^{2+} level is well maintained at 8.4–10.2 mg/dL. Ca^{2+} is absorbed from the small intestine (300 mg/day), into the blood, deposited in the bone (500 mg/day), and excreted from the kidneys (150 mg/day) or into feces.

In this section, the influences of gravity on bone structure and of hormones on bone formation and absorption are described.

5.1 Bone development and restructuring

Gravity influences the long bones of the lower extremities, e.g., the femur, tibia, calcaneus, and vertebrae, which support the body in the upright position. Bone tissue contains osteocytes, which develop from osteoblasts, and are changed into osteoclasts by the action of RANKL (also called osteoclast differentiation factor, short for receptor activation of NF-kappa B ligand).

Osteoblasts and osteoclasts are functionally closely related, as is the balance between bone formation and bone resorption. Thus, insufficient bone formation compared with bone resorption observed in spaceflight reduces the bone mass and bone strength, leading to fractures.

Two hormones, calcitonin and parathormone (PTH), and vitamin D play an essential role in Ca^{2+} metabolism. Calcitonin is secreted from C cells of the thyroid gland. The secretion of calcitonin is promoted by an increase in the blood calcium concentration and is suppressed by the decrease in the serum calcium level. Calcitonin acts on the calcitonin receptor in osteoclasts to suppress the release of Ca^{2+} from the bone and promotes the deposition of calcium and phosphate on the bone. Calcitonin also promotes the excretion of calcium and phosphate into the urine. As a result, the serum level of Ca^{2+} decreases.

PTH is secreted from the parathyroid gland, increasing the release of Ca^{2+} from bone. PTH binds to osteoblasts to increase the expression of RANKL and inhibits their secretion of osteoprotegerin (OPG), which competitively binds to RANKL, preventing RANKL from interacting with RANK (receptor for RANKL). The binding of RANKL stimulates osteoclast precursors to fuse, forming new osteoclasts. As a result, PTH increases bone resorption, thereby increasing the serum Ca^{2+} level.

Vitamin D (25-hydroxycholecalciferol) acts on the parathyroid gland and suppresses the synthesis and secretion of PTH. The intestinal tract promotes the absorption of calcium and phosphorus. Vitamin D is essential for bone formation, but its direct action on bone formation remains unclear. It is also necessary for the formation of osteoclasts, affects the bone action of PTH, and promotes bone resorption itself at a high concentration. In the kidney, vitamin D increases Ca^{2+} reabsorption in the distal tubule and promotes the Ca^{2+} reabsorption action of PTH.

5.2 Effects of microgravity on bone metabolism

The main factor of bone metabolism is mechanical impact. Gravity creates weight and is responsible for the pressure exerted on a large part of the skeleton, resulting in a mechanical impact on bones. These gravitational impacts provide mechanical constraints on the femurs, tibias, calcaneus, and vertebrae. Thus, decalcification and bone loss are observed as the result of bone resorption, and the disappearance of gravity from the body axis components induces bone loss and resultant osteoporosis [2, 40].

5.3 Effects of spaceflight on bone metabolism

In microgravity conditions, decalcification was observed in 12 of the astronauts on the Gemini and Apollo 7 and 8 flights in 1969 [8]. Based on bone density measured using X-rays, the bone Ca^{2+} loss was 3.2%.

The bone mineral density was measured before and after the Mir program. The changes were as follows: +0.6% in the skull, +0.1% in the arm, −1.07% in the spine, −1.35% in the pelvis, −1.16% in the femoral neck, −1.58% in the trochanter major, −1.25% in the tibia, and −1.50% in the calcaneus per month, with comparable results from the ISS [41]. The most affected bones during spaceflight are weight-bearing bones, e.g., the pelvis (os coxae), the trochanter major of the femur, the femoral neck, the tibia, and the calcaneus.

Mir studies using dual photonic densitometry demonstrated a mean decrease in bone mineral density of approximately 0.3%/month in the cortical bone and up to 0.9%/month in cancellous tibial bone. Decalcification only occurs in the weight-bearing bones, and demineralization is correlated with mission duration. During spaceflight, hypercalcemia and hyperphosphatemia develop due to demineralization, and Ca^{2+} excretion into the urine increases before stabilization at around the 30th day of flight [42].

The National Aeronautics and Space Administration (NASA) of the United States documented a bone mineral loss rate/month of 1–2% during spaceflight

(https://science.nasa.gov/science-news/science-at-nasa/2001/ast01oct_1), and bone density loss of 5–6% was reported in Apollo 15 crewmembers [43]. Bone loss is influenced by the spaceflight duration.

Tail suspension studies on rats demonstrated that simulated microgravity reduces bone formation, alters the Ca^{2+} balance, and inhibits the proliferation and differentiation of osteoprogenitor cells [44]. Osteocytes can also be affected by unloading stimulus in a bioreactor, with high expression of inhibitors of bone formation (sclerotin) and stimulators of bone resorption (RANKL) [45].

Osteoporosis can be irreversible. After returning to Earth without appropriate rehabilitation, the bone may be unable to return to normal activity under loads and may be weaker, easily inducing fractures. Reloading after a period of a week can ameliorate bone weakness, but even 2 weeks of bone restoration was not satisfactory. The length of spaceflight influences the bone density loss; however, another report stated that the bone loss at 1 month continued to increase after 6 months, suggesting that the length of spaceflight does not determine the bone density loss [42].

5.4 Effects of mechanical impact on hormonal influence

Wolff's law characterizes how the bone adapts to functionally withstand its mechanical environment [46]; however, several studies found that mechanical loading per se is not the direct stimulus for bone remodeling [47–51].

Then what are the effects of mechanical impact on calcitonin? Calcitonin receptors were not observed on osteoblasts [52] but were present on osteoclasts [53] and osteocytes [54]. Calcitonin was reported to inhibit apoptosis of osteoblasts and osteocytes, demonstrating a potential indirect influence on bone formation. These data confirmed that mechanical impact is not directly related to bone formation.

One notable property of PTH is that although chronic increases in PTH levels increase bone resorption, intermittent stimulation accelerates bone formation. PTH stimulates osteoclast formation by binding to PTH receptor 1 on stromal/osteoblastic cells and thereby increases the production of receptor activator of RANKL and macrophage colony-stimulating factor (M-CSF) and suppresses the RANKL decoy receptor osteoprotegerin. Moreover, PTH controls the production of osteoblasts through actions on osteocytes through Wnt signaling in osteoblastogenesis [55].

The action of osteocytes, which can directly sense a mechanical unloading stimulus, increased the expression of both inhibitors of bone formation (SOST/sclerotin) and stimulators of bone resorption (RANKL) through Wnt signaling [45]. These results support the hypothesis that intermittent mechanical impacts induce osteocyte action, which inhibits bone formation and stimulates bone resorption, and that an intermittent increase in PTH controls the production of osteoblasts.

5.5 Sympathetic alteration of bone metabolism during spaceflight

It has been reported that sympathetic neural traffic to bone inhibits the function of osteoblast and increases that of osteoclast, thus facilitating bone loss. Possible roles of the sympathetic nervous system in the mechanisms of bone loss in humans exposed to long-term spaceflight will be discussed.

Prolonged exposure to microgravity in space for 14 days increased sympathetic neural traffic in humans based on results from the Neurolab mission [14, 56–58], with comparable increases in noradrenaline spillover and clearance in space [14]. Concordant results were obtained during simulated microgravity, including dry immersion [28] or head-down bedrest [17]. In general, elderly people have a low bone density and high sympathetic neural traffic to muscles [59]. Our preliminary

study demonstrated that changes in sympathetic neural traffic to muscles after long-term bedrest of 20 days were significantly correlated with changes in the urinary secretion level of deoxypyridinoline [25, 60], which is used as a specific marker of bone resorption [61]. Based on these findings, exposure to prolonged microgravity may increase sympathetic neural traffic to the bone, which increases the noradrenaline level, thereby inhibiting osteogenesis and facilitating osteolysis through β-receptors to induce bone mineral loss.

5.6 Countermeasures for space-related osteoporosis

1. Physical factors

Exercise during weightlessness has been incorporated into the present countermeasure programs; however, exercise alone cannot prevent bone loss. The current exercise program for the ISS is a combination of aerobic and resistive exercise for 2.5 hours, 6 days/week. Data from spaceflight revealed that bone loss occurs mainly in the femur, tibia, calcaneus, and vertebrae. Therefore, exercise should be concentrated on these bones, and impact loading should be primarily provided rather than static loading [62].

2. Pharmacological factors

As bone mass is sufficient at the onset of the spaceflight, the optimal strategy for the pharmacotherapy against bone loss is the prevention of bone loss, not the acceleration of bone formation, when loading is removed during spaceflight. Several drugs have been proposed to prevent bone loss under microgravity.

3. Bisphosphonates

Bisphosphonates have two phosphonate (PO_3) groups and are similar in structure to pyrophosphate. They bind to hydroxyapatite in bone matrix and prevent bone loss by inhibiting osteoclastic bone resorption. Bisphosphonates have been demonstrated to be effective in preventing bone loss during bedrest studies [63–66]. Among several types of bisphosphonates, pamidronate has been confirmed to suppress bone mineral loss and to prevent the formation of renal stones during bedrest studies [67].

In 2010, LeBlanc and Matsumoto [68] proposed an experiment for the effectiveness of bisphosphonate as a countermeasure to spaceflight-induced bone loss. The astronauts chose either oral administration of alendronate at 70 mg once per week or intravenous administration of zoledronate at 4 mg before the flight, and their bone densities were examined by DXA, QCT, and pQCT, and bone metabolism markers, including bone formation and resorption markers, and renal stone formation were assessed. One of the co-investigators (Ohshima) reported successful suppression of spaceflight-induced bone loss and renal stone formation (Ohshima, personal communication).

The disadvantages of bisphosphonates are local irritation of the upper gastrointestinal (GI) tract and poor absorption from the GI tract. Therefore, the oral administration of bisphosphonates requires drinking 200 mL of water while remaining in an upright posture for at least 30 min until after their first meal of the day to facilitate delivery to the stomach. This poses a problem as there is no upright posture in space due to microgravity. Another potential problem is osteonecrosis of the maxilla and the mandible, although the incidence is low [69]. These osteonecrotic or osteolytic phenomena always accompany physiological stress (mastication), iatrogenic trauma (tooth extraction/denture injury), or tooth infection [70, 71].

Bisphosphonates are difficult to metabolize, and high concentrations of them are maintained in the bones for long periods. As bone formation is closely coupled with bone turnover, long-term use of the compound with resultant suppression of bone turnover can compromise healing of even physiological microinjuries within the bone. Osteonecrosis of the maxilla and mandible likely results from the inability of the hypodynamic and hypovascular bone to meet the increased demand for repair and remodeling because several alterations are associated with this necrosis.

4. Parathormone

Parathormone has anabolic effects on the bone and also functions in the kidney to stimulate the reabsorption of $Ca2^{+}$ and increase the synthesis of vitamin D. In this sense, parathormone may stimulate bone formation, increase vitamin D synthesis, and stimulate $Ca2^{+}$ reabsorption. As suppressing bone reabsorption is favorable for stimulating bone formation during spaceflight, the administration of parathormone is strategically unfavorable.

In conclusion, it is favorable to administer bisphosphonate orally under artificial gravity with exercise in order to prevent osteoporosis in space. Monitoring the blood and urine samples on the ISS or spacecraft by a simple method is necessary to assess the effectiveness of the countermeasure.

6. Immunology and hematology

6.1 Space anemia

The circulatory blood volume is 5 L on average and contains plasma and cellular components, including erythrocytes, leukocytes (neutrophils, eosinophils, basophils, and lymphocytes), and platelets [72–74]. Among them, reduction in cellular components, especially erythrocytes (RBC), is associated with anemia, whereas the function of leukocytes is related to immunological response.

In the early stages of space development, cases of "space anemia" (hematocrit reduction) were reported on Gemini, Apollo, Skylab, and Shuttle missions and in the cosmonauts in the Salyut and Mir missions. However, in spaceflight, microgravity causes cephalad fluid shift, meaning this "space anemia" was actually a misinterpretation of symptoms. The true effects of microgravity can be measured through the total RBC count calculated from the hematocrit and plasma volume measurements. In this way, "space anemia" corresponded to a reduction in the total RBC count.

After 10 days aboard Spacelab-1, the total RBC count was reduced by 9% and by 15% after several weeks. After returning to Earth, the total RBC count did not recover even after 6 weeks and, in the case of Skylab astronauts, had not recovered after more than 3 months.

This suggested that microgravity is responsible for "space anemia," and many investigations were carried out to reveal whether anemia is the result of an increase in RBC destruction or a decrease in their production. Using labeled RBC by the uptake of ^{14}C glycine, RBC destruction was found to be three times greater in rats having flown aboard Cosmos-782 than in the control rats. On the other hand, reduced RBC production is unlikely because the number of stem cells measured by the number of cellular colonies that developed in vitro from samples of bone marrow taken from rats that flew aboard the Soviet Biosatellite—2044 for 14 days—was unchanged.

Human studies carried out by [75] on Shuttle missions for 9 to 14 days demonstrated that space anemia is due mainly to a lower production of RBC, causing

increased plasma volume, reduced hemoglobin concentration, and increased serum erythropoietin. This reflects a decrease in the RBC life span and slower production.

Rizzo et al. [76] analyzed the cause of the shortened RBC life span and reported altered cell membrane composition and an increase in lipid peroxidation products. They suggested that antioxidant defense systems in the erythrocytes were induced, with a significant increase in glutathione content.

The mechanism underlying anemia was also confirmed by measuring the erythropoietin (EPO) level [77]. Radioimmunoassay revealed that the EPO level decreases after 24 hours of flight and is reduced by 30–40% on the third day compared with preflight levels. This low secretion of EPO will inhibit RBC maturation and cause hemolysis due to suppressed erythropoiesis.

Other changes in leukocytes (WBC) are in their polymorphonuclear characteristics. The composition of WBC is changed such that there is a slight increase in neutrophils and decrease in eosinophils. The percentage of lymphocytes, especially T cells, decreases, whereas that of monocytes slightly increases. These changes quickly disappear upon returning to 1 G on Earth.

6.2 Immunological changes during weightlessness

Recent studies confirmed dysregulation of the immunological response in humans and the reactivation of latent herpes virus, which persisted for the duration of a 6-month orbital spaceflight [78]. Blood samples from ISS crew members demonstrated that long exposure to microgravity reduced their T lymphocyte counts, suggesting the attenuation of cytotoxic function and viral reactivation in the space environment.

As the immune system is highly sensitive to different types of stressors, including psychological, physical, and local environmental stressors (e.g., oxidative and radiation exposure), exposure to the space environment suppresses T helper cells, which leads to susceptibility to viruses.

7. Artificial gravity as a total countermeasure for spaceflight deconditioning

For human space voyages lasting several years, such as those envisioned for the exploration of Mars, astronauts will be at risk of catastrophic consequences should any of the systems that provide air, water, food, or thermal protection fail. Beyond that, astronauts will face serious health and/or safety risks resulting from marked physiological deconditioning associated with prolonged weightlessness [1, 79]. The principal physiological deconditioning risks are related to physical and functional deterioration, and the loss of regulation of the several systems, including blood circulation, decreased aerobic capacity, musculoskeletal systems, and altered sensorimotor system performance. These physiological effects of weightlessness are generally adaptive to spaceflight and present a hazard only following G transitions upon returning to Earth or landing on another planet [80]. Among them, bone mineral metabolism will be greatly affected during prolonged spaceflight.

7.1 Why artificial gravity

Space biomedical researchers have been working for many years to develop "countermeasures" to reduce or eliminate the deconditioning associated with prolonged weightlessness. Intensive and sustained aerobic exercise on a treadmill, bicycle, or rowing machine coupled with intensive resistive exercise has been

used on US and Russian spacecraft to minimize these problems. The procedures were uncomfortable and excessively time-consuming for many astronauts, and their effectiveness for maintaining bone, muscle, and aerobic fitness has not been demonstrated due in part to the low reliability of the devices flown to date. Furthermore, they have had inconsistent effects on postflight orthostatic hypotension or sensorimotor adaptive changes. With the exception of fluid loading before reentry, other countermeasures (e.g., diet, lower body negative pressure, or wearing a "penguin suit" to force joint extension against a resistive force) either have been marginally effective or presented an inconvenience or hazard.

To succeed in the near-term goal of a human mission to Mars during the second quarter of this century, the human risks associated with prolonged weightlessness must be mitigated well beyond our current capabilities. Indeed, during nearly 45 years of human spaceflight experience, including numerous long-duration missions, no single countermeasure or combination of countermeasures that is completely effective has been developed. Current operational countermeasures have not been rigorously validated and have not fully protected any long-duration (>3 months) astronauts in low Earth orbit. Thus, it is unlikely that they will sufficiently protect astronauts journeying to Mars and back over a 3-year period.

Although improvements in exercise protocols, changes in diet, or pharmaceutical treatments of individual systems may be of value, they are unlikely to eliminate the full range of physiological deconditioning. Therefore, a complete research and development program aimed at substituting the missing gravitational cues, and loading in space is warranted.

The urgency of exploration-class countermeasures is compounded by the limited availability of flight resources for validating a large number of system-specific countermeasure approaches. Furthermore, recent evidence of the rapid degradation of pharmaceuticals flown aboard long-duration missions, putatively because of radiation effects, raises concerns regarding the viability of some promising countermeasure development results. Although the rotation of a Mars-bound spacecraft will not be a panacea for all human risks of spaceflight (artificial gravity cannot solve the problems associated with radiation exposure, isolation, confinement, and environmental homeostasis), artificial gravity does offer significant promise as an effective, efficient, multi-system countermeasure against the physiological deconditioning associated with prolonged weightlessness. Virtually all of the identified risks associated with cardiovascular deconditioning, myatrophy, bone loss, neurovestibular disturbances, space anemia, immune compromise, and neurovegetative state may be alleviated by sufficient artificial gravity.

7.2 Why artificial gravity with exercise

Although a short-radius centrifuge has been proposed several times, loading with artificial gravity has not been demonstrated to be effective at preventing spaceflight deconditioning on its own. Making a human-powered short-arm centrifuge is an effective method to create exercise loads for astronauts. Considering the size of the ISS, it is appropriate to employ a short-radius centrifuge rather than a large-radius human centrifuge; however, it may be beneficial to rotate the spacecraft itself to provide the artificial gravity for long-duration spaceflight such as Mars expeditions.

In 1999, Iwase proposed the creation of artificial gravity by ergometric exercise, and it was installed at Nagoya University [33]. Several studies were performed using this short-radius centrifuge with an ergometer. In 2002, a bedrest study was carried out to evaluate the effectiveness of artificial gravity with ergometric exercise. In 2005, the facility was moved to Aichi Medical University, and bedrest studies

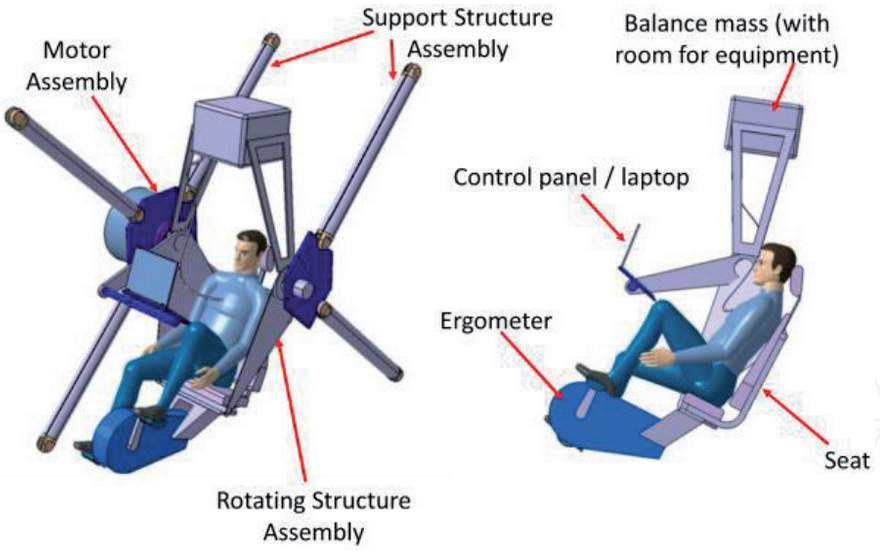

Figure 1.
Structure of the device of artificial gravity with exercise for AGREE project 2012.

were performed to finalize the protocol. This daily AG-EX step-up protocol was confirmed to be effective at preventing cardiovascular, musculoskeletal, and bone metabolism deconditioning in 2006, whereas the alternate-day (every-other-day) protocol (loading the AG-EX every other day) did not improve the spaceflight deconditioning associated with the microgravity exposure analogue of −6° head-down bedrest.

The authors applied for the installation of a short-radius centrifuge facility on the ISS and proposed it as a method to prevent spaceflight deconditioning, including bone loss. This project, Artificial Gravity with Ergometric Exercise (AGREE project), was promising to prevent space deconditioning during spaceflight, but it was canceled halfway through (**Figure 1**).

8. Conclusion and summary

Several deconditioning states in the neurovestibular, cardiovascular, ocular, musculoskeletal, bone metabolic, hematological and immunological, and central nervous systems have been documented, and efforts to ameliorate the symptoms have been made. In the near future, space medicine will play an increasingly important role in missions to the Moon and Martian expeditions as well as in future deep space exploration.

Historically, space medicine examined early adaptation to microgravity and early readaptation to the terrestrial 1 G state. However, the philosophy of the authors is to avoid adaptation to microgravity using artificial gravity. Under this scenario, short exposure to microgravity is permitted, but longer adaptation will be unnecessary. With this philosophy, the authors believe that the humans will achieve safe and comfortable spaceflight without deconditioning.

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