Human Research Program
Human Health Countermeasures Element

Evidence Report

Artificial Gravity

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I. ARTIFICIAL GRAVITY FOR PROTECTION OF HUMAN HEALTH DURING LONG-DURATION SPACEFLIGHT

The most serious risks of long-duration flight involve radiation, behavioral stresses, and physiological deconditioning. Artificial gravity (AG), by substituting for the missing gravitational cues and loading in space, has the potential to mitigate the last of these risks by preventing the adaptive responses from occurring. The rotation of a Mars-bound spacecraft or an embarked human centrifuge offers 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 bone loss, muscle weakening, cardiovascular deconditioning, and sensorimotor disturbances might be alleviated by the appropriate application of AG. However, experience with AG in space has been limited and a human-rated centrifuge is currently not available on board the ISS. A complete R&D program aimed at determining the requirements for gravity level, gravity gradient, rotation rate, frequency, and duration of AG exposure is warranted before making a decision for implementing AG in a human spacecraft.

II. EXECUTIVE SUMMARY

The urgency for exploration-class countermeasures is compounded by the limited availability of flight resources for performing the validation of a large number of system-specific countermeasure approaches. The notion of creating a substitute for gravity was introduced early in the conception of human space travel. The surest AG solution is clearly one that produces a gravitational environment close to that on Earth. Although AG could theoretically be created by several methods (e.g. using constant linear acceleration or magnetic field) centrifugation is the most realistic technique regarding vehicle design/engineering costs.

From a physiological countermeasure perspective, a good solution might be to provide AG continuously throughout the mission by spinning the crew compartment at a g-level sufficient to replicate terrestrial stresses on the bone, muscle, cardiovascular, and sensory-motor systems; a rotation rate low enough to have minimal impacts on sensorimotor coordination and human factors; and a radius sufficient to minimize the g-gradient effects on cardiovascular loading. An alternative approach would be to provide AG intermittently by spinning crewmembers periodically aboard a centrifuge within the habitable environment. This solution would likely provide periodic AG at a g-level substantially higher than terrestrial stresses on the bone, muscle, cardiovascular, and sensory-motor systems; a rotation rate high enough to affect sensorimotor coordination and human factors; and a radius short enough to create substantial g-gradients. While not expected to be as efficient a solution from a physiological standpoint, intermittent AG may prove effective, and the engineering costs and design risks might be lower.

The benefits of continuous or intermittent AG on physiological responses are not well known to date. Further physiological research is needed to determine the effects of gravity level, rotation rate, gravity gradient, as well as frequency and duration of exposure. Note that the physiological responses to continuous Mars g exposure are also unknown. If it turns out that substantial physiological deconditioning occurs at Mars gravity, then AG may also be required to protect crews during long stays on the surface of Mars.

III. INTRODUCTION

Much of this information is drawn from the author's contributions to previous reviews on this topic, including Clément & Pavy-Le Traon (2004), Paloski *et al.* (2006), Clément & Bukley (2007), Young *et al*. (2009), and Paloski & Charles (2014).

A. Why Artificial Gravity?

Human space exploration has been limited thus far to *low Earth orbit* (LEO) and to short visits to the Moon. These missions typically lasted only a few days to a few weeks, with the exception of extended stays on Mir and the *International Space Station* (ISS). For these short-duration missions, the adverse effects of weightlessness on the human body are minimal. However, once we begin extended exploration of the Moon, asteroids, Mars, and beyond, mission durations will increase significantly, thus exposing the crews to the long-term detrimental effects of weightlessness. The consequences of long-term weightlessness include undesirable physiological adaptations that impede the ability of astronauts to function efficiently upon the return to an environment with gravity.

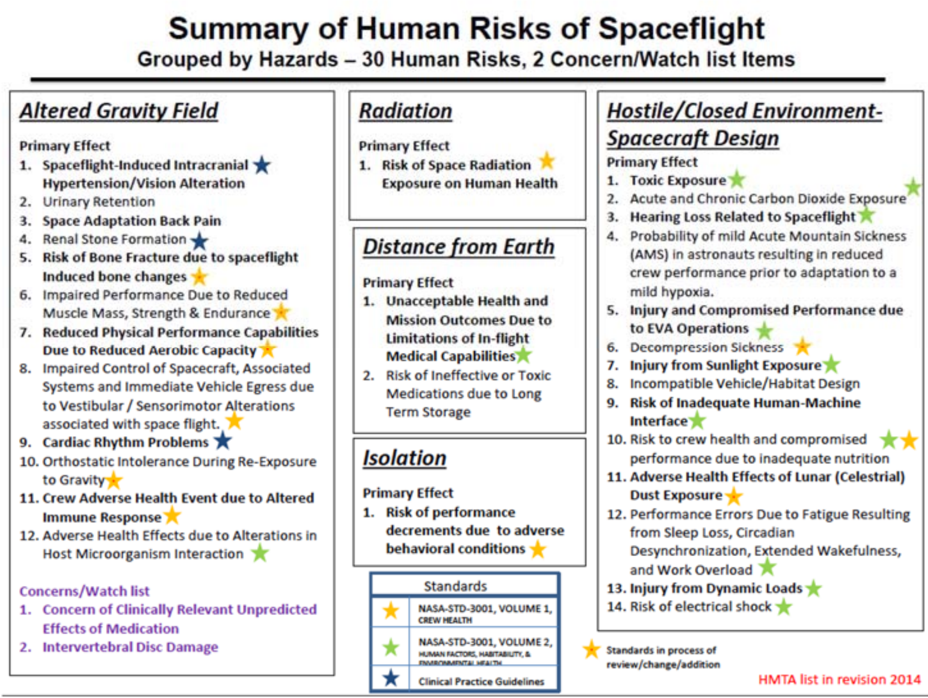

Figure 1. List of human risks that NASA is concerned about during spaceflight, and which the Human Research Program is aiming at mitigating (Paloski 2014a).

The physiological systems affected by each of the spaceflight environmental hazards are summarized in Figure 1. Altered gravity field is associated with 12 identified human risks and 2 concerns that affect most of the physiological systems such as the cardiovascular system (orthostatic intolerance), muscle atrophy, sensorimotor performance, bone demineralization, immune deficiencies, back pains, renal stone formation, etc. Lately, special focus of concern is changes in vision acuity in astronauts onboard the ISS, which is hypothesized to be caused by weightlessness-induced fluid shifts to the upper body leading to intracranial hypertension (Mader *et al.* 2011). Even though vision disturbances have been reported earlier on the shorter Shuttle flights, it is of more concern now, because if the hypothesis is confirmed, it could be an impediment for future long duration deep space missions. Thus an effective countermeasure against intracranial hypertension will be required, and since it concerns re-establishing gravity induced hydrostatic gradients, AG might be the most efficient.

Countermeasures that reduce the effects of weightlessness have been developed and some are commonly employed. For example, resistive and aerobic exercise, extra dietary calcium, and other pharmaceuticals are used to mitigate physiological adaptations to weightlessness due to changes in weight-bearing structures and tissues, redistribution of body fluids, and functioning of the gravity receptors. Those countermeasures currently used focus, however, on certain organ systems and symptoms and most require specially adapted therapeutic equipment. These techniques are time consuming and demand a high degree of individual discipline. In addition, they provide moderately successful physiological protection for six-month missions in LEO.

Because the primary factor affecting physiological deconditioning during spaceflight is the loss of gravitational loading and stimulation, there is little doubt that the most effective physiological countermeasure would be to bring gravity along. *Artificial gravity* (AG) could replace terrestrial gravity with inertial forces generated by centrifugation or sustained linear acceleration. Today's approach to countering the deleterious effects of microgravity is piece-meal, whereas AG provides an integrated countermeasure affecting multiple physiological systems.

Artificial gravity is an old concept, having gotten its start in the late in the 19th century when Konstantin Tsiolkovsky realized that the human body might not respond well to the free fall of orbital spaceflight. To solve this problem, he proposed that space stations be rotated to create centripetal accelerations that might provide inertial loading similar to terrestrial gravitational loading. Einstein later showed in his equivalence principle that acceleration is indeed indistinguishable from gravity. Subsequently, other individuals of note, including scientists like Werner von Braun as well as artists like Arthur C. Clarke and Stanley Kubrick, devised elaborate solutions for spinning vehicles to provide AG that would offset the untoward physiological consequences of spaceflight.

By 1959, concerns about the then-unknown human responses to spaceflight drove NASA to consider the necessity of incorporating AG in its earliest human space vehicles. Of course, owing in part to the relatively short durations of the planned missions, AG was not used in the early NASA programs. We learned from these early missions that humans could tolerate short periods of weightlessness, but a fear remained that longer exposures would lead to more profound effects, and that eventually an exposure threshold would be reached, beyond which crew health, safety, and performance might be compromised to the point of placing individual crewmembers or entire missions at unacceptable risk. Therefore, throughout the 1960s, NASA sponsored many forums to debate the need for AG on longer duration human spaceflight missions.

During the 1970s, we learned from the Skylab program that humans could tolerate many weeks of weightlessness exposure without reaching any untoward thresholds. During the last two decades, we have learned from the Mir and ISS programs that system-specific countermeasures (e.g., resistive and aerobic exercise emerged) can provide moderately successful physiological protection for six-month missions in LEO. But recently, NASA's thoughts have turned to more distant destinations, focusing first on long-duration stays on the Moon and moving on to 1000-

day missions to Mars or other locations well beyond LEO. These goals have led to a renewed interest in AG, stimulating workshops to develop international consensus on the open AG research issues and engineering studies of feasible designs for spinning transit vehicles.

In fact, many Mars mission designs in the early days of the space program recommended such an approach. Most concepts called for the use of spinning transit vehicles, which were not implemented in part due to technical issues associated with system complexity and cost in mass and energy. There remain many unknowns as to how humans can adapt to a rotating environment and then re-adapt to a non-rotating environment (e.g., when they arrive on Mars). Recent studies suggest that humans can adapt to high rates of rotation at short radius. Therefore, an alternative to rotating the entire habitat is to provide a short-radius centrifuge within the habitat and deliver therapeutic doses of AG. This would result in an overall simpler and more affordable design.

Exercise is currently the dominating countermeasure during spaceflight to maintain primarily muscle and cardiovascular fitness and bone strength. However, it probably also benefits other physiological systems such as the sensorimotor system. It has been shown that introduction of systematic resistive exercise on ISS improves muscle and bone strength and that the success depends on the intensity and load applied. The most efficient exercise prescription, however, has yet to be finally determined. But the so-called Sprint protocol is currently being tested, where a combination of aerobic and resistive exercise prescription is tested using a cycle, treadmill, and the *Advanced Resistive Exercise Device* (ARED) that can apply up to 600-pound loads. Preliminary results show that using ARED improves muscle strength and bone mineral density and strength (Ploutz-Snyder 2014).

The current evidence for using exercise as a countermeasure during long duration space missions in low Earth orbit indicates that astronauts can probably travel to Mars and back with exercise alone as a countermeasure for maintaining muscle and bone strength, and muscle performance as well as aerobic capacity using a Sprint-like protocol. However, it is likely that adding AG will make the exercise programs more efficient and reduce in-flight resources including crew time. AG could prove to be a determining factor for mission success should the exercise equipment fail during the mission.

During long duration deep space missions, exercise prescriptions will be required, but the question is whether adding AG will make it possible to reduce in-flight mass, power and time and make exercise more efficient. As space agencies seek to undertake human missions to distant destinations, they must inevitably consider AG designs and the physiological and human factors research necessary to develop optimal AG prescriptions for their crews. This report provides the evidence of what is currently known on AG research.

B. Principle of Artificial Gravity?

1. Definitions

Artificial gravity (AG) is defined as the simulation of gravitational forces aboard a space vehicle that is in orbit (free fall) or in transit to another planet. An important point is AG is not gravity at all. Rather, it is an inertial force that is indistinguishable from the normal gravity experience on

Earth in terms of its action on any mass. In a rocket with a constant linear acceleration, an inertial force proportional to the mass that is being accelerated is experienced rather than a gravitational pull. Similarly, in a rotating device, a centrifugal force proportional to the mass that is being accelerated centripetally is experienced rather than a gravitational pull. Although the effect of AG on a human body differs from that of true gravity, the effects are equivalent for any given mass. Therefore, one can think of AG as the imposition of accelerations on a body to compensate for the forces that are absent in weightlessness during spaceflight.

Other mechanisms could, in theory, be used to develop AG. For example, one could install an ultra-high density core into a spacecraft so that it would generate its own gravitational field. One could also implement large magnets or spinning superconductors that would produce a powerful gravitational field. However, the constraints in terms of mass, power, and thermal requirements make these solutions difficult for implementation on a spacecraft.

Continuous AG could also result from a continuously thrusting rocket that would accelerate a spacecraft at a constant rate for the first half of the journey to Mars and then decelerate at that same constant rate for the second half of the journey. As an added bonus, such a constantly accelerating vehicle could provide relatively short flight times through the solar system. Theoretically, a propulsion system employing very high specific impulse fuel and the key characteristic of a high thrust-to-weight ratio could accelerate for long periods of time. However, this technology is not currently mature enough for interplanetary travel. Finally, astronauts routinely experience intermittent impulsive linear acceleration during orbital adjustments of orbital spacecraft when the thrusters are fired. However, the duration of this AG is only a few seconds and is too short to be considered as a potential countermeasure.

To date, the most acceptable solution for generating continuous AG is centripetal acceleration generated by circular motion, or centrifugation. Using centrifugation, AG could be generated: (a) by spinning a spacecraft about its axis; (b) by rotating two spacecraft connected by a tether about the system center of mass; or (c) by using a short-radius centrifuge on board a spacecraft.

2. Gravity Level

The minimum artificial gravity level, normally measured at the rim of a centrifuge, is the key parameter in the design of an artificial gravity system. The centripetal acceleration (expressed in m/s^2) is referred to as the ***gravity level***. The centripetal acceleration is a vector quantity, having a magnitude and associated direction that is derived using vector multiplication by taking the cross product of the tangential velocity and the angular velocity. Accordingly, if an astronaut is standing on the rotating floor of a spinning vehicle, or is lying on an internal short-radius centrifuge with her feet outward, the gravity level at her feet is: $AG = \omega_c^2\, r$, where ω_c is the rotation rate of the centrifuge (in rad/s) and r the distance from her feet to the axis of rotation (in m).

Investigations on AG on Earth are hampered by the presence of the steady gravitational pull. On Earth, gravity adds to the centripetal acceleration vectorially and produces a net specific *gravitoinertial acceleration* (GIA) that is tilted relative to the horizontal. In weightlessness, the gravity level is only due to the centripetal acceleration (Figure 2).

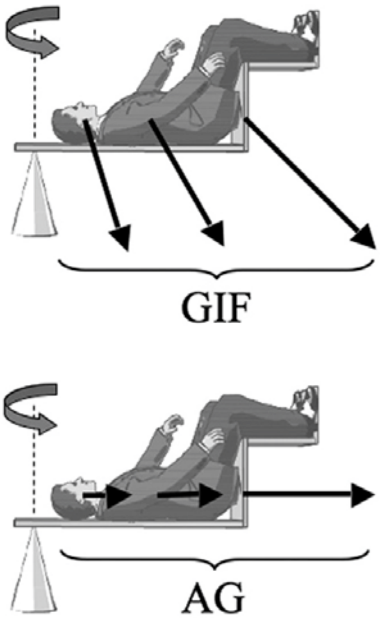

Figure 2. These drawings illustrate the difference between the physical effects of centrifugation on Earth (top) and in weightlessness (bottom). On Earth, the gravitoinertial force (GIF), i.e., the resultant of the gravitational force and the centrifugal force, is tilted relative to the plane of rotation, and its amplitude is always larger than 1 g. In weightlessness, the artificial gravity level (AG) is equivalent to the centrifugal force only and is aligned with the plane of rotation (Clément & Bukley 2007).

3. Gravity Gradient

Because the gravity level varies along the radius of the centrifuge, an astronaut lying in a centrifuge along a radius with her feet positioned at the rim will have her head closer to the axis or rotation than her feet. The head will have a smaller radius of rotation. Consequently, the gravity level at her head will have a lesser magnitude than the gravity level at her feet (see Figure 2, bottom). The variation in artificial gravity level as a function of distance from the center of rotation is referred to as the ***gravity gradient***.

For an astronaut of height *h*, lying in a centrifuge along a radius with her feet positioned at the rim and her head pointing towards the center of rotation *r*, her head has a radius of rotation equal to $r - h$. The ratio of head acceleration to foot acceleration can be simply expressed as $A_{head}/A_{foot} = \omega_c^2\, r\, (r - h) / \omega_c^2\, r = (r - h) / r$. By way of example, for an astronaut of height $h = 1.8$ m in a rotating environment with a radius of 100 m, this ratio is 98%, which corresponds to a gravity gradient of 2%. An individual would not likely perceive a difference of only 2%. However, for radii of rotation less than 10 m, the gravity gradient ranges from 20 to 100%, which may be perceived as a bent posture.

The gravity gradient also has an effect on the hydrostatic pressure along the longitudinal body axis. The hydrostatic pressure influences the circulation of blood to the head and from the lower extremities and therefore affects the functioning of the cardiovascular system. In a standing person on Earth there is a linear variation in the hydrostatic pressure, p (Pa) = $h\, d\, g$ where h is the height of fluid column (m) at which the pressure is measured, d is the density of liquid (kg/m^3) and g the gravitational acceleration (m/s^2). On a centrifuge, the hydrostatic pressure varies with the squares of the distances: $p = d / 2\, (h^2 - r^2)\, \omega_c^2\, r$, where h and r are the distances between the axis of rotation of the centrifuge and the feet and the head, respectively. This relationship shows large variations of hydrostatic pressure for a centrifuge radius of up to 12 m

(Stone *et al.* 1970). It is not known if these variations have any critical influences on the cardiovascular system. It is interesting to note, however, that at a given AG level, a shorter radius would have less influence in challenging the cardiovascular system that a larger radius.

4. Coriolis Force

Although subjects at rest in a rotating system feel only the sensation of weight, that is, the gravity level generated by the centrifugal force, when they move, other forces are felt. The effects of linear motions in a rotating environment must be distinguished from the effects of angular motions in a rotating environment. The former are due to Coriolis acceleration, the latter to cross-coupled angular accelerations. The Coriolis acceleration is a direct result of any linear movement within the rotating reference frame, i.e. when someone or something moves tangentially or radially in the rotating environment. The Coriolis acceleration (A_c) is equal to twice the cross product of the centrifuge angular velocity vector ω_c and the linear velocity vector v of the moving object, person, or body part: $A_c = -2\ (\omega_c \times v)$. The direction of the Coriolis acceleration is perpendicular to the plane formed by ω and v in a right-hand-rule sense in accordance with vector calculus. Of course, the resulting force is obtained by multiplying the mass m of the moving object or person by the acceleration, so the magnitude of the **Coriolis force** is: $F_c = -\ 2m\ (\omega_c \times v)$.

In non-vector terms, at a given angular velocity of the observer, the magnitude of the Coriolis force of the subject will be proportional to the linear velocity of the subject in the rotating frame as well as to the sine of the angle between the direction of movement of the subject and the axis of rotation. Relative to self-locomotion inside a rotating space station when an observer moves radially on the floor she will feel pushed to the side; when she moves tangentially she will feel heavier or lighter depending on the direction of motion. It is also important to note that the Coriolis force is independent of the radius of centrifugation. That is, its magnitude is the same at all distances from the center of rotation.

The Coriolis force acts in a direction that is perpendicular both to the direction of the velocity of the moving subject mass and to the axis of rotation. We can therefore make the following statements regarding the characteristics of the Coriolis force:

- When the velocity v (in the rotating system) is zero, the Coriolis force is zero.
- When v is parallel to the rotation axis, the Coriolis force is zero.
- When v is directed radially inward (outward) towards (away from) the axis of rotation, the resulting Coriolis force is aligned with (opposed to) the direction of rotation (parallel to the tangential velocity).
- If v is aligned with (opposed to) the direction the rotation (parallel to the tangential velocity), the Coriolis force acts radially outward from (toward) the axis of rotation.

5. Cross-Coupled Angular Acceleration

While a person is rotating, a head rotation in any other plane will produce an apparent rotation in a direction unrelated to what is actually happening. This causes a sensation of tumbling, rolling or yawing that is due to **cross-coupled angular acceleration**. The phenomenon results from simultaneous rotation about two perpendicular axes. The result is *angular* acceleration about a third, orthogonal axis equal to the product of the two original angular velocities. The general expression for the cross-coupled angular acceleration in a rotating environment is: CCAC = ω_c x ω_h where ω_c represents the angular velocity of the centrifuge, ω_h represents the angular velocity of the head. The absolute angular acceleration of the head is equal to the sum of the angular acceleration of the head relative to the centrifuge and the cross product of the centrifuge angular velocity with the relative head angular velocity (Elias *et al.* 2007).

Cross-coupled angular acceleration is a phenomenon distinct from the Coriolis phenomenon, which refers to expected *linear* acceleration associated with translation within a rotating environment. It is also unrelated to the change in position after stopping of rotation, where the directions of aftereffects remain invariant in head coordinates. The mechanism behind the cross-coupled angular acceleration effect is related to head motions that reorient the three semicircular canals of the vestibular system in the inner ear. For example, if the head is vertical, and rotation is about the vertical axis, the semicircular canal in the horizontal plane registers the rotating motion. If rotation continues, a steady state is reached where the *yaw* canal no longer senses the rotation. If now the head is tilted forward, for simplicity say by 90°, then the yaw canal becomes vertical and this canal is no longer in rotation; its response begins to slow down. The subject then perceives this effect as counter-rotation about a horizontal axis, although actually no motion is occurring. At the same time, the *roll* canal previously in a vertical plane is brought into a position where it must register the rotation. The resulting apparent motion in yaw and roll can be highly disorienting and induce nausea.

IV. SPACEFLIGHT EVIDENCE

A. Summary of Existing Animal Flight Data

1. Artificial Gravity Animal Research prior to ISS Utilization

The Soviet space research community expressed an early and intense interest in artificial gravity. In 1961 Soviet scientists began testing rats and mice in reduced gravity during parabolic flight. The posture and locomotion of the animals appeared normal during brief periods of 0.3 g exposure, thus setting this as a minimum gravity level requirement for locomotion (Yuganov *et al.* 1962; 1964).

The first animals to be centrifuged in space were flown on the 20-day Cosmos-782 mission in 1975, when fish and turtles housed in containers were centrifuged at 1 g. The center of the containers was placed at 37.5 cm from the center of a platform rotating at 52 rpm. After the flight, the physiology and behavior of the centrifuged animals was indistinguishable from their 1-g ground and 0-g flight controls. Furthermore, turtles centrifuged at levels as low as 0.3 g showed none of the muscle wasting that is typically associated with exposure to weightlessness (Ilyin & Parfenov 1979).

In 1977, a significantly more extensive investigation was executed using rats that were centrifuged during the 19-day mission of Cosmos-936. The rats were kept in individual cages and were not restrained. Their cages were placed in a centrifuge with a radius of 32 cm. An artificial gravity level of 1 g was obtained by spinning the centrifuge at 53.5 rpm (Gurovsky *et al.* 1980). Results revealed that in-flight centrifugation had a protective effect on the myocardium and the musculoskeletal system, as compared to the animals that were exposed to microgravity and not centrifuged (Adamovich *et al.* 1980). However, there were some adverse effects of the in-flight centrifugation that were noted in the visual, vestibular, and motor coordination functions, such as equilibrium, righting reflexes, and orientation disorders. These deficits may have been the result of the high rotation rate of the centrifuge and the large magnitude of the gravity gradient (Borisova *et al.* 2004).

Other experiments with rodents in flight centrifuges showed that for g levels above 0.28 g the effects were like 1 g, and that for g levels below 0.28 g the responses of the animals were not different from those of the in-flight 0 g control animals (Shipov 1977). Therefore, 0.28 g seemed to be the minimum efficient g level. The Russian investigators choose this value initially, as it was proposed by Tsiolkovsky and experienced by humans in parabolic flight. In the 1970s, the Russians proposed an investigation of the minimal AG level required for alleviating bone and muscle loss in animals on board the Mir space station, but this experiment was never implemented. The proposal suggested starting with 0.5 g: if the effects were positive, the g level would have been reduced to 0.25 g; if negative it would have been increased to 0.75 g, etc. (Galle *et al.* 1974; Kotovskaya *et al.* 1980). The use of the rodent model, however, raises the following question: will the physiological impact of 0.28 g on an anatomy that is primarily oriented in the Gx axis in rodents) result in similar effects on an anatomy that is primarily oriented in the Gz axis in humans)?

In the U.S., a series of experiments involved rotating four rats on suborbital rockets during a 5-min period of free fall. The rocket was rotated about its longitudinal axis using a special motor at a rate of 45 rpm. The rotation created a variable artificial gravity field of from 0.3 to 1.5 g along the boxes that housed the rats. The movements of the rats were recorded on film and showed than one rat stayed in a position where the artificial gravity level was about 0.4 g, whereas the other three settled down where the artificial gravity level was 1 g (Lange *et al.* 1975).

Small radius high rotation-rate centrifuges have been flown in the Spacelab of the Space Shuttle and in the Skylab, Salyut, and Mir space stations to conduct experiments on bacteria, cells, and other biological specimens. Results indicate that microgravity effects, especially at the cellular level, may be eliminated by artificial gravity (see Clément & Slenzka 2006 for review).

The original plans to install a 2.5-m-radius centrifuge on the ISS to carry up to eight modules for rodents, fish, and eggs have been cancelled. This variable gravity animal centrifuge would not only have provided a 1-g control for the 0-g experiments, but would also have allowed exploring the entire range from 0.01 g to 1 g for a variety of species. Such a device would have afforded the opportunity to examine the adequacy of various levels of artificial gravity in protecting rodents during spaceflight. It is unfortunate that this centrifuge, which was the heart of the gravitational biology flight program, was eliminated from the ISS program. Not only was it essential for basic research, but it also formed the basis for understanding the physiological effects of short radius centrifugation in a manner needed for effective human AG prescription.

Finally, it is worth to mention the efforts of the students from the *Massachusetts Institute of Technology* (MIT), and the Georgia Institute of Technology who had proposed to study the effects of Mars gravity on mice on board an unmanned biosatellite. Their project, the *Mars Gravity Biosatellite,* was a 400-kg biosatellite carrying 15 mice housed in individual life support systems that rotate about its central axis, providing 0.38 g outwards against a curved floor. After 5 weeks in low Earth orbit, the re-entry capsule were to separate from the primary spacecraft to return the mice safely to a landing zone in the Australian desert. The biosatellite provided autonomous life support capabilities and data telemetry or storage from on-board experiments. The comparison between the deconditioning of the mice in the Mars Gravity Biosatellite and in previous microgravity space missions promised valuable data about the effects of partial gravity on physiological functions. Unfortunately, this project was cancelled too.

2. Science Plans for Rodent Research on board the ISS

The National Research Council's 2011 Decadal Survey Report, *"Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era,"* identified the highest priority research topics to be addressed in space life and physical sciences over the next ten years. Animal and rodent research is specifically called-out in six of the highest priority recommendations, and rodent research is also expected to make a significant contribution to eight additional highest priority recommendations.

The ISS is currently the only option for studying partial and intermittent gravity exposure on animals. There are several critical applications using the rodent model for AG research in space (Fuller 2014):

- Rodents are the only mammalian models that can be used to study long-term physiological effects without any interference from the obligatory countermeasures normally required for humans; this cannot be done with humans.
- Many procedures are possible with rodents that cannot be performed in humans, *e.g.* dissections, injection of infections agents and/or biomarkers. This makes it possible to utilize all available analytical tools to uncover the mechanisms of spaceflight adaptation on mammalian systems including spaceflight homeostasis, countermeasure efficacy and post flight re-adaptation. As a corollary to this, rodent studies can allow many physiological systems to be studied together to ascertain the integrated physiological responses due to spaceflight.
- Rodents (particularly mice) offer a vast array of genetically modified strains and special breeds that can target specific molecular and genetic pathways affected by spaceflight. Fourth, rodents are the most complex mammalian model that can be supported in space for multiple generations, offering incredible insight and new understanding into reproductive health, developmental biology, maturation and aging beyond the Earth over multiple lifetimes.
- Rodents offer a very well-established and widely-used Earth-based biomedical research model which can be used in unique ways, not only to help solve the problems facing long-term human health in space, but also to address human health issues and diseases on Earth where inactivity, disuse and degeneration mimic the human health conditions seen in space.

JAXA has started the development of an ISS/Kibo mouse habitat system that will include a 15-cm centrifuge for generating 1 g at a rotation rate of 77 rpm. The mice will live in this habitat for 1 to 6 months and could be returned to Earth using the Space-X vehicle. This habitat is expected to launch in September 2015, with a first 12-mice experiment to be conducted on board the ISS by April 2016. Six mice will be exposed to 0 g and six other mice to 1 g. The main focus of this experiment is to investigate bone and muscle preservation by AG.

NASA is nearing completion of the first-generation habitat for performing rodent research aboard ISS. This new facility, the *Rodent Habitat Mark I* (RHMk-I) will allow for fundamental and applied biomedical research on the ISS using rodent models. Early flights (2015-2016) will be for female mice only and will allow for mission durations of 30-60 days. Later flights will have increasing durations of up to 180 days and will also include the capability to support both male and female mice, as well as rats. RHMk-I is based on the NASA *Animal Enclosure Module* (AEM), which successfully flew rats and mice on 27 separate Space Shuttle missions, ranging in duration from 5 to 17 days. RHMk-I builds on this Shuttle legacy with an inflight animal video system and improved lighting and access to animals to increase mission duration up to 30 days. Longer duration flight experiments are possible with an improved waste filter system and by transferring animals into fresh habitats every 30 days.

3. Benefits of AG Animal Research for Human Health Protection

These benefits include the following:

- Use of animals both on ground and in space should play an important role in facilitating the development of a human AG countermeasure and be part of a translational science based approach.
- Even though mice seem to readily adapt to centrifugation in space, the suggested rodent to be used should be the rat primarily because of its larger size, enabling some tissue analyses that cannot be performed in mice.
- AG in space can be also beneficial for more fundamental animal research for understanding the adaptation of the vestibular system to gravity level transitions. Despite the permanence presence of 1 g in their evolution, animals immediately sense exposure to a novel, non-1 g environment and adaptive mechanisms are quickly initiated. Short-term compensatory mechanisms are presumably confined to the peripheral sensory receptors, the central nervous system, or both. For longer exposures to a non-1 g environment, structural modifications of the end organ may also result. How these functional and structural changes within the otolith organs affect performance and neurovestibular perception, particularly following transitions between gravity levels, are yet to be determined, but clearly pose severe challenges to long-term missions. Artificial gravity tools used either intermittently or on a prolonged basis on the ISS are essential and can directly address these challenges.
- It is therefore urgent to define if additional facilities are required on board the ISS for AG studies.
- Collaboration between international partners and research programs is highly desired, because the partners do have in-flight animal facilities (e.g. the JAXA Mouse Habitat Unit).

The applicability of using a centrifuge on board the ISS to counteract the effects of microgravity and to study partial and intermittent applied gravitational forces in rodents has been recently addressed by the Space Biology group at NASA Ames Research Center (Boyle *et al.* 2014). The requirements include the possibility for crew access to the animal housing for treatment and/or removal of animals for inflight procedures, for on-orbit operational capability of up to 180 days on the ISS, and for return of live rodents for conducting post-flight experiment and recovering tissues within six hours post-landing.

The onboard centrifuge will have a radius of 0.5 m and the habitat will be arranged so that the “floor”| will be oriented perpendicular to the radius of the centrifuge with the animal’s feet nominally pointed away from the center of rotation. The gravity levels will be set at 0.16 g, 0.38 g, 0.66 g, and 1 g along the animals’ legs. The gravity gradient within the volume of the animal housing will not exceed 10% (Boyle *et al.* 2014).

Because rodent behavior, physiology and health is affected by their circadian rhythms that are triggered by exposure to light, the light cycle within the animals’ habitat will be controllable during both flight operations and transport.

B. Summary of Existing Human Flight Data

1. Anecdotal Observations

During the first 40 years of the space age, no formal human artificial experiments were performed in space. In the early years of human spaceflight, the only major physiological disturbance involved space motion sickness and this was of concern only for the first few days in orbit. After the Apollo missions, the NASA flight surgeon position was, "*The magnitude of the motion-sickness problem experienced by astronauts to date does not appear to suggest clearly the need for design and incorporation of artificial gravity system in near-future space vehicles*" (Berry 1973). The debilitating effects of weightlessness on the bone, muscle, and cardiovascular system were demonstrated on the longer Skylab missions in the early 1970s and later on the long-duration Salyut and Mir flights. However, it was believed that in-flight exercise, augmented by resistance training and fluid loading, would solve the problem. As time passed, the opportunities for human centrifuges or rotating spacecraft in orbit disappeared.

a. Gemini

The Gemini-11 mission in 1966 offered the first chance to turn artificial gravity science fiction into fact. The Gemini program was, however, halfway completed before NASA got around to planning tethered vehicle flights. When tethered flight was identified as a mission objective, NASA planners first thought of it as a way of evaluating the tether as an aid to station keeping. However, tethering might also be a means of inducing some degree of artificial gravity. The minimum rotation rate depended on whether the tethered activity was intended primarily for formation flying or for achieving gravity. NASA decided to attempt both, although it would settle for "an economical and feasible method of long-term, unattended station keeping", and chose a 36-meter Dacron line (Wade 2005).

An astronaut connected a tether to an orbiting Agena rocket casing and to the Gemini-11 spacecraft during a spacewalk. The two vehicles were then rotated at about 0.15 rpm. At a distance of about 19 m from the center of rotation, the Gemini cabin and its crew (astronauts Gordon and Conrad) experienced 0.0005 g of artificial gravity. When the astronauts put a camera against the instrument panel and then let it go, it moved in a straight line to the rear of the cockpit and parallel to the direction of the tether. However, the crew, themselves, did not sense any physiological effect of gravity. After they had been rotated for 2½ orbits around the Earth (about four hours), the pilots ended the exercise by jettisoning the spacecraft's docking bar. All in all, they reported it had been "an interesting and puzzling experience" (Wade 2005).

It is now known that Sergey Korolev also had a project for an artificial gravity experiment in 1965-1966 (Harford 1973). As mentioned above, his plan was to deploy a tether between a Voskhod vehicle and the spent last stage of its booster, and rotate both vehicles, thus providing artificial gravity in the crew compartment. The flight was supposed to last for 20 days and clearly upstage the Americans. The crew would have included a pilot and a physician (Volynov & Katys), and artificial gravity experiments would have been conducted for 3-4 days during the flight. However, after the unexpected death of Korolev in January 1966, the Soviet space program was in crisis. This mission was postponed to February 1966, with the deletion of the artificial gravity experiment, before being finally cancelled outright (Wade 2005).

b. Skylab

As an example, during the Skylab missions the crew took advantage of the large open compartment to run around the curved circumference, imitating the jogger in Stanley Kubrick's film. The astronauts produced self-generated artificial gravity by running (see the video at the following URL site: http://www.artificial-gravity.com/Skylab-clip2.mpg). The angular velocity of the astronauts was estimated to be about 5.3 rpm and the radius of the circumference to be about 3.3 m, so the gravity level was approximately 0.1 g. The astronauts reported no difficulty with either locomotion or motion sickness during this exercise (Conrad & Klausner 2005). One of the crewmembers, Joe Kerwin, felt "*no sensation of gravity, just a controlled bounce*" (John Charles, personal communication).

c. Apollo

The astronauts who visited the lunar surface were exposed to a reduced gravity level of 0.16 g on the Moon for several hours or days during their 12-day space missions. They reported having "*difficulty in determining just what straight up and down was*". During Apollo-11, the lunar module floor on the Moon surface was, in fact, tilted 4.5 deg from the horizontal. The crew did not perceive this tilt. During their spacewalks on the Moon, the astronauts lost their balance several times, in most cases because they could not evaluate the slope of the terrain. They also reported this problem "caused our cameras and scientific experiments sometimes not maintaining a level attitude we expected" (Godwin 1999).

Interestingly, less decrease in heart size and less increase in heart rate were found post flight in the Apollo astronauts compared with Skylab and Shuttle astronauts (Johnston *et al.* 1975; Johnston & Dietlein 1977; Nicogossian *et al.* 1994). Unfortunately, there was no comparison between the results obtained on those astronauts staying on the Moon and those who stayed in orbit around the Moon. Therefore, it cannot be concluded that the exposure to lunar gravity during the course of their exploration missions was helpful in reducing cardiovascular deconditioning. All of the Apollo astronauts were highly trained jet fighter pilots in exemplary physical condition. Their long hours flying high-g maneuvers in jet aircraft may have increased their orthostatic tolerance and promoted the development of adaptive protection in these individuals, as compared to other Skylab and Space Shuttle astronauts (Clément & Pavy-Le Traon 2004).

d. Soyuz

During its 2-day transit to the Mir station and now the ISS, the Soyuz spacecraft is constantly rotating about its yaw axis to maintain solar panel illumination. The rotation axis of the spacecraft is near the base of the reentry module, so the crew in the orbital module place above is at a distance of about 4 m from the axis of rotation. At this distance a rotation rate of approximately 0.42 rpm generates a gravity level of 0.0008 g. This AG was not perceived by the crew (Reinhold Ewald, personal communication).

e. Shuttle

During the STS-32 Shuttle mission in 1990, six of the 44 reaction control system (RCS) jets fired, causing about six slow pinwheel somersaults in about ten minutes. The crew that was sleeping through the incident did not feel this slow yaw rotation. The crew compartment was located at approximately 20 m from the axis of rotation and the rotation rate was about 0.6 rpm, thus generating 0.008 g.

In a recent study, Bukley *et al.* (2007) investigated two scenarios wherein gravity levels ranging from 0.2 to 0.5 g could be created on board the Space Shuttle. One possible means of accomplishing this is by rotating the vehicle about an eccentric roll axis (in this case, the baseline orbital trajectory of the vehicle) at a constant angular velocity. This roll maneuver would create artificial gravity in the +Gz direction of the vehicle, such as when the vehicle is on Earth. The other means is by rotating the vehicle in pitch about its center of gravity. This pitch maneuver would create artificial gravity in the +Gx direction of the vehicle, so that astronauts could "stand" on the middeck lockers.

A feasibility analysis of the eccentric roll maneuver was executed beginning with the simple dynamics of a point mass in a central gravitational field to ascertain the force levels required to execute the proposed maneuver. Once the force levels were determined, they were then compared to the capability of the Space Shuttle orbital control system. Results of the analysis indicated that the force levels needed to generate an artificial gravity environment in the +Gz direction of the Space Shuttle vehicle exceed the capability of its orbital control system. A lighter vehicle with a more robust thruster system may have the capability to fly such a trajectory.

The pitch maneuver is considerably simpler. Rather than altering the orbital trajectory of the Space Shuttle center of gravity, the vehicle is simply pitched about the center of gravity, nose-over-tail, at a rate sufficient to generate AG at the forward bulkhead of the middeck (approximately 16.8 m away), which would be now be used as the "floor" by the astronauts. Gravity levels ranging from 0.1 g to 0.5 g could be generated by rotation rates ranging from 2.3 to 5.2 rpm, which was within the capability of the Shuttle reaction control system. In fact, the Space Shuttle actually flew this rotational pitch maneuver during the return-to-flight missions STS-114 and STS-121 prior to docking with the ISS. This maneuver allowed the crew on board ISS to capture photographs of the heat shield on the belly of the Shuttle. However, the rotation rate during this 360-degree back-flip maneuver was 0.125 rpm, thus generating artificial gravity in the Space Shuttle crew compartment of 0.0003 g, a level far too low to be perceived as AG by the crew.

f. International Space Station

The main drivers cited in the literature for AG research are: (a) life science and physiological understanding; (b) prevention of physical deconditioning; (c) human factors; and (d) habitability, and these were considered by members of the astronaut office. Gravity is one of the basic elements that have shaped human development along with atmospheric pressure and composition. Understanding the role of gravity for fundamental physiological processes is required for understanding spaceflight physiology and adaptation and thus for development of the most efficient countermeasures. In this context, the gravitational dose-response curve should be defined and for this purpose AG is pivotal. In particular the dose-response curve between 0 and 1 g is required and can only be investigated by AG in space (Barratt 2014).

Regarding physical deconditioning, AG will target most physiological systems, but even so exercise will be combined with AG. Just as astronauts are required to exercise as part of their non-flight career, AG alone will not suffice to maintain a desired level of crewmember fitness during flight. An important driver for introducing AG may be as a countermeasure for the *Vision Impairment due to Intracranial Pressure* (VIIP) syndrome (which might also be called the *Microgravity Cerebral Syndrome* pending a complete mechanistic understanding). The hypothesis is that the weightlessness-induced fluid shift is the precipitating factor inducing impairment in cerebrospinal fluid re-sorption and central nervous system venous drainage. It is possible that interventions like venous limb occlusion and lower body negative pressure could play a preventive role, and these are being investigated. However chronic AG might be the most efficient countermeasure. For the time being, we do not fully understand all ramifications of the VIIP syndrome and whether it has long-term effects on brain function. However, the VIIP syndrome is currently of the highest concern for long duration missions. The possible role of AG in VIIP prevention should be investigated.

According to Barratt (2014) AG employed as a countermeasure during a weightless coast phase will probably not play a significant role in protecting crews during entry, but may be of benefit for the immediate post-flight adaptation to a gravitational load on a planetary surface in regard to orthostatic intolerance and sensorimotor performance. From his astronaut perspective, short-arm centrifuges for intermittent AG exposures during flight may not be preferred over continuous spinning of the entire craft, because astronauts generally prefer less complexity in machines on which they depend. From a habitability standpoint, spacecraft are inherently small; the limited volume becomes more usable in weightlessness, an aspect that might be sacrificed for a spacecraft or module that was spun for AG. Human factors represent a nearly even trade. Some tasks are easier and some more difficult in weightlessness, and the crew office does not see human factors as a driver for AG. From a behavioral health and performance perspective, benefits will depend on the method of providing AG.

In 2009, a human centrifuge project called AGREE (*Artificial GRavity with Ergometric Exercise*) was selected to fly on board the ISS as a result of an International Life Science Research Announcement. The *Principal Investigator* (PI) of this project was Dr. Satoshi Iwase with members of the investigation team from the US, Canada, Belgium, France, and Germany. The objective of AGREE was to test, for the first time, the acceptability and effectiveness of AG generated by short-radius centrifugation as a countermeasure to human deconditioning on orbit. This centrifuge combined with ergometric exercise capability was to be constructed by ESA, launched by JAXA HTV, and placed at the end of the *Multi-Purpose Logistics Module* (MPLM). Unfortunately, AGREE was cancelled in 2013 following a stress analysis showing that the ISS nodes would have been structurally compromised by the generated vibration loads.

2. Vestibular Experiments using Centrifugation or Linear Acceleration in Orbit

Although these experiments were not originally designed for testing artificial gravity in orbit, they provided the first reports of human thresholds for perception of linear acceleration in weightlessness, as well as anecdotal perceptual reports by the crew during controlled, although short-lasting, linear accelerations.

a. Motion Sickness

Several studies have shown that most crewmembers perceive a passive complex motion to be less provocative on landing day than preflight. This was first observed for passive yaw rotation and active head movements that generated Coriolis forces and cross-coupling angular accelerations following Apollo (Homick & Miller 1975) and Skylab (Graybiel *et al.* 1977) missions. In the latter experiment, eight crewmembers performed head and body movements during yaw rotation ranging from 12.5 to 30 rpm. After 3 weeks into the flight, when the first test was performed, all crewmembers had less motion sickness than preflight, and 7 out of the 8 crewmembers had even less symptoms post-flight.

In another study, 58 crewmembers participating in 16 flights of the Space Shuttle (duration < 11 days) were also tested during Coriolis maneuvers (12.5-30 rpm) and during off-vertical axis rotation (20 rpm up to 30º tilt) before and immediately after their space flight. Most crewmembers tested immediately after flight showed reduced motion sickness compared to before flight (Thornton *et al.* 1987). Clément & Wood (2013a; 2013b) also observed less motion sickness in astronauts during OVAR and during centrifugation immediately after landing compared to baseline.

Lackner & DiZio (2000a; 2000b) performed a series of parabolic flight experiments when subjects were exposed to Coriolis forces and cross-coupled angular accelerations. They conclude that “the severity of side effects from Coriolis forces during head movements is gravitational force-dependent, raising the possibility that an artificial gravity level less than 1 g would reduce the motion sickness associated with a given rotation rate”.

Apart from the Coriolis and cross-coupled effects due to angular motion of the centrifuge the altered gravity level itself may induces neurovestibular adaptation problems. After continuous exposure to a hypergravity stimulus of 3 g (Gx) in a human centrifuge for one hour, head movements provoke symptoms similar to space motion sickness (Bles *et al.* 1997). It is interesting to note that the centrifuge run itself is not experienced as uncomfortable, but that it is the head movements that trigger the symptoms for several hours after centrifugation. The susceptibility of 12 astronauts to this centrifuge-induced sickness was highly correlated to their susceptibility to space motion sickness in actual spaceflight. This result suggests that it might be the gravity level *transition* rather that the weightless environment that causes neurovestibular problems (Groen *et al.* 2008). The finding that a centrifuge run of limited duration (1 hour) may have dramatic impact on the astronaut’s well being and performance for a much longer time (> 8 hours) should certainly be considered in the design of AG protocols.

b. Threshold for Perception of Linear Acceleration

The threshold of acceleration detectable by the human has been measured by a number of researchers and methods. According to these studies, the subjective perception of motion ranges from 0.002 to 0.01 g. The large variability is due to factors such as differences among subjects, differences in orientation of the subject with respect to the direction of motion, and differences in interpretation of the data, e.g., some subjects determined merely the presence or absence of motion, whereas others determine the direction of motion as well.

The first investigation on the threshold of perception of linear acceleration during adaptation to microgravity was performed using a chair that was manually moved by an operator while the subject reported the beginning and direction of perceived motion using a joystick. The *Body Restraint System* (BRS) flew on board the first Spacelab mission in 1983 and valuable data were collected (Benson *et al.* 1986). The thresholds estimated from this study were confirmed later in 1985 during another study utilizing a servo-controlled sled on board the Spacelab-D1 mission (Arrott *et al.* 1990). In this experiment, subjects were oscillated in a sinusoidal fashion on a linear sled at frequencies between 0.18 Hz and 0.8 Hz generating a peak linear acceleration of 0.2 g. The acceleration could be in either the inter-aural (Gy) or the longitudinal (Gz) direction, with ±Gy directed to the right or left shoulder, respectively, and the ±Gz directed head-to-foot or foot-to-head, respectively (Figure 3).

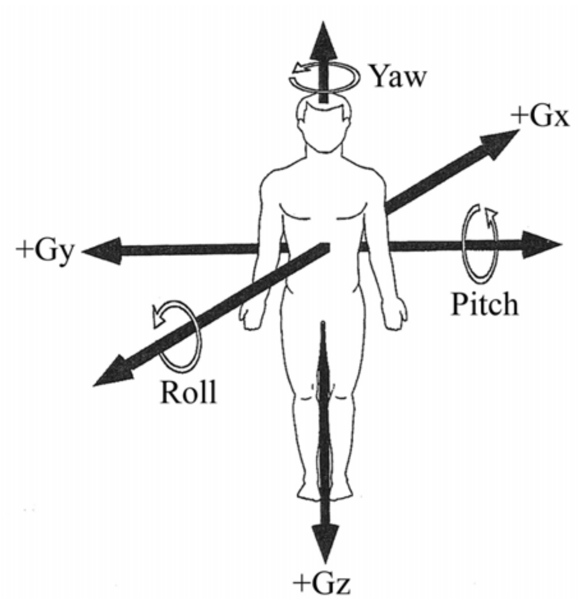

Figure 3. The cardinal axes of the human body are defined as x, y, and z. Rotation about these axes is roll, pitch, and yaw, respectively. The positive directions of the gravitoinertial forces (G) along these axes are chosen to be chest-to-back (+Gx), left-to-right (+Gy), and head-to-foot (+Gz), respectively. Note that the positive directions of the corresponding accelerations along these axes would be back-to-chest, right-to-left, and foot-to-head, respectively (Clément & Bukley 2007).

Using the methods of limits with a staircase procedure, results showed that detection thresholds for linear-oscillatory motion at 0.3 Hz in all three orthogonal axes of the body were higher (i.e., the crewmembers were less sensitive) during flight than before. On Earth, thresholds were higher for vertical (z-axis) accelerations (0.077 m/sec^2) than for anterior-posterior (x-axis) or lateral (y-axis) accelerations (0.029 m/sec^2) (Benson *et al.* 1986). Neurophysiological evidence supports such a lower gain for z-axis stimulation, as the sensitivity (spikes per second per g) in neurons signaling motion in the z-axis is 30% less than in neurons signaling motion in the x- and y-axes (Fernández & Goldberg 1976). Threshold variability increased during and after flight, and thus the changes were not consistent. Threshold determinations typically require more data than can be collected in space experiments, which may account for this variability.

A second measure of sensitivity, the time to detect acceleration ranging from 0.001 to 0.08 g indicated no consistent pattern of change in threshold, except for increased variability in response (Young *et al.* 1986; Arrott & Young 1986). Four members of the Spacelab D-1 crew, however, had a consistently shorter time to detect acceleration in weightlessness, with no significant differences between the axes tested (Arrott *et al.* 1990). Before and after the SLS-1 mission, four crewmembers were also translated sinusoidally at 1 Hz and 0.25 Hz with a peak acceleration of 0.5 g and with a series of low acceleration steps. The results indicated a "pattern of confused and erratic responses to early post-flight accelerations" (Young *et al.* 1993). There was an increased sensitivity to lateral (y-axis) linear acceleration, but a reduced sensitivity for longitudinal (z-axis) linear acceleration.

In another experiment, subjects were asked to null their movement using a joystick on a sled during sinusoidal oscillations along the y- and z- axes at high (1 Hz) and low (0.25 Hz) frequencies, or pseudorandom linear motion (0.036-0.451 Hz) with peak acceleration ranging from 0.2 to 0.5 g. In about half of the subjects tested the ability to complete this task was generally improved post-flight by comparison with the preflight performance, and this improvement decayed gradually over the ensuing week (Arrott *et al.* 1986; 1990; Merfeld *et al.* 1994). In this closed-loop test, the linear accelerations were large enough (0.2-0.5 g) to potentially generate a perception of tilt ranging from 15-27 deg preflight. After adaptation to microgravity, however, tilt was no longer perceived but reinterpreted as translation, and the performance of nulling-out pure linear acceleration then improved (Merfeld *et al.* 1996). This explanation remains a hypothesis, though, since simultaneous perception of tilt and translation was not recorded in these experiments.

c. Perception of Tilt during Linear Acceleration

The mean threshold for detection of static tilt is lower than for detection of linear acceleration. This threshold is about 0.005 g or 0.25 deg of tilt from the vertical, with the head in the normal upright position. However, these values are far worse when the head is tilted or the body inverted. This decrease in precision is related to the decrease in the sensitivity of the utricles with increasing tilt of the body. It is also interesting to note that subjects are able to detect when a visual line is tilted as little as 0.5 deg from the vertical or the horizontal (Howard 1982; Mittelstaedt 1986).

Astronauts returning from short-duration missions generally experience a larger sense of tilt when exposed to passive linear acceleration or when passively tilted relative to gravity (Clément *et al.* 2001; 2007; Glasauer & Mittelstaedt 1998; Clément & Wood 2013a, 2013b). It is interesting to note that the motion perception of astronauts when exposed to linear translation, centrifugation, or off-vertical axis rotation is fundamentally different post-flight compared to preflight, whereas the eye movements, in particular torsion, are not. This dissociation between otolith-driven eye movement and perception during passive vestibular stimulation after space flight suggests that eye movements and orientation perception are governed by qualitatively different neural mechanisms. Ocular torsion is primarily a response of otolith activation by low-frequency linear acceleration along the interaural axis, whereas perception of tilt is primarily governed by the integration of graviceptive cues, including somesthetic, presumably centrally processed through neural models of the physical laws of motion. The peripheral vestibular organ would experience little or no changes after short-duration space flight, but the central processing of graviceptors inputs and the outputs of internal models for spatial orientation are likely to be affected (Young *et al.* 1986). This dissociation would explain why otolith-driven eye movements appear relatively unaffected by microgravity, while perceptual responses depending on central vestibular processing can be greatly disrupted (Clément & Reschke 2008).

One interesting result from the sled experiment during the D-1 mission was that the test subjects in microgravity did not perceive linear Gz accelerations of less than 0.2 g in magnitude as artificial gravity (Arrott *et al.* 1990). In another experiment conducted during the Spacelab *International Microgravity Laboratory* (IML-1) mission that was flown on STS-42 in 1992, four subjects were spun on a rotator in pitch and in roll. The head of the subjects was 0.5 m off-center and experienced an acceleration of 0.22 g in the –Gz direction, while the feet were on the other side of the rotation axis, experiencing an acceleration of 0.36 g in the +Gz direction. No unusual inversion phenomena were reported, indicating that the artificial gravity stimulus of –0.22 g at the head did not provide a vertical reference in any of the test subjects (Benson *et al.* 1997).

During the Neurolab mission flown on STS-90 in 1998, a systematic evaluation of the effects of artificial gravity in humans was conducted using the ESA off-axis rotator, a short-radius centrifuge with a variable radius of 0.5 to 0.65 m that was capable of generating artificial gravity levels of 0.5 and 1 g. The artificial gravity forces were applied through the subject's ±Gy or –Gz axis for seven minutes at a time. Eye movements and perception recorded during the artificial gravity events provided both objective and subjective data. The experiment indicated that the test subjects perceived sustained levels of 0.5 g and 1 g as artificial gravity (Clément *et al.* 2001).

Although the threshold for perception of linear acceleration in humans is on the order of 0.007 g (Benson *et al.* 1986) the threshold for perception of artificial gravity by astronauts in space is, based on the data we have so far, somewhere between 0.22 and 0.5 g. Perhaps it is not necessary to *perceive* artificial gravity at the cognitive level for it to be effective as a countermeasure. However, for purposes of defining the comfort zone of astronauts in an artificial gravity environment (whether it's a rotating spacecraft or on-board centrifuge), it would indeed be useful to determine the threshold value of perceived artificial gravity. Unfortunately, there are no plans to put a human centrifuge on board the ISS, at least in the near term.

Interestingly, the four subjects tested intermittently in the on-board centrifuge during the Neurolab Spacelab mission mentioned above, seemed to have achieved some measure of resistance to post-flight orthostatic instability and did not show the usual decrease in vestibular sensitivity to tilt. The other three crewmembers on that mission had orthostatic intolerance. Based on the result that about 64% of astronauts experience profound post flight orthostatic intolerance (Buckey *et al.* 1996), the probability that four crewmembers on the same flight do not exhibit orthostatic intolerance by chance is approximately 1 in 60 (0.364) (Moore *et al.* 2005). During the flight, the centrifuge runs were executed during about 10 min at 0.5 g or 1 g every other day, for a total duration ranging from 45-60 min during the 16-day mission. Because of the limited number of subjects, more experiments are needed to validate these results.

3. Vestibular Experiments in Parabolic Flight

In late 1960s, tests were also conducted in parabolic flights to define artificial gravity requirements for a space station and to assure that the crew could perform well in reduced gravity. Parabolas were flown at 0.1 g, 0.2 g, 0.3 g, and 0.5 g during about one-half minute each. The tests subjects, who had previously flown several hundreds of parabolas in reduced gravity, carried out certain predefined tasks. These tasks included walking while carrying small and large containers, tightening bolts, connecting and disconnecting electrical equipment, and pouring water back and forth between two containers. These tests, although preliminary in nature, indicated that 0.2 g provided a much better environment for such tasks than did 0.1 g. At gravity levels greater than 0.2 g, very little gain in performance was indicated. Furthermore, the test subjects reported that at 0.5 g they felt as sure of themselves and as comfortable as they did at 1 g (Faget & Olling 1968).

Recent studies have investigated how the *subjective vertical* (SV) was affected by reduced gravity levels during the first European Parabolic Flight Campaign of Partial Gravity. In normal and hypergravity, subjects accurately aligned their SV with the gravitational vertical, thanks to the contribution of visual, otolith, and somatosensory inputs (Mittelstaedt 1992; Mittelstaedt & Fricke 1988). However, when gravity was below a certain threshold, subjects aligned their SV with their body longitudinal axis. The value of the threshold varied considerably between subjects, ranging from 0.03 to 0.57 g, with an average at 0.3 g (Winkel *et al.* 2012) (Figure 4). In other studies performed in parabolic flight as well as in subjects lying supine, it was found that the relative contribution of vision to the subjective vertical decreased below 0.17 g (Dyde *et al.* 2009; Harris *et al.* 2012; Harris *et al.* 2014).

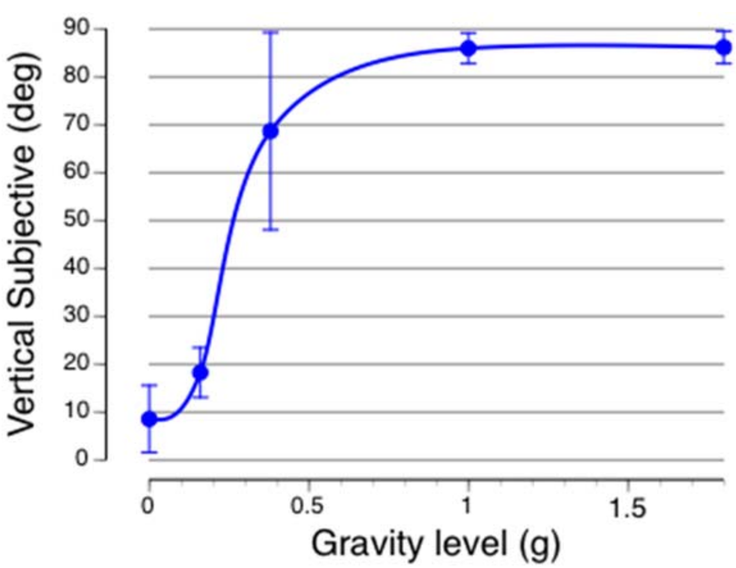

Figure 4. Dose-response curve of perception of verticality versus gravity level in parabolic flight. Mean of 6 subjects, 15-60 trials per subjects. Subjects lying on their side were able to accurately align their subjective vertical with the gravitational vertical for gravity levels ranging from 0.3 g to 1.8 g. Below 0.3 g they aligned their subjective vertical with their long body axis. Adapted from Winkel et al. (2012).

As indicated above, for whole body linear acceleration, the sensory threshold of the vestibular system is around 0.02 g (Guedry 1974; Janssen *et al.* 2011), so g-levels of 0.17 g, 0.2 g, and 0.22 g are well above this threshold. Yet the data from orbital and parabolic flight experiments suggest otherwise. This discrepancy between physiological and functional thresholds is not surprising. The equivalent situation in the visual system would be trying to predict the amount of light needed to recognize, for example, a face (perception) from knowing the minimum amount of light that can be detected in a dark room (sensation) (Harris *et al.* 2014). Dissociation between compensatory responses of the vestibular system, such as the eye movements by comparison with the sensation of tilt, has frequently been observed in astronauts during vestibular stimulation after space flight (Clément & Wood 2013a).

4. Loading in Space

a. Penguin Suit

A number of countermeasures have been developed and used in an attempt to prevent muscle and strength loss during spaceflight. In addition to treadmills, cycle ergometers, and resistive exercise devices, the Russian Cosmonauts have used a passive stretch garment known as the Penguin suit. This suit has rubber bands woven into the fabric, extending from the shoulders to the waist and from the waist to the lower extremities, to produce tension on antigravity muscles (Convertino 1996). The tension of elastic elements of the suit produces a “compressive” loading along the longitudinal axis of up to 40 kg. This axial loading compensates for the insufficient loading of the musculoskeletal system as well as the lack of weight-bearing and proprioceptive inputs in the space environment. A recent development of the Penguin suit includes the addition of a *Load Measuring System* (LMS). The LMS is an automatic system that measures the extension of the suit bungees through an interface with an onboard computer. This measuring system provides objective data regarding the loads provided by the suit to the cosmonaut’s body (Yarmanova & Kozlovskaya 2010).

More recently, the concept of a *Gravity Loading Countermeasure Skinsuit* (GLCS) that induces loading on the body to mimic standing and, when integrated with other countermeasures, exercising on Earth has been proposed (Waldie & Newman 2011). Comfort, mobility and other operational issues were explored during a pilot study carried out in parabolic flight for prototype suits worn by three subjects. Compared to the Russian Penguin suit, the elastic mesh of the GLCS can create a loading regime that gradually increases in hundreds of stages from the shoulders to the feet, thereby reproducing the weight-bearing regime normally imparted by gravity with much higher resolution. Modeling shows that the GLCS requires less than 10 mmHg (1.3 kPa) of compression for three subjects of varied gender, height and mass. Negligible mobility restriction and excellent comfort properties were found during the parabolic flights, which suggests that crewmembers should be able to work normally, exercise or sleep while wearing the suit. The suit may also serve as a practical 1 g harness for exercise countermeasures and vibration applications to improve dynamic loading (Waldie & Newman 2011).

b. Subject Loading

A "body-weight-equivalent loading" is routinely used while jogging on a treadmill in microgravity to preserve leg strength and aerobic capacity. A shoulder-hip harness assembly attached to bungee cords produces a vertical load equivalent to a crewmember's body weight (Hayes *et al.* 2013). In the early treadmill models used on the Shuttle the downward force provided by the bungees was not measured, and tension was adjusted for the highest load within crew comfort. Beginning with STS-64, the actual user loads could be set and measured during flights using *Subject Load Devices* (SLDs). The SLDs used torsion springs coupled to a cable-feed pulley to produce the loads. Using the keypad on the control panel, the subject would enter the desired loading and a microprocessor sent commands to the SLDs. The SLD motor control circuit would then command the motor to increase or decrease the preload on the torsion springs to provide the required subject load. A linear potentiometer in each SLD provided feedback on motor position and, therefore, subject loading. With both subject load systems activated, the runner could select up to 100 kg-force on the harness (Hayes *et al.* 2013).

Subject load devices on the ISS treadmill are using the same mechanical principle. A detailed biomechanical analysis of locomotion is currently on going on board the ISS to collect quantitative data during in-flight treadmill exercise. One objective of this study is to determine how combinations of subject loading system loads and exercise speed influence joint torque. The overall goal of the advanced exercise prescription being provided to the crewmembers is to increase loading at the joints in order to provide a greater stimulus for the preservation maintenance of bone and muscle tissue. External forces applied to the body will be measured with instrumentation built into the treadmill. A subject-specific computer model will be developed and provides joint kinetic approximations. These approximations are used to assess the effectiveness of the exercise prescription and allow for an iterative approach in exercise prescription modification based on evidence. Furthermore, these data provide better understanding of how exercise speed and external load affects the forces experienced by the joints and muscles. Providing subject loading information for exercise prescriptions increases the effectiveness of the exercise prescription (De Witt *et al.* 2014a; 2014b).

c. Lower Body Negative Pressure (LBNP)

Procedures other than centrifugation that induce venous pooling in the extremities of a magnitude similar to that seen in going from a supine to standing position have been tested in orbit. Lower Body Negative Pressure (LBNP) induces venous pooling, as would a Gz force exerted in the head-to-foot direction. In a LBNP, the subject's legs are enclosed in a chamber or trousers (below the iliac crests) and exposed to negative pressure. Levels of about –40 to –50 mm Hg are considered to produce blood shifts very similar to those induced by the upright posture on Earth (Charles & Lathers 1994). LBNP has been developed to assess cardiovascular responses to orthostatic stress, and is commonly used in-flight and during head-down tilt experiments. LBNP is also used in-flight as a countermeasure to prevent the orthostatic arterial hypotension encountered after spaceflight.

Study	***Days***	***Intervention***	***Session Duration (min)***	***# Session per day***	***Daily LBNP Exposure (min)***	***Total LBNP exposure (%)***
Lee et al. 1997	5	BR + LBNP + Treadmill	30	1	30	2.1
Schneider et al. 2002	15	BR + LBNP + Treadmill	40	1	40	2.8
Watenpaugh et al. 2000	15	BR + LBNP + Treadmill	40	1	40	2.8
Sun et al. 2001	21	BR + LBNP	60	1	60	4.2
Guell et al. 1991	28	BR + LBNP	20	3-6	120-240	8.3-16.6
Guell et al. 1995	28	BR + LBNP + Squats	30	1	30	2.1
Maillet et al. 1996	28	BR + LBNP + Isokinetic Exercise	20	1	20	1.4
Hughson et al. 1994	28	BR + LBNP + Cycling, Isometric, Isokinetic, Exercise	30	1	30	2.1
Lee et al. 2007	30	BR + LBNP + Treadmill	45	1	45	3.1
Lee et al. 2009	30	BR + LBNP + Treadmill	45	1	45	3.1
Watenpaugh et al. 2007	30	BR + LBNP + Treadmill	45	1	45	3.1
Smith et al. 2003	30	BR + LBNP + Treadmill	45	1	45	3.1
Zwart et al. 2007	30	BR + LBNP + Treadmill	45	1	45	3.1
Guinet et al. 2009	60	BR + LBNP + Treadmill, Squat, Calf Press	50	1	50	3.5
Edgell et al 2007	60	BR + LBNP + Treadmill, Squat, Calf Press	50	1	50	3.5
Trappe et al. 2007a	60	BR + LBNP + Treadmill, Squat, Calf Press	50	1	50	3.5
Trappe et al. 2007b	60	BR + LBNP + Treadmill, Squat, Calf Press	50	1	50	3.5
Trappe et al. 2008	60	BR + LBNP + Treadmill, Squat, Calf Press	50	1	50	3.5
Smith et al. 2008	60	BR + LBNP + Treadmill, Squat, Calf Press	50	1	50	3.5

Table 1. Summary of studies assessing the efficiency of LBNP during bed rest. Total LBNP exposure is the total time during LBNP relative to the duration of the bed rest. Adapted from Kaderka (2010).

Skylab had a LBNP device that was used to periodically test the integrity of the cardiovascular system. A subsequent analysis of the Skylab data revealed that the periodic LBNP use did not significantly improve the post-flight performance of astronauts (Nicogossian *et al.* 1994). In Russian missions on board Mir, LBNP tests were performed in the "Chibis" suit (trousers) to assess orthostatic tolerance and evaluate the effects of countermeasures generally every two months. LBNP was also used (and is still used today on board the ISS) as a countermeasure during the last month of long-duration spaceflight. The pre-landing sessions begin 16-20 days before landing, and consist of a 20-min session every 4 days (Gazenko & Kasyan 1990). These sessions are performed on a day free of muscular exercise. There is a gradual increase in the negative pressure level during these four sessions, which provides a step-wise re-conditioning of the cardiovascular function to a simulated orthostatic stress. Two sessions lasting nearly 1 h each are performed the last 2 days before landing. These scheduled protocols can be adapted slightly according to the cardiovascular responses of each crewmember. This timeline is derived from many years of experience with long-duration spaceflight, although the detailed flight studies leading to this timeline are not published in international peer-reviewed journals. It is interesting to note that the standard Russian exercise countermeasure prescription also follows a gradual exercise cycle: e.g., day 1, low workload but high intensity effort; day 2, moderate workload at moderate intensity; day 3, high workload at low intensity; day 4 *ad lib* (Kozlovskaya & Grigoriev 2004).

Because of shorter duration flights, the use of LBNP during Shuttle flights is more limited. In a review of LBNP experiments during spaceflight, Charles & Lathers (1994) considered that the use of a LBNP stress in-flight was a fairly good predictor of the cardiovascular response to the actual entry and landing of the Shuttle. Several protocols using LBNP as countermeasure were tested. A "soak" protocol, i.e., spending 4 h at a constant pressure of –30 mm Hg with fluid load, did not show significant improvement of orthostatic tolerance in five subjects treated (Sawin *et al.* 1998).

LBNP has also been used during bed rest studies, either to assess cardiovascular tolerance or as a countermeasure to prevent cardiovascular deconditioning (Table 1). Guell *et al.* (1995) showed that daily regular LBNP sessions at –30 mm Hg (3-4 sessions per day, and up to 6 per day the last 3 days) had beneficial effects on orthostatic intolerance after a 30-day bed rest, mainly by maintaining plasma and extra cellular fluid volume (Gharib *et al.* 1992; Maillet *et al.* 1996), with some beneficial effects on vasomotor tone (Arbeille *et al.* 1992). LBNP sessions had no preventive effects on lower limb venous resistance or loss in lower limb muscles (Berry *et al.* 1993). Other bed rest studies indicated that to be efficient, LBNP sessions require a good compromise between duration and pressure level. A pressure of –30 mm Hg is well tolerated, but –40 to –50 mm Hg better simulate the upright position. However, a sustained LBNP exposure above –50 mm Hg is more susceptible to induce fainting, since hypotension depends on both the level of negative pressure and duration of exposure (Lightfoot *et al.* 1991).

One problem is that most of the bed rest studies used a combination of LBNP with other countermeasures, such as treadmill, squat, and calf press. These countermeasures also improved orthostatic tolerance, but the effects of LBNP and muscular exercise cannot be easily dissociated. Both bed rest and spaceflight reduce exercise fitness due to cardiovascular deconditioning and muscular atrophy. The bed rest experiments have confirmed the advantage of combining LBNP with other countermeasures, in particular exercise. LBNP combined with treadmill exercise in supine subjects provides both cardiovascular and musculoskeletal stimulation. Murthy *et al.* (1994) compared 5 min exercise in the supine position within an LBNP chamber (–100 mm Hg) to 5 min exercise in the upright position and concluded that exercise combined with LBNP produced the same musculoskeletal stress on the legs and greater cardiovascular stress than exercise in the upright position. Lee *et al.* (1997; 2007; 2009) showed that 30-min daily sessions of intense upright or supine exercise (treadmill) with LBNP (–50 mm Hg) were sufficient to maintain exercise muscular capacity after 5 days of bed rest. Watenpaugh *et al.* (2007) also reported beneficial effects on exercise performance of 40 min of supine exercise per day in a LBNP chamber (–58 mm Hg) in a 15-day bed rest. However, moderate exercise performed with

LBNP (–50 to –60 mm Hg) failed to protect muscular capacity and orthostatic tolerance after 15 days of bed rest (Schneider *et al.* 2002).

Other experiments showed that LBNP exposure during bed rest failed to improve tolerance to 2.5-3.0 g (+Gz acceleration) after bed rest (Yajima *et al.* 1992; 1994). Tests performed during the 7-day recovery period showed that fluid/electrolyte and body composition values returned to pre-bed rest levels with continued low acceleration tolerance times (Sandler *et al.* 1983)

V. GROUND-BASED EVIDENCE

A. Animal Studies

Just as the ISS has led to a new era of long-duration studies on human subjects, the duration of study for basic cellular and animal experiments has been extended as well. Conducting basic research on model organisms enables scientists to better understand the cellular and molecular workings of the human body.

Large bipedal primates, such as monkeys, can be restrained for days in a head-down tilt position that simulates the long-term effects of weightlessness on the cardiovascular, muscle, and bone function in a similar manner as in humans. However, bed rest cannot be used on quadrupeds, such as mice or rats, because they would still be standing and not immobilized. In these animals, a hind limb unloading model (or tail suspension) is generally used to simulate a fluid shift and hind limb immobilization (Morey-Holton *et al.* 2005). The animal retains some mobility with its front limbs; the bar to which the tail is attached can swivel 360 deg. This model has become a widely used spaceflight analog since it allows chronic unloading of the hind limbs in semi-ambulatory rodents. About 25% of the studies published focus on bone or calcium metabolism and often the SUS model is used as a precursor experiment modality to study the effects of musculoskeletal disuse.

Rodent models are commonly used in biomedical research on Earth due to the major commonalities with humans in physiological responses and genome elements. Genomes are well sequenced and understood, it's easy to create genetically altered strains to mimic human disease processes involving the cardiovascular, musculoskeletal, immune, nervous and other physiological systems. Their short reproductive cycles and lifespan make them well suited to aging and multigenerational research.

1. Hind Limb Suspension

In recent studies, rats were exposed to a single bout of *hind limb suspension* (SUS) for 28 days and tracked for 84 days after return to weight bearing. Other animals recovered for 56 days following an initial SUS and were then exposed to a second SUS plus 56 days of re-ambulation. A third study added exercise during the 56 days of recovery between two SUS exposures. Bone density was measured *in vivo* at 28-day intervals, and bone strength and bone density were assessed by *ex vivo* micro-CT in both the tibia and femur. Proximal femur mass and density of the rat exhibited similar loss and recovery trends as reported for ISS crewmembers (Lang *et al.* 2006). In particular, recovery of density lagged that of mass. However, the femoral neck of the rat did not match the human data as well, and this complicates interpretation of the biomechanical strength testing results.

For the double-SUS study, losses of both mass and density were milder for the second SUS. These results suggest that repeat missions for crewmembers may not be more detrimental as there was no evidence of an exacerbating effect. Adding exercise during recovery between the two SUS bouts enhanced bone mass and density to levels exceeding aging controls but did not alter the reductions due to the second SUS. The net effect remained positive, however, as the absolute values following the second SUS were higher due to the elevations from exercise pre-exposure. Exercise induced vigorous increases in trabecular thickness and mechanical properties. Both histology and serum markers confirmed severely reduced bone formation at the end of the second SUS (Shirazi-Fard *et al.* 2014).

A bone healing study was conducted comparing a five-day spaceflight with synchronous weight bearing and hind limb suspension controls using 7-month old rats. The four rats in each group were given a surgical right hind-limb mid-shaft fibular osteotomy five days before launch. Histologic examinations of all rat groups immediately post-flight showed a callus had formed after 10 days. Chondrogenesis (formation of cartilage) was more advanced in the weight-bearing rats than the other two groups supporting the view that healing was impaired during both SUS and spaceflight (Kirchen 1995).

Recent studies in mice using partial weight-bearing activity have shown proportional declines in bone mass to partial gravity. The authors used a partial weight suspension system, in which a two-point harness is used to offload a tunable amount of body weight while maintaining quadrupedal locomotion. Skeletally mature female mice were exposed to partial weight bearing at 20%, 40%, 70%, or 100% of body weight for 21 days. A hindlimb unloaded (SUS) group was included for comparison in addition to age-matched controls in normal housing. They found that total body and hindlimb bone mineral density, calf muscle mass, trabecular bone volume of the distal femur, and cortical area of the femur midshaft were all linearly related to the degree of unloading (Ellman *et al.* 2013).

In another study mice were subjected to 16% (i.e., simulated lunar gravity) or 33% (i.e., simulated Martian gravity) weight bearing for 21 days. Earth gravity and tail-suspended mice (simulated microgravity) served as controls to compare the effects of simulated lunar and Martian gravity to both Earth and microgravity. Animals experiencing one-third but not one-sixth weight bearing exhibited attenuated deficits in femoral neck mechanical strength associated with 0 g. These results suggest that partial weight bearing (up to 33% of body mass) is not sufficient to protect against bone loss observed with simulated 0 g but does mitigate reductions in soleus mass in skeletally mature female mice (Swift *et al.* 2013).

A variety of countermeasures have been developed for animals, with many being similar to their human counterpart, such as passive centrifugation (in the –Gx direction), standing or walking in 1 g, or head-up tilt. On the other hand, some countermeasures to simulate resistive exercise differ substantially from their human counterpart, such as dropping the animal from 58 cm (Hauschka *et al.* 1987); climbing a 85-deg grid (Herbert *et al.* 1988); mechanical stimulation where the rat leg undergoes external loading (Innman *et al.* 1999); or electrical stimulation to trigger muscle contraction (Haddad *et al.* 2006). The methods used for these ground-based studies are summarized in Table 2.

For example, standing or walking of rats during suspension significantly lessened the soleus type I fiber CSA and soleus peak force. In addition, pure AG or standing maintained the soleus wet weight; however, none of the countermeasures maintained the gastrocnemius wet weight. Most of the rat studies have concentrated on the soleus muscle. While this is advantageous because human studies have mostly analyzed the quadriceps, which results in little overlap, rat studies should also examine effects of countermeasures on the quadriceps so common measures between animal and human can be compared (Kaderka 2010).

2. Centrifugation

Primates, such as Rhesus monkeys, are especially useful because their bipedal nature closely links their physiology with *Homo sapiens*. Belozerova *et al.* (2000) found that pure AG maintained fast-twitch vastus lateralis CSA. In another AG study analyzing Rhesus monkeys, Korolkov *et al.* (2001) found that extracellular fluid, interstitial fluid, and blood flow to the medial gastocnemius was maintained with centrifugation.

Numerous long-duration hypergravity studies have also been performed on animals. Chickens, mice, rats, dogs, rabbits, turtles, snails and many more species have been exposed to continuous hypergravity for weeks, months or even over a year. These studies have provided important information on the adaptation to higher accelerations. The obvious targets were the vestibular and musculoskeletal systems, but the immune and cardiovascular systems also showed clear plasticity to a hypergravity environment.

Study	*Days*	*Animal*	*Intervention*	*AG level (g)*	*Session Duration (min)*	*Number Sessions per day*	*Daily AG Exposure (min)*	*Total AG exposure (%)*
Haddad et al. 2006	6	Rat	SUS + Isometric Exercise	1.0	27	1	27	2.6
D'Aunno et al. 1990	7	Rat	SUS + AG or Standing	1.5-1.6	60-120	1	60-120	4.2-8.3
D'Aunno et al. 1992	7	Rat	SUS + AG or Standing	1.2	15	4	60	4.2
Pierotto et al. 1990	7	Rat	SUS + Walking	1.0	10	4	40	2.8
Hauschka et al. 1988	7	Rat	SUS + Walking	1.0	10	4	40	2.8
Graham et al. 1989b	7	Rat	SUS + Walking	1.0	10	4	40	2.8
Herbert et al 1988	7	Rat	SUS + Climbing	1.0	1.5	4	6	0.4
Widrick et al. 1996	14	Rat	SUS + Standing	1.0	10	4	40	5.5
Zhang et al. 2000	21	Rat	SUS + AG or Standing	1.5-2.6	60-240	1	60-240	4.2-16.7
Korolkov et al. 2001	28	Monkey	BR + AG	1.2-1.6	30-40	1	30-40	2.1-2.8
Zhang et al. 2003	28	Rat	SUS + AG or Standing	1.0-2.6	5-20	1	5-20	0.4-1.4
Zhang et al. 2008	28	Rat	SUS + Standing	1.0	60	1	60	4.2
Sun et al. 2003	28	Rat	SUS + Head-up tilt 45 deg	1.0	120-240	1	120-240	8.3-16.7
Sun et al. 2004	28	Rat	SUS + Standing or Head-up tilt 45 deg	1.0	60-240	1	60-240	4.2-16.7
Thomason et al. 1987	28	Rat	SUS + Standing or Walking	1.0	90-240	1	90-240	6.2-16.7
Graham et al. 1989b	28	Rat	SUS + Walking	1.0	10-90	1	10-90	0.7-6.2
Hauschka et al. 1987	28	Rat	SUS + Drop	2.0	5	1	5	0.4
Fluckey et al. 2002	28	Rat	SUS + Squats	1.0	10	1	10	0.7
Belozerova et al. 2000	30	Monkey	BR + AG	1.2	5-20	1	5-20	0.4-1.4
Inman et al. 1999	42	Rat	Immobilized Leg	1.0	24	1	24	100

Table 2. Summary of AG level, duration, and frequency used during bed rest (BR) or hind limb suspension (SUS) in animals. Total AG exposure is the total time during AG/Standing/Walking/Head-up Tilt relative to the duration of the intervention. Adapted from Kaderka (2010).

The last decade has seen increasing interest in the application of centrifugation in the study of neuroplasticity, metabolism, general animal behavior, and cognitive function. Exposure to hypergravity for long duration increases bone and specific muscle masses, alters the size of the vestibular otoconia, and reduces fat mass in animals. Such an observation provides an important clue to explore sustained hypergravity in humans in light of the current societal issue like obesity and ageing.

Investigators have used centrifugation to study acute responses and chronic adaptation to increased gravity environments (Wade 2005). Responses of pregnant and lactating rats to centrifugation are generally similar to those observed in non-reproducing adult animals, namely, an initial decline in feeding, drinking, body mass, physical activity, and temperature, with a return, following 4-6 days of acclimation, to an approximate 8-15% reduction in body mass relative to controls (Ronca *et al.* 2000). Other studies have shown that body temperature, locomotor activity, and circadian rhythms were altered during adaptation to chronic (14 days) exposure to 1.25 g, 1.5 g, and 2 g (Holley *et al.* 2003).

Behavioral responses of animals have also been investigated under partial-gravity conditions on Earth. In a recent study, rat behavior was monitored by video cameras during parabolic flights generating two cycles of seven partial gravity levels: 0.4 g, 0.3 g, 0.2 g, 0.15 g, 0.1 g, 0.05 g, and 0.01 g. Comparisons were made between partial-gravity levels and the frequency of occurrence of some predominating behavior patterns in rats. Gravity-dependent behavior patterns were observed. In the conditions of 0.4 g through 0.2 g, rats showed startle and crouching. Hind limb stretching emerged at 0.15 g and was more frequently observed toward 0.01 g. The authors of this study noted that different thresholds could exist for emotional and balance/posture-related behaviors (Zeredo 2012).

Growing evidence suggests that dose-response relationships exist across gravity levels exceeding 1 g (Phillips 2002; Wade 2005). Thus, some biological systems respond to increased and decreased gravity with responses that are opposite in directionality. For example, antigravity muscles undergo hypotrophy in weightlessness, but hypertrophy in response to gravitational loading (Vasques *et al.* 1998). A strong ($R^2 = 0.98$) linear relationship has been observed between mammary metabolic activity and gravity loading at 0 g, 1.25 g, 1.5 g, 1.75 g, and 2 g (Plaut *et al.* 2003) (Figure 5). In other cases, responses appear in an “all or none” fashion at and above a certain gravity threshold, with no response observed below that threshold. Still other systems may show the same response to deviations from 1 g in opposite directions. In this case, a biological system that evolved for maximal efficiency at 1 g may show a degraded efficiency at g loads that are sufficiently greater or less than the Earth’s 1 g (Ronca 2007).

For example, results for bone show enhanced mineralization under hypergravity conditions as compared to 1 g, and reduced mineralization under hypogravity conditions. At first glance, this is suggestive of hypergravity being capable of preventing, or even reversing, the detrimental effects of microgravity upon bone, as though there was a continuum: microgravity–normal gravity–hypergravity. Unfortunately, animal studies do not support this simplistic view.

Rittweger (2007) has drawn the following conclusions from past research on the effects of hypergravity on bone in animals:

- Hypergravity effects upon the skeleton are not just opposite to those by microgravity (Smith 1975).
- Hypergravity leads to a reduction in body mass (Wunder *et al.* 1960).
- In rodents, this reduction is related to an inhibition of longitudinal growth. Evidence suggests a crucial role of the growth plate, which may be subject to mechanical damage (Vico *et al.* 1999). This may allude to a failure to cope with hypergravity.
- Conflicting results are observed with respect to bone mass. In rodents it seems to decline with hypergravity, but data in dog suggest an increase (Oyama 1965; 1967; 1975).
- Very interestingly, there seems to be a consistent alteration in long bone geometry, with the shaft assuming a more circular cross section in response to hypergravity exposure (Smith 1975).

In another study, Vico & Gnyubkin (2012) have investigated the skeleton of 7-week mice exposed to 2 g and 3 g for 21 days. At 2 g they found a 5% increase in bone formation and a 3% decrease in bone resorption. These effects were opposite during exposure at 3 g, i.e. a 5% *decrease* in bone formation and a 5% *increase* in bone resorption. These results confirm the need for establishing the curve of dose versus response for several gravity levels as this relationship is not always linear.

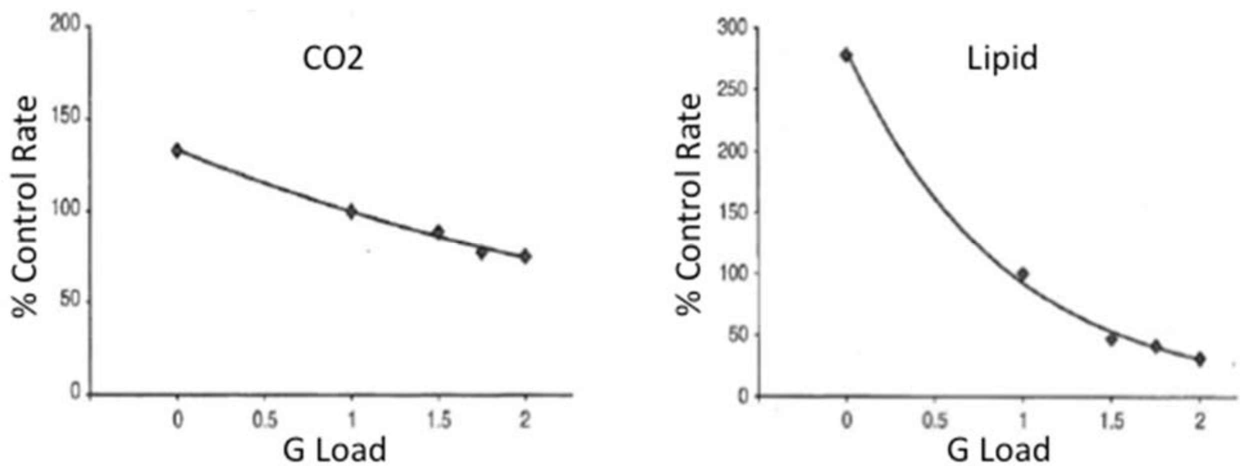

Figure 5. Changes in glucose oxidation into carbon dioxide (left) or incorporation into lipids (right) across gravity levels. Adapted from Ronca (2005) and Plaut et al. (2003).

Walton et al. (2005a; 2005b) demonstrated that the normal 1 g environment is required for the development of motor function. The microgravity environment alters surface righting and swimming behavior in a time-dependent manner. Motor function is permanently altered in rats exposed to microgravity for16 days (P14-P30), but is transient in rats exposed to microgravity for nine days (P15-P24). Different gravitational environments may affect the development of the gravity sensor and the vestibular system; this may, in turn, influence vestibular-mediated motor function. Motor coordination was also found to be impaired in rats born and reared in a 2 g environment and remained impaired even after one week of unloading. These results indicate that hypergravity impaired both the vestibulo-cardiovascular reflex and motor coordination. The vestibulo-cardiovascular reflex was only impaired temporarily and partially recovered following one week of unloading. In contrast, motor coordination did not return to normal in response to unloading (Abe *et al.* 2008).

Exciting new opportunities for long-duration bioculture research on the ISS are emerging, which will be useful for studying the effects of a continuum of gravity from 0 g to 1 g at cellular level. A new bioculture system set to launch soon on the ISS will contain and grow cells, microorganisms and tissues over many months. New experiments will be accommodated that assess the space environment effects on biochemistry physiology, genetics and gene expression of these living systems. “Omics” studies with cells and tissues grown in space can be used for drug discovery, countermeasure analyses and infectious disease processes. Related studies can be conducted on tissue engineering, regeneration and wound healing. The bioculture system is designed so it can be used in conjunction with animal research on the ISS in a new way. Biosamples from flown animals can be transferred onboard to the bioculture system for maintenance and analysis thus avoiding complications from gravity effects associated with animal reentry and landing (Ronca 2014).

Another new opportunity is the *geneLab* research effort, which focus on defining the “physiome” through omics systems biology approaches utilizing cutting-edge omics technologies. These technologies allow the simultaneous examination of multiple, complex changes in DNA, messenger RNA, proteins, metabolites and methylation states. Omics approaches generate vast datasets on the protein, transcript, lipid, and metabolite status of cells, paving the way for new areas of inquiry in genomics, transcriptomics, proteomics, metabolomics, and epigenomics. These exciting new approaches for describing changes in the animal and human physiome are rapidly advancing thereby significantly advancing our perspectives and understanding of biosystems and their functional integration. Next generation deep sequencing machines promise to yield whole genome and organ specific expression profiles at unprecedented speeds, thereby enabling impressive scientific achievements and novel biological applications. The geneLab initiative will allow the science community to input and freely access omics data generated from spaceflight and ground-based experiments (National Research Council 2011).

B. Human Studies

1. Long-Radius Centrifuges

Compared to the static supine position, centrifugation is responsible for an increase in ADH, renin, and cathecholamine secretion, a decrease in diuresis and mineral excretion, and restoration of circulating blood volume to normal. It is also known that changes in the ECG under the influence of gravity levels are linked to an increase in sympathetic tone (Cohen & Brown 1969).

Large-radius human-rated centrifuges are used to simulate the acceleration stress encountered by pilots flying high-performance jets (Offerhaus & Dejong 1967). These centrifuges have usually a radius ranging from 6-12 m, and can generate a centripetal acceleration of up to 30 g at an onset rate ranging from 5-8 g/s, depending on the drive motor used. These centrifuges are primarily used to investigate the physiological effects experienced by a pilot exposed to a rapid onset, high-g environment and to investigate methods to provide the pilot protection thus maintaining his performance in this environment. Other uses include testing equipment designed to provide aircrew g-protection, medical evaluation of flight personnel, training aircrew for improving their tolerance to high-g environment, and acceleration physiology research.

Figure 6 shows the human tolerance to acceleration (time to gray-out) on a long-arm centrifuge. Tolerance to acceleration varies widely depending on body axis, onset rate, and individuals (Gillingham 1987). It also varies from day to day and is modified by body build, muscular tone, gender, and experience. Tolerance can be increased by continued exposure and experience. On the other hand, tolerance to higher gravity levels is decreased as a result of poor health, physical deconditioning, and fatigue (Burton &Whinnery 2002).

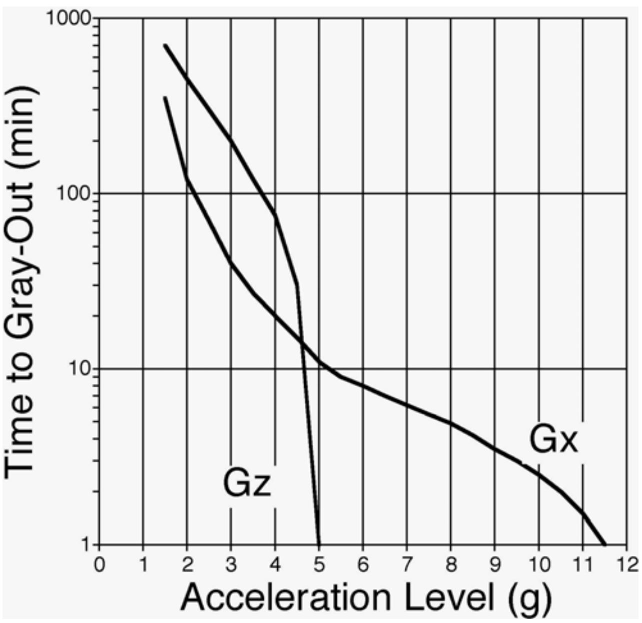

Figure 6. Human tolerance to acceleration on a long-arm centrifuge (Gz: force directed from head to foot; Gx: force directed from chest to back). Time to gray-out is the elapsed time between the start of a constant g level (reached with onset rate <1 g/s) and loss of peripheral (80 deg) vision determined with a standard light array. Adapted from Webb (1964) and Gillingham (1987).

Acceleration tolerance time proves to be a sensitive test for the deconditioning process of the cardiovascular system. This tolerance is measured by exposing subjects to +Gz acceleration levels starting at 2 g and increasing by 0.5-g increments to a gray-out point. This point is determined by peripheral vision loss with a standard light bar and by reverse blood flow in the temporal artery. Tests before bed rest, immediately following, and 5 days later showed that average +Gz tolerance decreases by 67% after bed rest in women (Newsom *et al.* 1977; Greenleaf *et al.* 1977b).

Acceleration tolerance time before and after bed rest varies with the level of physical fitness of the individuals. In a study performed on 20 men during a 10-day bed rest, with centrifuge testing before and after, all subjects showed an increase in heart rate, and a decrease in blood plasma volume during centrifugation. But the 13 men who were aerobically fit had a greater increase in heart rate, and a greater decrease in plasma volume than did the seven “sedentary” men. The seven men who were heavier, had more body fat, and started with lower maximal treadmill oxygen uptake, showed higher orthostatic tolerance after bed rest (Ludwig *et al.* 1987; 1998). Before the bed rest, both subject groups could handle an average of 370 s of 3 g (+Gz) in the centrifuge before they started to gray out. After a week, the physically fit men could only tolerate the acceleration for 111 s. Two of these prime specimens could barely handle the initial rise to 3 g. The overweight crew could go an average of 203 s—almost twice as long—without graying-out (Shulzhenko *et al.* 1979).

Although there is ample evidence of a decrease in tolerance to Gz acceleration after bed rest (Greenleaf *et al.* 1973), little is known about tolerance to acceleration after spaceflight. Russian cosmonauts returning from the ISS have been tested in a centrifuge in the Gagarin Cosmonaut Training Center in the day following landing. The results of these tests are not published yet.

Test subjects' work capacity and tolerance to acceleration on short-arm centrifuges are known to be less than on centrifuges with relatively long arms (Nyberg *et al.* 1966). This is presumably related to the higher gravity gradient in short-radius centrifuges. However, studies revealed that with little training, human tolerance to g levels with short-arm centrifuges could reach that with long-arm centrifuges (Shulzhenko & Vil-Viliams 1992; Vil- Viliams *et al.* 1997). Vil-Viliams (1994) tested subjects at different levels of +Gz acceleration exposure alone at 0.8, 1.2, and 1.6 g on a 2-m centrifuge before and after dry immersion, and combined with exercise. After 7 days of immersion without countermeasures, the mean acceleration tolerance of the subjects was decreased by 28%. When subjects were centrifuged (0.8-1 g) and exercised intermittently during immersion, a restoration of the pre-immersion level of acceleration tolerance was observed.

2. Short-Radius Centrifuges – Continuous Exposure

Extensive tests during continuous exposure to short-radius centrifugation were conducted in the *Naval Aerospace Medical Research Laboratory* (NAMRL) in Pensacola, beginning in 1958. The *Slow Rotating Room* (SRR) had a 5-m-radius with complete living facilities, in which subjects could live for periods ranging from one day to three weeks. Rotation rates ranged from 1 to 10 rpm, with the floor of the SRR staying horizontal. When subjects turned their heads about any axis that was not aligned with the rotation of the environment, they experienced vestibular illusions of rotation. The illusions were approximately proportional in magnitude and direction to the vector product of the angular velocities of the environment and the head. The resulting mismatch between the vestibular and visual senses of motion is believed to be a major cause of motion sickness (Graybiel 1977). The authors stated "*In brief, at 1 rpm even highly susceptible subjects were symptom-free, or nearly so. At 3 rpm subjects experienced symptoms but were not significantly handicapped. At 6 rpm, only subjects with low susceptibility performed well and by the second day were almost free from symptoms. At 10 rpm, however, adaptation presented a challenging but interesting problem. Even pilots without a history of air sickness did not fully adapt in a period of twelve days.*"

Research was also performed to examine the problem of adapting the postural system to a 3-rpm rotation rate. As is the case for motion sickness, the subjects' balance control was initially disrupted on entering the SRR, but recovered within three to four days. Subsequently, most subjects were able to walk on thin rails about as well as in the Earth-normal environment, throw darts, and pour coffee without having to think about motor control. They also performed watch-keeping tasks within normal limits (Guedry *et al.* 1964).

When the SRR was stopped after 12 days, subjects experienced after-effects and erroneous motion sensations during head movements. Their balance control was again disrupted for three to four days. These effects were stronger after runs at 10 rpm than after runs at 3 rpm (Graybiel *et al.* 1965). The investigators concluded from these studies that humans can adapt to rotation rate of 3 rpm and that a 14-day period of rotation at this velocity causes no significant changes in general condition or performance. In contrast, no adaptation took place when subjects were rotated at 10 rpm for 12 days, implying that a 10-rpm rotation rate is close to the upper threshold of endurance.

As a next step, ways of adapting humans to rotation at 10 rpm were investigated through incremental increases in rotation rate over time. Increasing the rotation rate in nine stages of approximately two days each over the course of 16 days mitigated the symptoms of motion sickness and generated less balance problems even at 10 rpm (Graybiel *et al.* 1969). Results also indicated that executing a series of specific head movements could significantly shorten the time needed to adapt. The higher rotation rate, the more difficult the adaptation, but adaptation to 10 rpm was possible as long as the rate-increase increments were held to 1-2 rpm with a period of 12-24 hour at each increment (Guedry 1965; Reason & Graybiel 1970). The time needed for this adaptation might therefore prove to be too long for practical use during spaceflight. However, anti-motion sickness drugs could then be used to attenuate motion sickness while the terminal velocity is more rapidly achieved (Lackner & DiZio 2000b).

Periodic stops of 10 to 15 min were required during the long-duration SRR runs for re-provisioning. Over time, the on-board experimenters who helped in this activity made transitions between the stationary and SRR rotation without experiencing motion sickness or disruptions of movement control. They manifested perfect *dual-adaptation* (Kennedy & Graybiel 1962; Lackner & Graybiel 1982; Cohn *et al.* 2000), thus indicating that is possible to be simultaneously adapted to rotating and non-rotating environments. Furthermore, there was retention of the adaptation to the SRR for several days in all the subjects, which implies that transitions from weightlessness to rotation should be acceptable under certain conditions (Graybiel & Knepton 1972).

The *Institute for Biomedical Problems* (IBMP) in Moscow conducted a major ground-based research program on artificial gravity beginning in the 1960s. Their earliest tests in the *MVK-1* small rotating chamber were executed at speeds up to 6.6 rpm and involved continuous rotation of one or two subjects for up to a week. The *MVK-1* was followed up by the roomier 10-m radius *Orbita* centrifuge, capable of rotating two to three people for several weeks at speeds up to 12 rpm. The longest tests executed during this program were for 25 days at 6 rpm.

The initial rotation exposures produced the expected disturbances in dizziness, equilibrium, and coordination. Within an hour, the usual pattern of motion sickness symptoms occurred, including vomiting in some cases. In four to five hours, subjects also complained of drowsiness and headache. Three periods of vestibular adaptation were distinguished for these long-duration exposures. The first one or two days were characterized by severe motion sickness. This was followed by a week during which the nausea and related acute symptoms disappeared, but drowsiness and headache remained. Finally, after the first 7 to 10 days, subjects showed immunity to motion sickness, even when additional vestibular stimulation was provided.

As found in Graybiel's SRR studies in Pensacola, the severity of motion sickness symptoms and the time to adapt to prolonged rotation on the Russian small rotating room *MVK-1* were related mostly to rotation rate. There was an absence of any motion sickness symptoms at 1 rpm, moderate symptoms at 1.8 rpm, and marked symptoms at 3.5 rpm. On the larger *Orbita* centrifuge, however, symptoms appeared only above 1.8 rpm. Head movements brought on discomfort in all cases (Kotovskaya *et al.* 1981). The authors also report the following: "cardiovascular function remained within normal limits, […] no significant sleep disturbances were noted in the long-rotation environment, […] all assignments were completed even in the presence of pronounced illness, and no decline was noted in short-term verbal memory" (Shipov 1977).

Experiments were later conducted at IBMP by exposing humans to continuous rotation for up to one month using a 7.25-m centrifuge. In the first series of studies (Jupiter-1, 1979-1986), the SRR rotation rate was 15.3 rpm and the floor of the cabin was titled relative to the horizontal so that the resulting GIF was along the subject longitudinal axis. In the second series of studies (Jupiter-2, 1989 to 1992) the SRR rotation rate ranged from 6 to 9 rpm. Despite the occurrence of motion sickness, the main conclusion of these studies was that "*it is possible for humans to live in a rotating environment at a constant rotation rate*" (Orlov & Koloteva 2014).

Much of the research into the human factors of rotating habitats is thirty or forty years old. Over the past four decades, several authors have published guidelines for comfort in artificial gravity, including graphs of the hypothetical "*comfort zone*" bounded by values of gravity level, rotation rate, and head-to-foot gravity gradient (Thompson 1965; Stone 1970; Hall 1997; Young 1999) (Figure 7).

These experiments employing long-radius centrifugation suggest that all of the undesirable sensations are proportional to the rotation rate. Almost all subjects can adapt quickly to work in a 3-rpm rotating environment. With higher rotation rates, however, the subjects will all experience symptoms of motion sickness and disturbances in postural equilibrium, the extent of which are a function of the rotation rate. Nevertheless, adaptation can be achieved under these conditions in six to eight days, and the remainder of the stay in a rotating environment is characterized by normal health and performance.

However, the recommended limits for rotational environment are often discordant across studies. For example, Clark & Hardy (1960) concluded that the rotation rate of a space station providing AG should not exceed about 0.1 rpm to stay completely below the threshold of vestibular illusions and nausea due to Coriolis forces and cross-coupled angular accelerations when moving the head. At 0.1 rpm, a 1-g spinning station would require a radius of approximately 90 kilometers! Later, Stone (1970) assumed acceptable cross-coupled angular acceleration for up to three times the nausea threshold predicted by Clark & Hardy (1960), giving a maximum station rotation rate of 6 rpm. This is 60 times the maximum rotation rate proposed by Clark & Hardy (1960) and brings the radius of a 1-g station down to only 25 meters. However, this solution could still only be achieved by a large spinning station or by a tether connecting two sections of a spaceship, possibly a habitat module and a counterweight consisting of every other part of the spacecraft. Recent data indicate that these earlier limits on rotation rate for eliciting Coriolis motion sickness are overly conservative (Diamandis 1987). For example, Young *et al.* (2001) have recently shown that subjects can quickly adapt to motion sickness induced by rotation of the head during centrifugation at 23 rpm. Higher rotation rates permit a shorter radius to obtain a specified gravity level, and make it possible the use of embarked short-radius centrifuges.

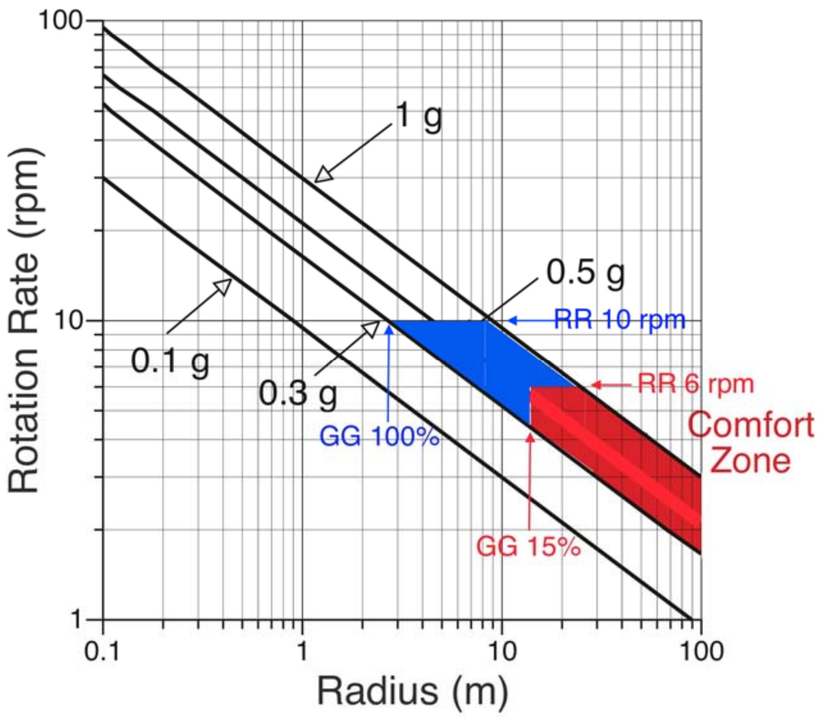

Figure 7. The rotation rate is plotted as a function of the radius of rotation for four gravity levels. Slow rotation rooms studies performed in the 1960s referred to the "comfort zone" as the red area delimited by gravity levels ranging from 0.3 g to 1 g, rotation rate < 6 rpm, and gravity gradient GG < 15% (radius > 12 m). However, recent data indicate that these limits are overly conservative: rotation rates of up to 10 rpm can be tolerated with incremental exposures, and large gravity gradient during intermittent short-radius centrifugation does not seem to be a critical factor (blue area).

3. Short-Radius Centrifuges – Intermittent Exposure

a. Rationale

Ground-based studies investigating AG as an intermittent countermeasure in humans have either had subjects passively ride the centrifuge or have coupled centrifugation with exercises such as cycling. In addition to centrifugation *per se*, some investigations have used standing or walking (in 1 g) to simulate the AG countermeasure.

In the past few years, dedicated centrifuges for investigating the effects on centrifugation on physiological deconditioning during bed rest studies have been developed in the US, Europe, Russia, and Japan (Table 3). The objective of these studies is to place test subjects in a 6-deg head-down bed-rest position for duration lasting up to 60 days, which simulates the long-term effects of weightlessness on the cardiovascular, muscle, and bone function. Typically, one group of test subjects is placed in the supine position on these ~3-m-radius centrifuges and subjected to various gravity levels and duration along their longitudinal axis throughout the bed rest to periodically simulate a +Gz gravitational environment. Another group of subjects is not exposed to centrifugation. After the bed rest, comparison between the deconditioning of both subject groups allows to determine the effectiveness of centrifugation as a countermeasure. These studies help to develop appropriate prescriptions for using a centrifuge to protect crews and to understand the side effects of artificial gravity. The baroreflex regulation, blood pressure, and venous tone, especially in the legs, are the principal cardiovascular reactions of interest for centrifugation studies during bed rest (Zander *et al.* 2013).

Immersion in water has also been used to simulate physiological deconditioning as water offloads much of the effect of gravity. The Russians have had vast experience with this method and first utilized wet immersion, in which the subject is submerged to the neck, as a deconditioning measure (Vil-Viliams 1980). However, subjects cannot stay submerged in water for extended periods of time and this method was only used for very short studies on the order of a few days or less. Water immersion is performed with the subjects in the upright-seated posture with the water level up to the neck, whereby the hydrostatic water pressure upon the body counteracts the intravascular hydrostatic pressure gradients thus resembling what is expected to occur during weightlessness (Norsk 1992). The reference control posture outside the water is also upright seated. Because the reference control posture in bed rest studies is horizontal supine (Pavy-Le Traon *et al*. 2007), there is often confusion when comparing the effects of the two models.

In both simulation models, blood and fluid shifts occur, however, with larger shifts to the intra-thoracic vessels during water immersion as indicated by larger increases in central venous pressure and cardiac output (Shiraishi *et al.* 2002). The shifts to the regions above the thorax, however, are more pronounced during head-down bed rest, because during water immersion the head is still in a vertical position outside the water so that the hydrostatic Gz-pressure gradients persist, whereas they are much less during head-down bed rest. There are also differences between the cardiovascular effects of water immersion and head-down bed rest because water immersion induces a negative pressure breathing effect (Gabrielsen *et al.* 1993). This negative pressure breathing effect is caused by the pressure of the water around the chest, which is higher than the atmospheric breathing pressure adding further to the increased venous return. Mean arterial pressure decreases acutely during head-down bed rest compared to an upright-seated control position, whereas this is not the case during water immersion (Norsk 2014).

A suitable modification to wet immersion for longer duration studies is dry immersion in which the subjects are immersed in water to the neck in a reclining, semi-supine position separated from the water by a sheet to avoid water contact with the skin but still maintaining the hydrostatic effects on the body's fluid distribution (Navasiolava *et al.* 2011). Nevertheless, the major limitation of the bed rest and immersion models is that the gravitational stress still persists. Other limitations are that it is not even known today, how accurate the models are, because it has not been possible to make direct comparisons in the same subjects during same operational conditions between the acute and more chronic effects of weightlessness in space with those of the simulation models (Norsk 2014).

Name	Location	Radius	Max g	Axes
Unilateral Centrifuge	Antwerp U, B	0.4 m	0.2 g	±Gy
Unilateral Centrifuge	Charite Campus, Berlin, D	0.4 m	0.2 g	±Gy
NASA JSC centrifuge	NASA, Houston, USA	0.5 m	1.0 g	–Gz
ESA Neurolab Off-Axis Rotator	Antwerp U, B	1.0 m	1.0 g	±Gy, –Gz
Short-Radius Centrifuge	Mt Sinai School of Med, New York, USA	1.0 m	1.0 g	±Gx, ±Gy
Human Centrifuge	Brandeis U, Waltham, USA	1.2 m	3.0 g	±Gy
Short-Arm Human Centrifuge	Nihon U, Nishi-Funabashi, Japan	1.8 m	3.0 g	+Gz
Centrifuge-Induced Artificial Gravity	Nagoya University, Japan	1.8 m	3.0 g	+Gz
Short-Radius Human Centrifuge	CNRS, Marseille, F	2.0 m	3.0 g	+Gz
Short-Radius Human Centrifuge	Aichi Medical U, Japan	2.0 m	5.7 g	+Gz
NASA Human Performance Centrifuge	NASA Ames, Moffet Field, USA	2.0 m	5.0 g	+Gz
Space Cycle	UC Davis, USA	2.0 m	3.0 g	+Gz
Artificial Gravity Sleeper	MIT Cambridge, USA	2.5 m	3.0 g	+Gz
Short-Radius Centrifuge	IBMP, Moscow, Russia	2.5 m	5.0 g	+Gz
ESA Short-Arm Human Centrifuge (SAHC)	MEDES, Toulouse F DLR, Cologne, F	2.8 m	3.5 g	+Gz
Twin-Bike System	University Udine, I	3.0 m	1.0 g	+Gz
NASA IMAG Short-Arm Centrifuge	UTMB, Galveston, USA	3.0 m	3.5 g	+Gz
DLR :envifuge	DLR Cologne, D	3.8 m	6.0 g	+Gz
TNO Desdemona	Soesterberg, NL	4.0 m	3.3 g	±Gx, ±Gy, ±Gz
Slow Rotation Room	Brandeis U, Waltham, USA	6.7 m	4.0 g	±Gx, ±Gy, ±Gz
Slow Rotation Room	NAMRL, Pensacola, USA	7.0 m	3.0 g	±Gx, ±Gy, ±Gz
Slow Rotation Room	NASA Ames, Moffet Field, USA	7.9 m	2.0 g	±Gx, ±Gy, ±Gz
Long Radius Centrifuge (ESA access)	Karolinska Institut, Stockholm, S	7.2 m	9.0 g	+Gz
NASA 20 g Centrifuge	NASA Ames, Moffet Field, USA	8.8 m	20.0 g	+Gz
Long Radius Centrifuge	IBMP, Moscow, R	9.0 m	30.0 g	+ Gz

Table 3. Centrifuge facilities used worldwide in research projects on artificial gravity, with their radius, the maximum gravity level (usually at the subject's feet) and the direction in which this level is exerted. This list is not exhaustive.

Bed rest and water immersion have been shown to affect the cardiovascular system, muscle and bone structure and strength, and, to a lesser degree sensorimotor performance. One of the most persistently measured parameters to gauge cardiovascular deconditioning and the efficacy of countermeasures is orthostatic tolerance time under cardiac stress, i.e. an integrated performance measure that depends on hydration status, vascular status, and the integrity of the sympathetic nervous system. Orthostatic tolerance is measured through a test that stresses the cardiovascular system. This test usually takes the form of a head-up tilt to 60-80 deg from a supine starting position. However, Russian and Japanese investigators have used a +3Gz (measured at the feet) overload to provide cardiac stress, while other investigators have used a graded LBNP test as the stressor. These tests are terminated when either the subject reaches a preset time limit or the subject undergoes pre-syncope. Pre-syncope itself is defined in a variety of manners, though the most common definition is any occurrence of the following: sudden drop in heart rate (>15 beats per min), systolic blood pressure >25 mm Hg or diastolic blood pressure > 15 mm Hg, sweating, nausea, or clammy skin. Orthostatic tolerance time is the measured by the time elapsed in the stress test (Kaderka 2010).

Cardiac function is measured through an *electrocardiogram* (EKG) analysis. Stroke volume is calculated as the product of the aorta *cross sectional area* (CSA) and the integral of the beat-to-beat aortic outflow (measured by ultrasound). Cardiac output is generally calculated as the product of the stroke volume and heart rate. *Total peripheral resistance* (TPR), i.e. the sum of resistances in the systemic vasculature, is calculated as the mean arterial pressure divided by the cardiac output. The degree of change in TPR with cardiovascular stress is indicative of the baroreflex function (Kaderka 2010).

Aerobic capacity is usually measured by a graded exercise test on a cycle ergometer or treadmill until the subject underwent volitional fatigue. Total exercise time (i.e. a measure of endurance) is the total elapsed time of the exercise test. Gaseous exchange studies include measurements of the *rate of oxygen uptake per minute* (VO2), the *volume of air inhaled in one minute* (VE) or minute ventilation, and the ratio between exhaled CO2 and inhaled O2 (*Respiratory Exchange Ratio*, RER). Several degrees of these measurements are made with the exercise test, including a resting measurement, possible submaximal measurements, and maximum measurements (the maximum measurements usually occur close to volitional fatigue) (Kaderka 2010).

Blood samples drawn from subjects provide the means to measure hematocrit, i.e., the fraction of packed erythrocyte volume of blood. Blood assays are performed to measure catecholamines, such as epinephrine and norepinephrine. Plasma volume – the liquid component of blood – is estimated through the carbon monoxide rebreathing method.

A relatively new technique to measure the autonomic nervous system is through heart rate variability. A frequency analysis of heart rate variability divides the response into low frequency (LF) and high frequency (HF). The high frequency (0.15-0.04 Hz) is indicative of parasympathetic activity, while the low frequency is affected by both sympathetic and parasympathetic activity. The ratio LF/HF reveals the sympathetic activity alone (Kaderka 2010).

The primary concern with muscle deconditioning lies with the antigravity muscles (Trappe *et al.* 2009). Therefore, measurements in experiments are almost exclusively performed on the soleus, gastrocnemius, and quadriceps (Spector *et al.* 2009). Non-invasive techniques include the use of dynamometers to measure muscle strength and *maximum voluntary contraction* (MVC) data. *Magnetic resonance imaging* (MRI) is also used to determine the muscle CSA as well as the whole muscle volume. Invasive techniques include muscle biopsies to determine muscle fiber characteristics. In many studies, the biopsy is stained to differentiate between type I, type IIa, and type IIx fibers. From these biopsies are obtained fiber CSA (or diameter), fiber type ratios, and fiber performance characteristics such as fiber shortening velocity, and peak force.

Measurements for changes in bone density are using include *Dual energy X-ray absorptiometry* (DEXA) scans, and *peripheral quantitative computed tomography* (pQCT), which can make volumetric measurements and distinguish between cortical and trabecular bone. Measurements are usually performed on the weight-bearing bones, with special attention given to areas of the hip such as the femoral neck and greater trochanter. Other sights of interest are the calcaneus, lumbar spine, and tibia. Bone can also be assessed through an array of bone markers found in blood serum and urine, although they tend to be extremely variable depending on the time of day the sample is taken. To reduce variability as much as possible, a 24-hour sampling period is performed. One downside of bone marker measurements is that they represent the entire skeletal homeostasis and are not capable of translating site-specific information. Bone markers can represent either osteoblast or osteoclast activity (Kaderka 2010).

b. Centrifugation during Bed Rest Studies

A lot of the excellent material published by Justin Kaderka (2010) in his MSc thesis in Aeronautics and Astronautics at MIT is used in this section. The studies and the methods used for testing the effects of AG during bed rest or immersion studies are summarized in Table 4.

In a pioneering study, Vernikos *et al.* (1996) demonstrated that intermittent (real) gravity loading could effectively reduce the deconditioning associated with prolonged bed rest in healthy human males. She showed that intermittent standing or controlled walking during otherwise continuous bed rest prevented post-bed rest orthostatic intolerance and attenuated decrements in peak oxygen uptake, plasma volume, and urinary calcium excretion.

Other studies found that intermittent centrifugation supplemented or not with concurrent aerobic exercise during bed rest could completely protect respiratory and cardiovascular responses to upright exercise, improve orthostatic tolerance time, suppress plasma volume loss, prevent fluid volume shifts, and reduce the elevated heart rate, muscle sympathetic nerve activity, and exaggerated responses to head-up tilt after bed rest. The results of these studies are summarized thereafter.

Orthostatic Tolerance Time

White *et al.* (1965) showed that intermittent exposure to 1 g or 4 g on a 1.8-m-radius centrifuge was effective in alleviating orthostatic intolerance. Exercise produced little additional benefit. A series of experiments using intermittent centrifugation with a radius of 1.7 m during bed rest were conducted at IBMP from 1973 to 1979. Results showed that intermittent AG ranging from 1- 2 g was protective for what was described as "gravitation stability" (orthostatic intolerance) in combination with exercise and water-salt supplements (Orlov & Koloteva 2014).

Similar studies by Iwase (2005), Watenpaugh *et al.* (2007), Guinet *et al.* (2009), Schneider *et al.* (2002) and Shibata *et al.* (2010) also found that centrifugation was successful at either preventing the degradation of orthostatic tolerance time or increasing tolerance time from baseline following bed rest. In all these studies, most of the traditional countermeasures (resistive exercise, aerobic exercise, lower body negative pressure, or some variation of these) did not protect orthostatic tolerance (Zhang 2001).

Results of very recent studies on human subjects in the ESA *Short-Arm Human Centrifuge* (SAHC) at MEDES in Toulouse have shown that during 5 days of bed rest, 5 x 6 min of centrifugation per day with 1 g at the center of body mass interrupted by 3-min intervals protects better against orthostatic intolerance and was better tolerated by the subjects than 30-min continuous centrifugation treatment per day (Linnarsson *et al.* 2015).

It was debated for quite some time as to whether intermittent centrifugation conditioned only the passive motor tone or whether the active baroreflex that counters the effects of gravity on blood pressure, was also affected. Burton & Meeker (1992) used a 1.5-m-radius centrifuge intermittently to show that the baroreceptors are adequately stimulated by the centrifugal force. Their slow compensation for the hydrostatic pressure drop during rotation permits the tolerance to gradual onset acceleration to exceed that to rapid onset acceleration.

Blood Volume

Beyond the benefits derived from intermittent acceleration on cardiovascular responses, positive effects on blood volume were also seen. Normally, weightlessness or head-down bed rest produces a fluid shift toward the head that in turn leads to fluid loss, including plasma, and a resulting increase in hematocrit. Plasma volume is not a direct indicator of orthostatic intolerance, though it might serve as a triggering mechanism for further dysfunction (Platts *et al.* 2009).

Plasma volume seems to be maintained with an exercising countermeasure. However, the results are sometime conflicting. For example, the cycling countermeasure of Greenleaf *et al.* (1989) prevented a decrease in plasma volume during bed rest while a similar exercise regime of Shibata *et al.* (2010) did not prevent the decrease. The treatment group in the Greenleaf study had two bouts of 30 min of cycling for five days per week at an intensity that varied from 40% to 90% of their pre-bed rest VO2 max. On the other hand, the treatment group of the Shibata study had three bouts of 30 min of cycling seven days per week at a constant intensity of 75% of their pre-bed rest maximum heart rate. Even though the treatment was of longer duration in the Shibata study, the higher intensity exercise of subjects in the Greenleaf study may be the reason plasma volume was maintained (Kaderka 2010).

Study	***Days***	***Intervention***	***AG level (Feet)***	***AG level (Heart)***	***Session Duration (min)***	***Nb of Sessions per day***	***Daily AG Exposure (min)***	***Total AG Exposure (%)***
Shulzhenko et al. 1979	3	Wet Immersion + AG	1.6		40	3	120	8.3
Vil-Viliams et al. 1980	3	Dry Immersion + AG	1.6		40	3	120	8.3
Vernikos et al. 1996	4	BR + Walking	1.0	1.0	15	8, 16	120, 240	3.3-6.7
Yajima et al. 1994	4	BR + AG		2.0	60	1	60	4.2
Iwasaki et al. 2001	4	BR + AG		2.0	30	2	60	4.2
Sasaki et al. 1999	4	BR + AG		2.0	30	2	60	4.2
Lee et al. 1997	5	BR + Running	1.0	1.0	30	1	30	2.1
Linnarsson et al. 2015	5	BR + AG		1.0	30, 5	1, 6	30	2.1
Mulder al. 2014	5	BR + Walking		1.0	30, 5	1, 6	30	2.1
White et al. 1965	13	BR + AG		1.0-4.0	7.5-11.2	4	30-45	2.1-3.1
Grigiriev et al. 1979	13	Dry Immersion + AG	0.6-2.0		60-90	1	60-90	4.2-6.2
Iwasaki et al. 2005	14	BR + AG + Cycling		1.2	30	1	30	2.1
Iwase 2005	14	BR + AG + Cycling		1.2	30	1	30	2.1
Katayama et al. 2005	20	BR + AG + Cycling	1.0-5.0	0.3-1.4	40	1	40	2.8
Akima et al. 2005	20	BR + AG + Cycling	1.0-5.0	0.3-1.4	40	1	40	2.8
Paloski & Young 2009	21	BR + AG	2.5	1.0	60	1	60	4.2
Vil-Viliams & Shulzenkho 1980	28	Dry Immersion + AG + Cycling	0.8-1.6		60-90	1	60-90	4.2-6.2
Vil-Viliams 1992	28	Dry Immersion + AG + Cycling		1.2-1.9	120	1	120	8.3

Table 4. Summary of AG level, duration, and frequency used during bed rest (BR) or dry/wet immersion studies in humans. Total AG exposure is the total time while during AG/Walking/Running relative to the duration of the intervention. Adapted from Clément & Pavy-Le Traon (2004) & Kaderka (2010).

Nevertheless, centrifugation alone was not found to be effective at counteracting the loss of plasma volume. It's only when centrifugation was coupled with more than 30 min of cycling that is proved effective in maintaining plasma volume (Iwasaki *et al.* 1998, 2001, 2005; Vernikos *et al.* 1996; Lee *et al.* 1997).

Total peripheral resistance, a measure of vascular status, is known to increase after bed rest due to increased vasoconstriction because the heart tries to maintain systemic blood pressure with lower plasma volume. Exercise is usually not effective at maintaining TPR, except when cycling is combined with Dextran (Shibata *et al.* 2010). Dextran is administered at the end of the bed-rest as a fluid loading measure. The AG analog of standing for two hours was an effective countermeasure in preserving CSA of the anterior tibial artery and CSA of its media thickness (Kaderka 2010).

Spectral analysis of high frequency heart rate also indicated that centrifugation during bed rest prevented the decrease in parasympathetic activity. However, the increase in sympathetic activity, or HF/LF heart rate was only prevented with centrifugation coupled with cycling (Iwasaki *et al.* 2005). The baroreflex is a sympathetic response that is indirectly assessed with the orthostatic tolerance time. Only centrifugation or LBNP are capable of stimulating the baroreflex in deconditioned individuals and thus maintaining its effectiveness.

An important function of the vestibular system is the *otolith-sympathetic reflex* (OSR) that is enacted by otolith stimulation (Yates & Miller 1994). In humans the OSR has been found to be a mediator of cardiovascular stress (Sauder *et al.* 2008) and to even have shorter response latency than the baroreflex (Kaufman *et al.* 2002). Voustianiouk *et al.* (2006) have suggested that the OSR could play a vital role in maintaining astronaut orthostatic intolerance. Little is known about the OSR during space flight. AG may be a suitable countermeasure to protect the integrity of the OSR. Anecdotal evidence of the efficacy of AG is given in a study that analyzed the hemodynamic responses from the four Neurolab mission astronauts who had undergone centrifugation against the two astronauts who had not (Moore *et al.* 2005). This study found that one of the non-centrifuged astronauts exhibited signs that were indicative of orthostatic intolerance, which suggests that centrifugation of the OSR and baroreflex was a successful countermeasure. Although this evidence is extremely circumstantial, it nevertheless represents the only space-based evidence that AG in orbit assisted in mitigating orthostatic intolerance after space flight.

Exercise Capacity

Exercise capacity is the second facet of the cardiovascular system that must be maintained. A meta-analysis of bed rest studies using centrifugation and traditional countermeasures has shown that centrifugation is as effective as traditional countermeasures in measurements of VO2 max, minute ventilation, and exercise time to exhaustion. Specifically, both centrifugation only and centrifugation coupled with cycling were effective at maintaining the former two measurements, while only centrifugation coupled with cycling studies have measured exercise time to exhaustion and have consequently maintained this parameter (Kaderka 2010).

The interaction between the cardiovascular fitness enhancement of regular exercise and the tolerance built up during centrifugation has also been studied. For example, Katayama *et al.* (2004) showed that cardiovascular fitness could be protected by intermittent AG exposure in individuals exposed to 20 days of head down bed rest.

Muscle & Bone

Only a couple of bed rest studies have examined AG as a countermeasure to human skeletal muscle deconditioning. Caiozzo *et al.* (2009) showed that AG alone significantly attenuated the decrease in soleus CSA, possibly because of ad lib calf presses that subjects were instructed to perform to maintain the muscle pump during centrifugation. No similar benefit was observed for the other muscles, as they did not degrade significantly from before the bed rest (Adams *et al.* 2003). It is important to note that most of the traditional bed rest studies that examine muscle are of a longer duration (35-119 days) than the AG studies to date (Bamman *et al.* 1998). Because of the slow changes in muscle structure and strength, potential benefit from AG require longer duration studies, especially with regards to knee extensor muscle volume and MVC.

In a recent study aimed at evaluating the suitability of intermittent short-radius centrifugation as countermeasure against musculoskeletal deconditioning, 11 healthy male subjects participated in three campaigns of head down tilt bed rest for 5 days, with preceding baseline data collection and recovery phases. Bed rest without AG was used as control condition, and the interventions included AG with 1 g at the center of mass applied for 30 min continuously or for 6 bouts of 5 min. This 5-day bed rest format yielded succinct catabolic effects upon muscle and bone metabolism that were not prevented by AG. Bone resorption markers (CTX, NTX and DPD) revealed increases by approximately 25% towards the end of bed rest, and nitrogen balance was negative by approximately –3 g/day without any protection by AG (Kos *et al.* 2013). However, a decrease in vertical jump height after bed rest with no countermeasure was prevented by either of the AG protocols. The preservation of vertical jump performance by AG is likely to be caused by central nervous rather than by peripheral musculoskeletal effects (Rittweger *et al.* 2014).

No bed rest studies combined with centrifugation have examined the structural integrity of muscle fibers (i.e. CSA and distribution by fiber type) after deconditioning while this has been performed in many of the traditional countermeasure studies. These measurements can be used to assess the degree of slow-to-fast fiber transition as well as peak force and shortening velocity of the fiber. Future AG studies should analyze global muscle parameters (e.g. muscle volume, MVC, endurance, etc.) as well as individual muscle fibers by fiber type in order to better understand any salutary effects of centrifugation on skeletal muscle.

Like the skeletal muscle studies, AG bed rest studies that analyze bone are very few in number. Smith *et al.* (2009) performed the only comprehensive AG bone study and they did not found significant changes in bone mineral density. However, this AG study was only 21-day long whereas the traditional countermeasure studies were of much longer duration, e.g. 30 days (Zwart *et al.* 2007) to 117 days (Shackelford *et al.* 2004).

Urinary and serum calcium are two markers of calcium balance that were commonly studied in these bed rest studies. Dietary calcium is generally fixed at 1 ± 0.1 g/day during bed rest studies.

Shackelford *et al.* (2004) showed that a daily vitamin pill, which contained a 400IU vitamin D dose, had additional beneficial effects on urinary and serum calcium levels when coupled with exercise. Based on the WISE-2005 study performed on female subjects, a nutritional countermeasure alone was not found to be effective in protecting the cardiovascular or musculoskeletal deconditioning (Guinet *et al*. 2009; Trappe *et al.* 2004; 2007a; 2007b; 2008; Smith et al. 2003; 2008).

Traditional resistive exercise countermeasures use high loading of the lower skeleton through the use of supine squats (Bamman *et al.* 1998). No comparable loads were attained in the AG studies combined with exercise so far. Proof-of-concept studies without human deconditioning have been performed that compare squats during centrifugation to upright squats and have found that squats during centrifugation can provide high foot forces that are analogous to squats in 1g (Yang *et al*. 2007a). In addition to providing high loading for bone, AG squats might also be beneficial for the skeletal muscle as previously discussed.

Unfortunately, due to the shortage of AG bed rest studies that have examined muscle and bone and due to the relatively short duration of those AG studies, few conclusions can be made regarding its efficacy as a countermeasure for muscle and bone deconditioning.

Sensorimotor Performance

It has been long known that after prolonged bed rest, subjects exhibit some dysfunctions in their vestibular responses, such as in postural instability during standing, and gait changes (Pavy-Le Traon *et al.* 2007). A study performed at *University of Texas Medical Branch* (UTMB) analyzed the neurovestibular effects following bed rest and centrifugation. It was found that centrifugation did not impact balance control or ocular counter-rolling compared to the control group (Jarchow & Young 2010). In the same study, error in subjective visual vertical was significantly different from zero in the treatment group and not different in the control group; however, this effect was short-lived (Moore *et al.* 2010).

Investigators also tested whether intermittent standing or a combination of heel raising, squatting and hopping exercises was sufficient to prevent alteration in balance and gait following a 5-day bed rest. A cross-over design study was performed with 10 male subjects during 6-deg head down tilt: (a) with no countermeasure; (b) while standing 25 min per day; (c) during locomotion-like activities 25 min per day. Gait was evaluated by grading subjects' performance during various locomotion tasks. Equilibrium scores were derived from peak-to-peak anterior-posterior sway while standing on a foam pad with the eyes open or closed or while making pitch head movements. When no countermeasure was used, head movements led to decreased postural stability and increased incidence of falls immediately after bed rest compared to before. When upright standing or locomotion-like exercises were used, postural stability and the incidence of falls were not significantly different after the bed rest from the baseline. These results indicate that daily 25-min of standing or locomotion-like exercise proves useful against postural instability following a 5-day bed rest. The efficacy of these countermeasures on locomotion could not be evaluated, however, because gait was not found to be altered after a 5-day bed rest (Mulder *et al.* 2014).

Integrative Physiology

In 2006-2007 a comprehensive study was conducted at UTMB as part of the *International Multidisciplinary Artificial Gravity* (IMAG) project to investigate the effects of intermittent short-radius centrifugation during a 21-day bed rest. Several physiological systems were evaluated (bone, muscle, cardiovascular, central nervous, immune, and metabolic) and psychological and clinical assessments were performed as well. Head-down bed rest was done at –6 deg and AG was provided in –6 deg head-down supine participants for 1 hour daily, with 1 g at the heart and 2.5 g at the feet along the Gz direction (Warren *et al.* 2007).

The IMAG study was the first of its kind to study the effects of centrifugation across all physiological systems. The results showed that bone density, muscle mass and strength, orthostatic tolerance, aerobic capacity, sympathetic nervous responses and proprioceptive reflexes were improved by centrifugation compared to bed rest subjects, who had not undergone centrifugation (Figure 8). Some systems, however, were not protected by centrifugation, in that the immune systems did not respond to either bed rest or centrifugation and there were no effects on cognitive performance. Even though centrifugation protected against bone mineral density, bone homeostasis was still changed by bed rest and centrifugation, which could indicate insufficient loading effects of the daily 1-hour centrifugation procedure (Young & Paloski 2007).

The test subjects in the IMAG study were all males, so it was recommended to repeat this study with females and over longer deconditioning periods. Other recommendations for future studies included increasing the number of test subjects and adjusting the prescription parameters (gravity level, rotation rate, radius, duration, frequency) for optimal effectiveness and/or efficiency, and adding exercise capability (Young & Paloski 2007). Other physiological responses that need to be studied are the changes in circadian rhythms (Fuller *et al.* 1996).

	main hypotheses*	**finding**	**comment**
Bone	↔ bone mineral density ↑ bone homeostasis	as expected not supported	short duration insufficient loading?
Muscle	↑ strength ↑ fiber-type homeostasis ↓ muscle atrophy	supported supported supported	
Cardio	↑ orthostatic tolerance ↑ sympathetic response ↑ aerobic capacity	supported supported supported	
Neuro	↔ CDP, OCR ↔ SVV ↑ proprioceptive reflexes	as expected not supported supported	no adverse response spatial disorientation?
Immuno	↑ stress marker response	not supported	no Δ either group
Psych	↔ cognitive performance	supported?	↓ trend, but low n

Figure 8. Main results of the IMAG study on effects of short-radius AG during 21 days of head-down bed rest in male subjects. CDP: computerized dynamic posturography. OCR: ocular counter rolling. SVV: subjective visual vertical (Paloski 2014b).

4. Human Powered Short-Radius Centrifuges

The rationale behind a human powered centrifuge is that exercising while being centrifuged may further reduce the daily minimum exercise time needed for counteracting muscle deconditioning (Chou *et al.* 1998; Greenleaf *et al.* 1999). This approach is similar to that of another countermeasure used by the Russians, the so-called "*Penguin suit*", a suit that does not provide any g force, but has internal bungee cords that provide passive stress on antigravity muscle groups. This constant loading provides partial compensation for the absence of gravity by opposing movement, and simulates the terrestrial load on muscles of the legs and trunk (Kozlovskaya *et al.* 1995).

Only recently have several research groups begun to explore the potential benefits of artificial gravity generated by a human powered centrifuge. With respect to skeletal muscle, data suggest that muscles must be mechanically loaded to maintain or increase muscle mass. Similarly, mechanical loading (e.g., microstrain) of bone is essential for maintaining or increasing bone density (see Clément 2011 for review). Given these perspectives, several authors suggest that passive centrifugation on a short-radius centrifuge will not be effective in maintaining skeletal muscle mass and bone density during long exposures to microgravity. Hence, as a complement for passive centrifugation, they have pursued the development of active centrifugation, where the subjects exercise while being centrifuged, as potential multipurpose countermeasures to microgravity. This system has the capacity for studying the effects of centrifugation on muscle mass, bone density, and orthostatic tolerance.

Currently, two general types of ground-based designs have been described in the literature. The first of these designs has been referred to as a *human powered centrifuge.* Both the NASA Ames Research Center and the University of California Irvine groups have been actively pursuing research on this concept. The second approach has been described as a *Twin Bike System*, and was proposed by Di Prampero and his colleagues at the University of Udine, Italy.

The *Human Powered Centrifuge* developed by Greenleaf *et al.* (1977a; 1999) is a 1.9-m-radius centrifuge fitted with two recumbent rider seats, and can carry one or two subjects in the seated supine position with their heads near the centrifuge hub. The configuration allows for one active on-board subject to power the centrifuge using a modified cycle mechanism (Figure 3-20). The cycling activity of the rider is coupled to the rotation of the platform and, hence, the development of various gravity levels along the Gz axis. An additional passive rider can be carried on the centrifuge at the same time. Alternatively, an off-board operator can power the centrifuge by using an upright off-centrifuge bicycle. Centrifugal force up to 5 g at the subject's feet (Gz) is obtained during rotation at 50 rpm.

Similarly, in the *Space Cycle* concept, developed by the Irvine Medical Center at the University of California, subjects ride opposite one another, one on a bike and one on a platform (Caiozzo *et al.* 2004). However, both the bike and the platform are free to tilt. As one individual pedals, the cycle moves in a circular motion around a central pole. The motion generates a gravitoinertial force aligned with the riders along their long body axis. The rider on the platform can perform various types of resistance training exercises, such as running on a treadmill or performing squats.

Study	***Intervention***	***G level (Feet)***	***G level (Heart)***	***Number of Days***	***Sessions per day***	***Total # of Sessions***
Iwase et al. 2002	AG, AG + Cycling		1.2	1	4	4
Stenger et al. 2007	AG, AG + Cycling	1.0-2.5		21	5	105
Evans et al. 2004	AG, AG + Cycling	1.0-2.5		21	5	105
Caiozzo et al. 2004	AG, AG + Cycling	1.0-3.0		1	4	4
Iwasaki et al. 1998	AG		2.0	7	7	49
Greenleaf et al. 1999	AG + Cycling	2.2		1	4	4
Yang et al. 2007a	AG + Squats AG + Cycling	1.5-3.0		4	1	4
Yang et al. 2007b	AG + Squats AG + Cycling	1.5-3.0				
Edmonds et al. 2007	AG + Stair Stepper	0.7-1.3				
Duda 2007	AG + Squats	2.0				
Clément et al. 2014	AG	2.0-3.5	1.0-2.5	2	10	10

Table 5. Summary of AG level and frequency used during studies testing the tolerance to short radius centrifugation with and without exercise in humans. Adapted from Kaderka (2010).

Study	***Days***	***Intervention***	***Session Duration (min)***	***# of Sessions per day***	***Daily Exercise (min)***	***Total Exercise (%)***
Suzuki et al. 1994	20	BR + Cycling	60	1	60	4.2
Greeleaf et al. 1989	30	BR + Cycling	30	2	60	4.2
Shibata et al. 2010	18	BR + Cycling + Dextran	30	3	90	6.3
Greenleaf et al. 1989	30	BR + Isokinetic Exercise	30	2	60	4.2
Bamman et al. 1998	14	BR + Squat	30	1	30	2.2
Belin de Chatemele et al. 2004	90	BR + Squat + Calf Press	40	1	40	2.8
Alkner et al. 2004	90	BR + Squat + Calf Press	40	1	40	2.8
Trappe et al. 2004	90	BR + Squat + Calf Press	40	1	40	2.8
Tesch et al. 2004	30	BR + Squat + Calf Press	40	1	40	2.8
Koryak et al. 1997	35	BR + Squat + Arm Press	60	1	60	4.2
Shackelford et al. 2004	119	Resistive Exercises	60	1	60	4.2

Table 6. Summary of bed rest studies assessing the efficiency of exercise as a countermeasure. Total exercise exposure is the total time while exercising relative to the duration of the bed rest. Adapted from Kaderka (2010).

The *Twin Bike System* proposed by Antonutto *et al.* (1993) and Di Prampero (2000) envisions two bicycles mechanically coupled to one another in a counter-rotating fashion. Astronauts would ride the bicycles along the inner wall of a cylindrically shaped space module (see Figure 5-06). The angular velocity of cycling would then determine the amplitude of the centrifugal vector along the main body axis of the rider. Like the human powered centrifuge designs developed by Greenleaf and Caiozzo, the *Twin Bike System* approach also has the potential for overcoming the deconditioning effects of microgravity on the musculoskeletal and cardiovascular systems.

Other types of exercise, such squats or stair stepper, have been evaluated during short-arm centrifugation. Table 5 lists the studies that use centrifugation without another intervention (such as bed rest or water immersion) for physiological deconditioning. By comparison, Table 6 lists the studies that tested only exercise countermeasures during bed rest.

5. Partial Gravity Simulators

Another method for simulating partial gravity is through the use of suspension techniques. By unloading a portion of a subject's weight, a partial gravity environment can be replicated. Human single-leg suspension and limb immobilization have been used as analogs to simulate the effects of weightlessness on long-duration muscular deconditioning, but these conditions do not mimic bed rest or spaceflight effects on human muscle fiber function (Widrick *et al.* 2002). A common shortcoming of all suspension systems is the limited degrees of freedom provided for natural movement. This limits the realism in a subject's training, possibly confounding experimental results. Another problem is that the internal physiological effects of partial gravity are absent from the simulation. Although the subject may feel suspended, the 1-g force is still acting on all internal organs.

Two types of suspension system are generally used: body inclination and suspension with springs or counterweight. The body incline suspension system involves a person walking in a plane parallel to the surface of the Earth. The suspension configuration inclines the subject on their sides so they are elevated from the horizontal. For example, a 9.5-deg inclination results in a perpendicular 0.16 g force simulating lunar gravity. An inclined walkway is used for translation. This method of cable suspension was used extensively to simulate lunar conditions in the 1960s (Figure 9A). However, despite this ingenious concept, study showed that the left and right sides of the subject exhibited a statistical difference in hip motion. The second type of inclination system requires that the subject faces upward. A treadmill is mounted vertically on a wall and the subject is suspended in a supine position by elastic suspension cords (Newman *et al.* 1996; Schultz 1995).

The overhead suspension system operates on the normal suspension principle that reduced gravity can be simulated by unloading a portion of the subject's weight. The simulated reduced gravity environment can be accomplished by attaching a counterbalance on subjects. One problem is that this counterbalance adds momentum to the system and tends to keep the subject moving in the direction of the motion. Another problem is that keeping a constant force during vertical motion is complicated. For these reasons, this method has not been used extensively (Schultz 1995). Another method uses an overhead suspension system to partially or fully unload the subject's legs by means of cables, springs, and a bicycle harness. The vertical unloading force is produced by stretching two garage door springs in series along the wall. This simple set-up provides a satisfactory simulation of a partial gravity environment on the lower extremities. However, a non-vertical lifting force that occurs during locomotion and the discomfort of the bicycle harness are the main disadvantages of the system. The MIT partial gravity simulator, also known as the Moonwalker, is capable of simulating partial gravity as low as 0.05 g (Figure 9B). A recent study tested 12 healthy subjects before and after Martian gravity simulation to determine the effects of partial gravity adaptation on walking performance. Results showed that the subjects walked with an altered gait characterized by an increased downward center of mass acceleration, reduced muscle activity, and increased maximum joint angles after Martian gravity simulation (Wu 1999).

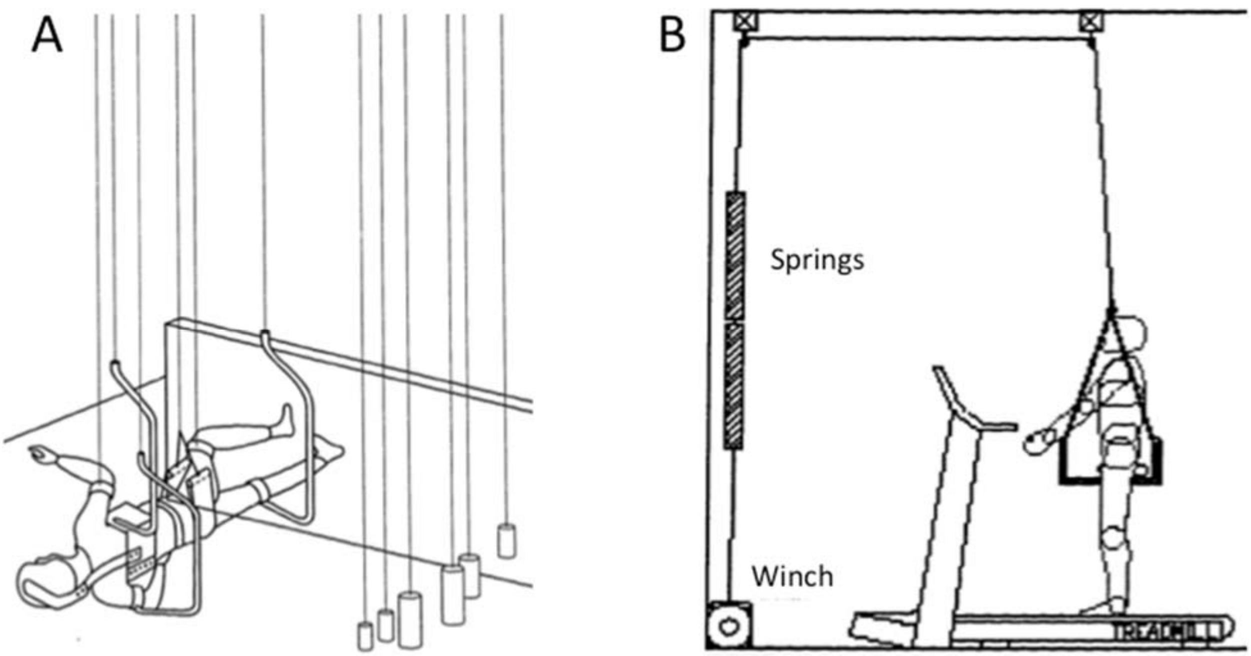

Figure 9. A. Body incline suspension system. The cables are attached to a crane located 10-20 m above the subject lying on his/her side. B. Body suspension system of an upright subject with cables, springs, and a bicycle harness. Adapted from Wu (1999).

6. Models

Various computational models exist or are in development to help predict and assess risks associated with physiological changes during spaceflight, and enhance exercise countermeasure development. For example, the NASA's *Digital Astronaut Project* (DAP) is working with bone specialists in the Human Research Program to establish a beta model of bone loss due to skeletal unloading in the femoral neck region. The model calculates changes in mineralized volume fraction of bone that can be related to changes in volumetric bone mineral density measured by Quantitative Computed Tomography. The model is governed by equations describing changes in bone volume fraction and rates of changes in bone cell populations that remove and replace bone in packets within the bone region (Pennline & Mulugeta 2014a).

The DAP bone model is being developed primarily as a research tool, and not as a clinical tool. As such it will not predict bone fracture. Its purpose is to provide valuable additional data via forward predictions during and after spaceflight missions to gain insight on: (a) the mechanisms of bone demineralization in microgravity, and (b) the volumetric changes at the various bone sites in response to in-flight and post-flight exercise countermeasures. These data can then be integrated with Finite Element Modeling to gain insight on how bone strength may change during and after flight. Such information could also contribute to optimizing exercise countermeasure devices and protocols designed to minimize changes in bone strength during flight. Preliminary validation analysis was carried out by comparing a set of simulation results against bone loss data from control subjects who participated in bed rest studies (Pennline & Mulugeta 2014b). Once validated during spaceflight and bed rest this model could be used for predicting the changes in bone volumes and strength during partial gravity and AG.

The *Renal Stone Formation Simulation Model* (RSFM) developed at the NASA Glenn Research Center is designed to evaluate the risk of developing a critical renal stone incident during long duration microgravity missions based on available astronaut biochemical data (Kassemi *et al.* 2014). Based on prediction for nucleation, growth and agglomeration of renal stones according to their sizes, and nephron geometry, the model predicts the effect of gravity on stone development and transport. Such a model can be useful to assess the impact of AG on stone transport. Recent bed rest studies have shown that intermittent centrifugation during a 21-day bed rest was partially effective against calcium excretion (Smith *et al.* 2009). The model predicts that AG may have an adverse effect on stone transport and agglomeration process due to the Coriolis and cross-coupled angular accelerations.

A *Multiscale Heart Model* (MHM) is also being developed to predict changes in heart shape and stress distribution in reduced gravity (Iskovitz *et al.* 2013). Changes in heart shape have been observed during orbital and parabolic flight (Summers *et al.* 2010; Levine & Bungo 2014). A 9% increase in sphericity of the left ventricle at the end of a diastolic cycle was captured with echocardiography in ISS crewmembers. It is likely that the shape and location of the heart changes during centrifugation as well, as a function of the AG level, but this has not been measured yet. Computational models can capture the heart shape change, but also predict the associated changes in the cardiac stress field during centrifugation that may be at the origin of cardiac remodeling. Models can also predict the effect of Coriolis force and gravity gradients on blood flow and blood vessel shape changes in various gravity levels. They could help determining the relationship between the gravity level and the extent of cardiac shape and stress change.

Motion sickness models are also being used to design adaptation protocol to facilitate head movements during centrifugation. The dynamics of motion sickness onset and progression have been described by Oman (1982) using a mathematical model in which the input is sensory conflict and the output is motion sickness. Sensory conflict is defined here as the difference between expected and actual sensory afference for a given motion (Guedry *et al.* 1998). The basic difference during AG arises due to the cross-coupled acceleration stimulus associated with a head movement in a rotating environment. This conflict can be mathematically described as a multi-dimensional vector, with the magnitude of the conflict assumed proportional to the magnitude of the vector. With a clearly defined sensory conflict and knowledge of motion sickness dynamics, such a model can be used to predict nausea trends for a given AG protocol. Data collected during a 3-day incremental adaptation protocol in which subjects made head movements during rotation at 30 rpm showed good agreement with model predictions and demonstrated the feasibility of adaptation to increasingly high rotation rates (Elias *et al.* 2007). Based on the results and modeling, it appears that adaptation to head movements at higher rotation rates is almost certainly possible. The authors suggest that this model could be employed in the design of experiments incorporating additional days of training to potentially reach rates corresponding to 1 g at heart level. The demonstrated capability for adaptation to high rotation rates presents a strong argument for short-radius centrifugation as a practical implementation of AG. Whether or not a short radius is ultimately desirable, it cannot at this point be ruled out based on concerns regarding vestibular adaptation to the rotating environment (Young 2003).

VI. GAPS

A. Gravity Level

Most studies on the physiological effects of centrifugation during bed rest or wet/dry immersion have used gravity level at the heart equivalent to 1 g (Gz) or higher. These gravity levels were successful to preserve orthostatic tolerance and sensorimotor performance after deconditioning ranging from 5-28 days. The protective effects on muscle and bone changes were inconclusive, mostly due to the limited duration of the bed rest/immersion. No studies have investigated the effects of centrifugation on intracranial pressure.

The G dose-response relationship between physiological variables and 0 g to 1 g is virtually unknown (Figure 9). Thus, we do not know for example, whether the Martian gravity level of 0.38 g is at all protective, and what gravity threshold is needed for maintaining musculoskeletal functions during long duration weightlessness. To define the physiologically protective gravity threshold of AG is one of the most important requirements for the engineers when developing AG in space.

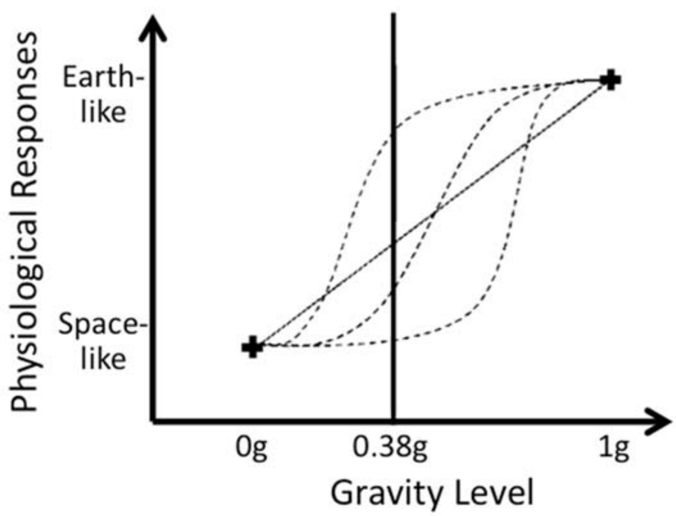

Figure 9. Hypothetical G dose-response curves of physiological responses versus gravity level between 0 g and 1 g. Adapted from Paloski (2014b).

We know that the threshold for the *perception* of AG by the crew in orbit is between 0.22-0.5 g. However, in some studies the subject's head was on-center whereas in other studies it was off-center, either on the same side as the feet or on the opposite side. The threshold for the perception of AG in ground-based centrifuges or in parabolic flight is also comprised between 0.16 g and 0.38 g.

The perception of AG is presumably subject-dependent. In a recent ground-based study Clément *et al.* (2014) attempted to determine the rotation parameters of a short-radius centrifuge so that subjects rotating in the dark would feel as if they were standing upright. Subjects were lying supine in a nacelle on a 2.8 m-radius centrifuge with their head closer to the axis of rotation and their feet pointing radially outwards. Subjects verbally reported body orientation for 26 combinations of centrifuge rotation rate and nacelle pitch tilt. ECG and respiratory responses were also recorded. Results showed that about half of the subjects felt like they were vertical when centrifugation elicited 1 g at their center of mass along their body longitudinal axis, whereas the other half felt they were vertical when they experienced about 1 g at ear level, regardless of the nacelle tilt angle. Heart rate variability varied with the subjects' perception of verticality. These results suggest that one group of subject was relying principally on the otolith organs for the perception of verticality, whereas the other group was also relying on extra vestibular somatosensory receptors. The crewmember's *perception of verticality* might therefore be a factor to take into account for the prescription for artificial gravity during space flight (Clément *et al.* 2014).

Based on long-duration centrifuge studies on Earth, Russian scientists suggest that the minimum level of effective AG in humans is about 0.3 g. They further recommend that a level of 0.5 g be induced to increase a feeling of wellbeing and normal performance (Shipov *et al.* 1981). Human factor studies in which subjects were suspended horizontally and allowed to "walk on the wall" of the rotating platform at NASA Langley suggested that walking in the direction of rotation at a simulated gravity level of between 0.16 and 0.3 g at the feet was found to be the most comfortable. At levels above 0.3 g, the subjects reported "sensations of leg and body heaviness", which became quite disturbing at 0.5 g. Consequently the lower limit of 0.3 g was preferred in most design studies for implementation of artificial gravity (Letko & Spady 1970).

As for the maximum level of AG in the +Gz direction, ground-based bed rest studies suggest that gravity levels up to 2 g at the feet are well tolerated. However gravity levels as high as 3-4 g at the feet are tolerable for less than 30 min in most subjects. Active exercise, such as bicycling, on human powered centrifuges is, however, well tolerated from a hemodynamic perspective at gravity levels up to 3 g at the feet (Caiozzo *et al.* 2004). Symptoms of grey-out begin at 2-3 g at the head level. Tolerance to acceleration is reduced after bed rest, but little is known about tolerance to acceleration after spaceflight. Consequently, tolerance to acceleration, especially during short-radius centrifugation, must be further investigated both after spaceflight and after bed rest.

B. Gravity Gradient

A gravity gradient makes limb movement and changing body positions awkward. Objects in a rotating environment have a different "weight", depending on their distance from the center of rotation. The larger the gravity gradient, the greater is this difference. This can greatly affect handling materials or moving objects. For example, for an astronaut standing on the rim of the centrifuge, objects become lighter as she "lifts" them toward the center of rotation. Furthermore, she becomes heavier when squatting down towards the rim and slightly lighter when she is standing on tiptoes (Stone *et al.* 1970).

Based on the observations in the slow rotation room experiments, a minimum radius of 12 m is generally specified to limit the gravity gradient to approximately 15%. This limit was imposed taking into account the human factors consideration of work efficiency (Piemme *et al.* 1966). Because of the virtual absence of data on the effects of gravity gradient (most the studies were performed on large-radius centrifuges that minimized the gravity gradient), the 15% upper limit was obviously a conservative value aimed at easing materials handling and reducing the potential risk of musculoskeletal injuries. As a matter of fact, recent experiments with subjects lying supine on short-radius centrifuges with gravity gradients of 25 and 50% did not reveal any significant effect on cardiovascular responses (Hastreiter & Young 1997; Clément *et al.* 2014).

C. Rotation Rate

The maximum rotation rate of a centrifuge or spinning spacecraft is limited by the Coriolis forces and cross-coupled angular accelerations encountered when walking, moving the head, or when moving the objects within the rotating environment. At body motion or centrifuge rotation rates that are of low magnitude, the effects of the Coriolis force are negligible, as on Earth. However, in a centrifuge rotating at several rpm, there can be disconcerting effects. Simple limb movements become complex, eye-head movements are altered, and nausea can occur. Motion sickness generally occurs at more than 3 rpm, although people can eventually adapt to higher rates after incremented, prolonged exposure.

Studies performed in slow rotation rooms in the 1960s suggested that the Coriolis forces should be kept to less than 25% of the AG level (Stone 1970). However, in the light of recent ground-based data showing a rapid adaptation to the vestibular conflict generated by Coriolis forces and cross-coupled angular accelerations (Young *et al.* 2001), this limit seems overly conservative. Also, during an experiment performed on board Skylab, it was observed that head movements made during rotation after six days in weightlessness failed to elicit motion sickness or disorientation (Graybiel *et al.* 1977). Lackner & DiZio (2000a) performed parabolic flight experiments indicating that *"the severity of side effects from Coriolis forces during head movements is gravitational force-dependent, raising the possibility that an artificial gravity level less than 1 g would reduce the motion sickness associated with a given rotation rate"*. Finally, restraining head movement during centrifugation also mitigates the nausea-inducing effects of Coriolis forces. Limiting head rotations to less than 3 rad/s (180 deg/s) provides acceptable cross-coupled angular accelerations for centrifuge rotation rates up to about 10 rpm.

It is important to note that the limits for the above comfort criteria address the issues of humans *walking* and *moving objects* in a rotating environment. Indeed, these limits were proposed at a time where large rotating stations were foreseen for space missions, as described in the next section. These comfort limits must obviously be re-evaluated for the case of on-board short-radius centrifuges, where body, limb, and head movements will be more restricted.

D. Exposure Duration & Frequency

Studies during bed rest and water immersion in humans have focused on centrifugation exposure during one or two daily sessions of 1 hour each. This regimen is presumably based on the current exercise regimens during long-duration space missions (2 daily 1-hour sessions). However, many crewmembers complain that the current 2-hour duration of imposed exercise is "much too long" given the other onboard constraints (work and rest schedules, boredom, absence of showers, vibrations, etc.). As suggested by Vernikos (1997), "the requirements (for intermittent centrifugation) should be provided in a manner that is practical and acceptable to the crews to encourage compliance". At this point, only a few studies on AG have reported subjects' comfort level and acceptability of the various interventions tested.

Relative to the full duration of the intervention (bed rest or immersion) the duration of intermittent AG during these ground-based tests ranged from 2.1% to 8.3% (see Table 4). Bed rest studies that assessed the efficiency of intermittent exercise typically used exercise durations corresponding to 2.2% to 6.3% of the total bed rest duration (see Table 6). LBNP exposures typically lasted about 20-60 min per day with a gradual increase in intensity, also corresponding to 2.1%-4.2% of the duration of the bed rest (see Table 1). By comparison, the Neurolab off-axis rotator exposed the crewmembers of the STS-90 mission to centrifugation for 20 min or less every other day (Moore *et al.* 2005), which corresponded to only 0.7% of the mission duration.

Very few studies have used multiple daily exposures. White *et al.* (1965) used 4 daily AG sessions of 7.5 or 11.2 min and showed that this was sufficient to reduce most of the physiological markers associated with orthostatic intolerance. Vernikos *et al.* (1996) used 8 to 16 daily sessions where the subjects were standing in place or walking, based on the concept that "in general physiological systems respond to signal and intensity changes rather than to the duration of a stimulus" (Vernikos 1997). More recently, Chouker *et al.* (2013), Linnarsson *et al.* (2015), Rittweger *et al.* (2015), and Clément *et al.* (2015) showed positive effects of AG for a total exposure equal to less than 1% the intervention. They compared the effects of 1 versus 6 daily AG/countermeasures sessions and concluded that 6 daily exposures of 5 min each were more efficient and better tolerated by the subjects that 1 single daily exposure of 30 min.

E. Centrifugation Combined with Exercise

AG coupled with cycling is effective for many of the cardiovascular parameters, including orthostatic tolerance time and VO2 max. The minimum duration and minimum intensity protocol that works is specifically two 20-min sessions of (a) centrifugation at 0.8-1.4 Gz at the heart (2.9-5.0 Gz at the feet), which is based on individual tolerance to centrifugation, coupled with cycling at a constant 60W; and (b) centrifugation at 0.3 Gz at the heart (1.0 Gz at the feet) coupled with cycling at variable levels based on pre-bed rest VO2max (2 min at 40% VO2max, 3 min at 60%, 2 min at 40%, 3 min at 70%, 2 min at 40%, 3 min at 80%, 2 min at 40%, and 3 min at 80%). This protocol was effective with alternating days of treatment (Kaderka 2010).

Although little musculoskeletal data exists for AG, soleus fiber CSA was preserved with 60 min of 2.5 g along Gz and calf presses. In animals, 60 min of standing or centrifugation at 1.5 g along Gz protects soleus muscle characteristics, vastus lateralis fiber CSA, and femur density and strength. Squats have been successfully performed in AG training studies without adverse effects from Coriolis forces or motion sickness (Duda 2007; Yang *et al.* 2007b). Foot forces and EMG activity are comparable when performing high intensity squats during centrifugation and during standing upright (Yang *et al.* 2007a).

VII. CONCLUSIONS

A. Physiological Requirements

Based on NASA flexible path for future manned deep space missions, design requirements for AG will be sought circa 2022. Regarding the physiological requirements for AG, the following scenarios are considered: (a) centrifugation inside the space vehicle (intermittently, autonomously, and/or human powered; radius less than 2.5 m); (b) centrifugation of part of the vehicle (chronic or intermittently; radius between 3 and 15 m); or (c) spinning the whole vehicle (chronically; radius 56 m or greater).

To help the final decision whether to conduct short- or long-radius centrifugations in space and whether whole or only part of the space vehicle should be spun, the following limitations of AG in humans should be determined:

1. Intermittent, short-radius centrifugation: What are the acceptable and/or optimal ranges for radius and rotation rate of an onboard centrifuge to avoid unacceptable crew health and performance consequences?
   - What are the physiological consequences of AG generated by short-radius centrifugation?
   - What are the physiological limits for rotation rate, gravity gradient, and duration of AG exposure?
   - What frequency of AG exposure is optimal for intermittent applications?

2. Continuous, long-radius centrifugation: What are the acceptable and/or optimal ranges for radius and rotation rate of a rotating transit vehicle to avoid unacceptable crew health and performance consequences?
   - What are the physiological consequences of AG generated by long-radius centrifugation?
   - What are the physiological limits for rotation rate?
   - What are the acceptable ranges of Coriolis forces and cross-coupled angular accelerations to avoid spatial disorientation and motion sickness when moving inside the rotating vehicle?

B. Tests Needed to Close the Gaps

1. More gravity level values along Gz within the range from 0 g to 1 g must be tested to reasonably conclude about the threshold, optimal stimulus-response, and saturation for the effects of centrifugation on cardiovascular, sensorimotor and musculoskeletal functions.

2. We do not know if increasing the intensity of the Gz stimulus actually reduces the time of exposure needed. Consequently, the effects of gravity levels higher than 1 g on physiological functions definitely need to be further investigated.

3. In the absence of a space-based human rated centrifuge, these tests would include deconditioning interventions during ground-based studies such as bed rest and wet/dry immersion in humans, and tail suspension in animals, with intermittent centrifugation.

Centrifugation could also be replaced by head-up tilt (as in animal studies) within the range of 0 to 90 deg.

4. Evidence of the effects of Mars gravity on physiological deconditioning could be obtained by performing bed rest studies using a 22.3-deg head-up tilt. In this position, there is a 0.38 g force exerted along the Gz direction. Measurements of cardiovascular and sensorimotor responses before, during, and after bed rest would be compared with those during head-down bed rest.

5. Evidence of the effects of Mars gravity on the recovery of physiological deconditioning in crewmembers following 6 months in weightlessness could be obtained by exposing ISS crewmembers returning from the ISS in a 22.3-deg head-up tilt. In this position, there is a 0.38 g force exerted along the Gz direction. Measurements of cardiovascular and sensorimotor responses in this head-up tilt position would be compared with those of crewmembers who are not exposed to head-up tilt after landing.

6. It is not yet certain that exposure to centrifugation for intermittent, short periods of time is as beneficial as continuous exposure to normal gravity. Experiments must compare if it is the total block of time in AG or the number of AG exposures per day that is the most effective.

7. To study the effects of AG on musculoskeletal system, ambulatory subjects could be exposed to intermittent centrifugation, say 2-3 times per week, for a period > 3 months. Centrifugation could be coupled with squats or other exercises. Measurements of the antigravity muscles and weight-bearing bones and biological markers would be taken before and after training. Comparison with control groups without centrifugation, and possibly with or without exercise, would help determine the benefits of centrifugation training on muscle and bone.

8. To assess whether AG can possibly mitigate the VIIP syndrome, studies could measure if Gz centrifugation has an effect on ocular anatomy, head and neck vascular parameters, and reduces intracranial pressure. Measurements would be performed while healthy subjects are being centrifuged at various gravity levels. Other studies could include patients with VIIP-like syndrome.

9. To assess whether AG can possible mitigate post-flight on decrease in performance, studies could evaluate if Gz centrifugation reduces (a) disorientation induced by galvanic vestibular stimulation, reversing prisms, or Coriolis motion sickness; and (b) impairment in locomotion and movement induced by an aging suit.

10. There is a lack of data on the effects of gravity gradient on physiological functions. A simple test would consist in comparing the responses to constant rotation rate in subjects placed at various distances from the axis of rotation on a long radius centrifuge. Because the magnitude of Coriolis forces is independent of the radius of rotation, the differences in response between radii would only be due to the gravity level and gravity gradient. The effects of gravity level will be known from the results of separate studies (see 1-4), so the delta could be attributed to the gravity gradient.

11. Inside a rotating vehicle, the artificial gravity level is constantly being distorted as the astronauts move about within the spacecraft, except when they move along an axis that is parallel to the axis of rotation. On a short-radius centrifuge, Coriolis forces act on the limbs during movements and after-effects occur when rotation stops. Only a few studies have taken into account the cognitive aspects of centrifugation (e.g., prediction, memory, dual-task). More research is needed in this area.

12. Although ground-based studies have the potential for determining a sound AG prescription, its validation can only be performed in space. No human-rated centrifuges that have been built specifically to counteract cardiovascular and musculoskeletal deconditioning have flown in space to date. Given the time constraints of this project, it is most likely that a full validation using an ISS-based human-rated centrifuge won't be feasible. Nevertheless, questions such as what are the impacts of centrifugation inside a space vehicle on the vibration level, motion sickness, or crew time could be answered using a simple, lightweight on-board centrifuge. Any positive results from this space centrifuge would also provide the impetus for further ground-based research.

C. Recommendations for Performing these Tests

In the review of existing studies assessing the effects of centrifugation, many confounding variables arose from the protocols themselves. Differences were observed in intervention duration, subject selection criteria, daily nutritional content and supplements (if any), fluid intake, and conventional countermeasures, etc. Standardizing the protocols should be the first step in future AG research as it would allow for a more compatible assessment across various studies. For this standardization we recommend the following:

1. Establish a set of standard conditions for AG interventions. For example, studies should clearly define the gravity level generated at the vestibular system (otolith), center of mass, heart, and feet. Studies should also clearly define the direction of AG in ground-based studies, as it can refer to the centripetal acceleration only or to the resultant of centripetal acceleration and gravitational acceleration.

2. Establish a set of standard biomedical measures specifically designed for comparison of gravity level, gravity gradient, and g exposure duration. These measures could be based on those developed with international input for coordinated bed-rest investigations, plus additional ones. The AG standard measures should be focused on medical events, countermeasure validation, and subject acceptance and comfort.

3. Establish a set of integrated investigations selected through an open international AG research announcement of opportunity to optimize science collaboration.

4. Establish subject, hardware, and data sharing agreements to permit the widest possible access to AG research results.

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IX. LIST OF ACRONYMS (LOA)

1 g	Earth normal gravity
AEM	Animal Enclosure Module
AG	Artificial Gravity
AGREE	Artificial Gravity with Ergometric Exercise
ARED	Advanced Resistive Exercise Device
ATV	Automated Transfer Vehicle
BR	Bed Rest
BRS	Body Restraint System
CAM	Centrifuge Accommodation Module
CNES	Centre National d'Etudes Spatiales
CNS	Central Nervous System
CSA	Cross Sectional Area
D-1	First German Spacelab Mission
DAP	Digital Astronaut Project
DEXA	Dual Energy X-Ray Absorptiometry
DLR	German Space Agency
EKG	Electrocardiogram
ESA	European Space Agency
GIA	Gravitoinertial Acceleration
GIF	Gravitoinertial Force
GLCS	Gravity Loading Countermeasure Skinsuit
HF	High Frequency
HPC	Human Powered Centrifuge
HRF	Human Research Facility
IBMP	Institute for Biomedical Problems
IMAG	International Multidisciplinary Artificial Gravity
ISS	International Space Station
JAXA	Japan Space Agency
LBNP	Lower Body Negative System
LEO	Low Earth Orbit
LF	Low Frequency
LMS	Load Measuring System
MVC	Maximum Voluntary Contraction
MHM	Multiscale Heart Model
MIT	Massachusetts Institute of Technology
MPLM	Multi-Purpose Logistics Module
MRI	Magnetic Resonance Imaging
MVI	Microgravity Vestibular Investigations
NAMRL	Naval Aerospace Medical Research Laboratory
NASA	National Aeronautics and Space Administration
OSR	Otolith-Sympathetic Reflex
OVAR	Off-Vertical Axis Rotation
PI	Principal Investigator
pQCT	Peripheral Quantitative Computed Tomography

RCS	Reaction Control System
RER	Respiratory Exchange Ratio
RHMk-1	Rodent Habitat Mark I
RPM	Revolution Per Minute
RSA	Russia Space Agency
RSFM	Renal Stone Formation Simulation Model
SAHC	Short-Arm Human Centrifuge
SLD	Subject Load Device
SRR	Slow Rotating Room
STS	Space Transportation System (Space Shuttle)
SUS	Hind Limb Suspension Model
SV	Subjective Vertical
TPR	Total Peripheral Resistance
UTMB	University of Texas Medical Branch
VE	Volume of Air Inhaled in One Minute
VIIP	Vision Impairment and Intracranial Pressure
VO2	Rate of Oxygen Uptake per Minute