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**Review Article**

# Centrifugation as a countermeasure during bed rest and dry immersion: What has been learned?

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**Abstract**

**Objectives:** We review the studies that have evaluated intermittent short-radius centrifugation as a potential countermeasure for cardiovascular, musculoskeletal, and sensorimotor deconditioning in simulated weightlessness. **Methods:** The findings from 18 experimental protocols that have used bed rest and dry immersion for comparing the protective effects of centrifugation versus standing upright or walking, and the effects of continuous vs. periodic exposure to centrifugation are discussed. **Results:** Centrifugation for as little as 30 min per day was found to be effective in mitigating orthostatic intolerance and strength in postural muscle after 5 days of bed rest, but it was not effective in mitigating plasma volume loss. **Conclusion:** To determine the optimal prescription for centrifugation as a countermeasure, we recommend further studies using (a) bed rest of longer duration, (b) individualized prescriptions of centrifugation combined with exercise, and (c) functional performance tests.

**Keywords:** Bed Rest, Dry Immersion, Centrifugation, Artificial Gravity

## Introduction – The gravity of the situation

Deconditioning of the cardiovascular and musculoskeletal systems has been observed during space flight[1-5], head-down bed rest[4,6-8], and dry immersion[9]. Evidence comes from reduced plasma volume, reduced exercise capabilities, and increased orthostatic intolerance, as well as muscle weakening and bone loss. The cause of this deconditioning is mostly attributed to the lack of both the static G force along the longitudinal body axis (z-axis) and the body's exertion against this $G_z$ force during movement and locomotion[10].

Artificial gravity generated by centrifugation has the potential to mitigate this deconditioning by mimicking a constant $G_z$ stimulus equivalent to the one experienced on Earth. A constant 1 $G_z$ stimulation elicited by spinning the whole spacecraft would be the most effective, but this solution requires additional costs in term of mass, power, and controls. A more affordable solution is periodic $G_z$ stimulation of individual crewmembers using an onboard short-radius centrifuge[11-13].

To date, only two human-rated short-radius centrifuges have flown in space, on board the Space Shuttle in 1992 and 1998. However, the primary objective of these experiments was to investigate not artificial gravity but spatial orientation (eye movements, motion perception) in subjects exposed to transient linear acceleration in space[14-15]. In the first experiment, four astronauts were positioned on a rotating chair so that their head and feet were off center by a few cm, generating -0.22 $G_z$ at the head level and a centripetal force of +0.36 $G_z$ at the feet. Duration of rotation was 1 minute every other day of a 7-d mission. None of the subjects perceived any sense of tilt relative to the +$G_z$ stimulus during the in-flight tests[14].

During the second experiment, subjects sitting upright or lying supine on a flight centrifuge were exposed to +1 $G_y$, +0.5 $G_z$, and +1 $G_z$ for about 10 min per day during a 16-d mission. They felt tilted relative to the direction of the G stimulus, so centrifugation was actually perceived as artificial gravity by the crewmembers[15]. No

| Abbreviations | |
|---|---|
| AGEG | Artificial gravity expert group |
| BR-AG1 | First bed rest and artificial gravity study |
| CON | Control condition |
| DI | Dry immersion |
| ESA | European space agency |
| FT | Field test |
| FTT | Functional task test |
| HDBR | Head-down bed rest |
| ISS | International space station |
| LRT | Locomotion replacement training |
| MVC | Maximum voluntary contraction |
| SAG | Simulated artificial gravity |
| SRC | Short-radius centrifugation |
| STA | Standing |

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sign of altered vestibular responses or orthostatic intolerance was observed during postflight tilt tests in any of the four crewmembers exposed to in-flight centrifugation. The other three crewmembers on that mission had orthostatic intolerance. Based on the result that 64% of astronauts experienced severe orthostatic intolerance after Space Shuttle missions[1], the probability that four crewmembers on the same flight would not exhibit orthostatic intolerance by chance is about 1 in 60[16].

So, except for this latter study, little is known about the effects of centrifugation on cardiovascular function in space. Head-down bed rest (HDBR) is a valuable analog for simulating some of the effects of space flight on this function[17]. HDBR is characterized by inactivity, confinement, and suppression of the $+G_z$ gravitational stimulus. Unloading the body's upright weight reduces proprioceptive stimulation and eliminates the need for musculoskeletal force to work against gravity, thus reducing the body's energy requirements. The upward fluid shift during HDBR, by acting on central volume receptors, induces a reduction in plasma volume that leads to orthostatic intolerance during head-up tilt and upright standing after HDBR. Multiple factors influence this orthostatic intolerance; they include decreased blood volume, decreased baroreceptor sensitivity, increased venous distensibility, decreased heart muscle strength, and altered autonomic function. In addition, bone resorption is increased by HDBR, leading to a sustained negative bone balance. Body weight, muscle strength, exercise endurance capacity, and aerobic power are also reduced in a manner similar to what happens during space flight. Over the past 20 years, HDBR has proved its usefulness as a reliable simulation model for most of the physiological effects of space flight[6].

Dry immersion (DI) consists of immersing a subject covered with an elastic waterproof fabric in thermo-neutral water. As a result, the immersed subject, who is virtually buoyant, remains dry. Russian investigators have reported that DI leads to the same changes as HDBR, but after a relatively shorter duration of exposure, presumably because of the lack of perceived body weight[9].

We have identified 18 experimental protocols that, in the past 50 years, have investigated the benefits and side effects of a $+G_z$ stimulation during HDBR and DI. This article summarizes what has been learned during these ground-based studies and recommends further research.

## Physiological effects of $G_z$ stimulation during bed rest and dry immersion

In these experimental protocols the $+G_z$ stimulus was periodically provided during HDBR and DI using four different methods: (a) short-radius centrifugation (SRC); (b) standing upright; (c) walking or running on a treadmill; and (d) physical exercise simulating locomotion (Table 1). For SRC the centrifuges had a short outer radius (1.5-2.5 m), and consequently the $+G_z$ stimulus at the feet was larger than at the heart. Note, however, that on Earth, the centrifugal force combines with the gravitational force (along the $G_x$ axis in a supine subject), and the resultant force is larger than 1 G. The smallest $G_z$ level tested was 0.38 $G_z$ at the heart, corresponding to the gravity level on the surface of Mars. The largest level tested was 2 $G_z$ at the heart, coupled with cycling. In average, studies utilized 1 $G_z$ at the heart (SD 0.4 $G_z$; median 1 $G_z$). This $G_z$ level was presumably chosen because the most obvious countermeasure in space would be to provide a 1 $G_z$ artificial-gravity environment.

The duration of the DI protocols (mean 9.7 d; SD 9.8 d; median 5.5 d) was shorter than the HDBR protocols (mean 11.4 d; SD 11.3 d; median 5 d). Overall both of these studies had a relatively short duration (mean 10.8 d; SD 10.5 d; median: 5 d). Short-duration analog studies have the advantage of being both practical and cost effective, especially in the case of a crossover experimental design in which the same individuals can be tested repeatedly using various $+G_z$ stimuli more frequently, and these treatments can be randomized. In addition, previous studies have demonstrated that orthostatic intolerance occurs within a few hours of HDBR or DI[9,37], maximal exercise capacity is reduced after 24 h, bone resorption starts to increase on the second day of bed rest[38], and diuresis occurs mostly during the first 48 h[39-41]. Although plasma volume is somewhat reduced by 24 h, this reduction is essentially maximal by 3 days[42].

The duration of exposure to the $+G_z$ stimulus during HDBR ranged from 25 min to 4 h per day (mean 1.0 h; SD 0.9 h; median 0.7 h). The SRC sessions were an average of 60% longer during the DI protocols (mean 1.6 h; SD 0.5 h; median 1.5 h). However, the SRC sessions were not performed every day (see notes in Table 1), so overall the duration of $G_z$ exposure for both HDBR and DI studies was comparable. This duration was presumably chosen for the purpose of comparing the effects of artificial gravity with the effects of traditional countermeasures, such as physical exercise that crewmembers in orbit also perform for about 1-1.5 h per day[43-44]. This comparison is difficult, however, because some of the HDBR and DI studies using SRC also had the volunteers perform aerobic exercise.

Details on the study protocols 1-15 listed in Table 1, including number and gender of subjects, and experimental design (crossover or with a control group), can be found in Kaderka[19]. Kaderka also performed a meta-analysis on the main results obtained with these protocols in terms of cardiovascular performance (orthostatic tolerance time, plasma volume, hematocrit measurement, stroke volume, heart rate, total peripheral resistance, VO2 max), muscle alteration (soleus and vastus lateralis cross-sectional area, muscle volume, knee extensor maximum voluntary contraction) and bone changes (bone mineral density on the lumbar spine, femoral neck, trochanter and total hip, bone resorption markers, bone formation markers, calcium in the urine, and serum).

Despite vast differences between these study protocols in terms of objectives, durations and measured physiological parameters, results have shown that a periodic circa 1 $G_z$ stimulus at the heart during HDBR and DI does the following: (a) improves post-HDBR and post-DI orthostatic tolerance time[32,34,36,45-47]; (b) reduces the exaggerated responses to head-up tilt after the interventions, such as elevated heart rate and increased muscle sympathetic nerve activity[27,48-49]; (c) attenuates plasma volume loss when SRC is combined with exercise[29-30,50]; and (d) maintains exercise capacity[31,51]. These benefits are not surprising given that cardiac performance and baroreceptor sensitivity are presumably optimized for functioning in a 1 $G_z$ environment. On Earth we spend about 8 h per day exposed to a 1 $G_x$ or 1 $G_y$ stimulus when sleeping and 16 h per day exposed to a 1 $G_z$ stimulus when sitting or standing (or more during locomotion). What is surprising is how little $+G_z$ exposure the human body needs per day to maintain adequate exercise capacity and orthostatic responses[10].

## Is shorter, more frequent $G_z$ stimulation best?

Only three studies have attempted to answer the question of how often $+G_z$ stimulation is needed to maintain normal physiological functions. These studies have compared the effects of generating the same duration of $+G_z$ stimulus during HDBR and DI in two, three, six, eight or sixteen daily sessions in the same subjects. It was hypothesized that several shorter centrifugation periods with rest in between would not only be better tolerated by the subjects, but also prove more efficient as a countermeasure. Support for this hypothesis comes from studies on hind-limb suspension in rats, and in-orbit exercises in astronauts that showed that repetitive short-duration, high-load exercise training was more effective in mitigating musculoskeletal deconditioning than longer, less intense sessions.

Vil-Viliams & Shulzhenko[26] compared SRC-generated $+G_z$ stimulation for 60 min twice a day and 40 min three times a day in subjects otherwise immersed in water. Both $+G_z$ treatments were equally effective, as shown by the same mitigating effects on orthostatic intolerance after water immersion.

During the first ESA First Bed Rest and Artificial Gravity (BR-AG1) study, Linnarsson et al.[36] compared daily SRC sessions generating 1 $G_z$ at the heart for 30 min continuously (1 x 30 min) and for 6 bouts

**Table 1.** List of the various experimental protocols used for investigating the effect of $+G_z$ stimulation using short-radius centrifugation (SRC) or standing/walking/running during bed rest (BR) and dry immersion (DI) in the past 50 years. LRT: locomotion replacement training (see text for details). Adapted from Clément & Pavy-Le Traon[18] and Kaderka[19].

| Study | Intervention | Days | $+G_z$ at heart | Number of daily sessions | Session duration (min) |
|---|---|---|---|---|---|
| 1. White et al.[20] | BR + SRC | 41 | 1.4 | 4 | 7.5 |
| 2. Nyberg et al.[21] | BR + SRC | 10 | 1.8 | 4 | 20 |
| 3. Kamenskiy et al.[22] | DI + SRC | 3 | 0.5, 0.6 | 1 | 60 |
| 4. Gale et al.[23] | DI + SRC | 4 | 0.5, 0.6 | 1 | 60 |
| 5. Grigoriev et al.[24] | DI + SRC | 13 | 0.6 | 1[a] | 60, 90 |
| 6. Vil-Viliams & Shulzhenko[25] | DI + SRC | 3 | 1.0 | 3 | 40 |
| 7. Vil-Viliams & Shulzhenko[26] | DI + SRC | 28 | 0.8, 1.2, 1.6 | 2[b]<br>3[b] | 60<br>40 |
| 8. Yajima et al.[27] | BR + SRC | 4 | 2.0 | 1 | 60 |
| 9. Vil-Viliams[28] | DI + SRC + Cycling | 7 | 0.8, 1.2, 1.6 | 1 | 120 |
| 10. Vernikos et al.[10] | BR + Standing + Walking | 4 | 1.0 | 8<br>16 | 15<br>15 |
| 11. Lee et al.[29] | BR + Running | 5 | 1.0 | 1 | 30 |
| 12. Iwasaki et al.[30] | BR + SRC | 4 | 2.0 | 2 | 30 |
| 13. Katayama et al.[31] | BR + SRC+ Cycling | 20 | 0.4, 0.8, 1.4 | 1[c] | 40 |
| 14. Iwase[32] | BR + SRC + Cycling | 14 | 1.2 | 1[c] | 30 |
| 15. Young & Paloski[33] | BR + SRC | 21 | 1.0 | 1 | 60 |
| 16. Yang et al.[34] | BR + SRC + Cycling | 4 | 0.4, 0.7 | 1 | 30 |
| 17. Mulder et al.[35] | BR + Standing + LRT | 5 | 1.0 | 1 | 25 |
| 18. Linnarsson et al.[36] | BR + SRC | 5 | 1.0 | 1<br>6 | 30<br>5 |

*Notes: [a] SRC was used on DI days 8-13 only. [b] SRC was used on DI days 9-14 and 23-28 only. [c] SRC was used during 3-4 days per week only.*

of 5 min (6 x 5 min) separated by 3 min of rest. HDBR without SRC was used as a control condition (CON). The effects of the two $+G_z$ treatments and the control condition were investigated in a crossover study design in which eleven subjects were each tested during three campaigns of 5-d HDBR, in a random order. The results of the various investigations using this protocol are summarized in Table 2.

The 6 x 5 min $+G_z$ treatment was found to be the most effective in preserving orthostatic tolerance after HDBR, and appeared equivalent to a continuous 60-min exposure to $+G_z$ stimulation in other studies[36]. However, neither the 6 x 5 min nor the 1 x 30 min $+G_z$ treatment attenuated plasma volume loss[54]. The interpretation for the observation that centrifugation has a beneficial effect on orthostatic tolerance without mitigating plasma volume loss is the following: first, the centrifugal force pushes the blood "down" to the feet and the venous return in the legs pushes it back to the heart. This reaction might take only a short period of time on the centrifuge because reflexes are usually reinforced with rather small time periods. The second effect of centrifugation is an attempt to maintain plasma volume, as well as muscle and bone integrity, by mimicking the continuous presence of Earth gravity, and this process is more time-consuming. The decrease in plasma volume is mostly due to urinary excretion in response to the fluid shift to the upper body that occurs during head-down tilt and spaceflight. This fluid shift is interrupted temporarily during SRC, but 30-60 min per day might not be sufficient. Nevertheless, if SRC were not sufficient, space travelers could perform fluid loading to compensate for plasma volume loss, as is currently done on the International Space Station (ISS) prior to returning on Earth[1].

The subjects reported fewer neurovestibular symptoms during the 6 x 5 min than during the 1 x 30 min $+G_z$ treatment[53]. The conclusion that a 6 x 5 min $+G_z$ treatment was less stressful was also supported by the subjects' neuroendocrine responses. Indeed, Choukèr et al.[55] found that the 6 x 5 min $+G_z$ treatment was associated with lower adrenocortical stress responses than the 1 x 30 min $+G_z$ treatment in the same subjects.

The 6 x 5 min $+G_z$ treatment also increased the maximal voluntary contraction (MVC) capability in the knee extensor and plantar flexor muscles, which was not the case for the 1 x 30 min $+G_z$ treatment[49]. On the other hand there were no significant differences between the two $+G_z$ treatments in aerobic power (peak VO2) after HDBR compared with the control condition[36].

Serum levels of bone formation markers decreased and serum levels of bone resorption markers increased towards the end of HDBR in control subjects, and these changes were attenuated in centrifuged subjects for both $+G_z$ treatments[52]. A decrease in vertical jump height after bed rest with no countermeasure was also prevented by both the 6 x 5 min and the 1 x 30 min $+G_z$ treatments[49].

In yet another study Vernikos et al.[10] used a crossover design for testing nine subjects across four treatment conditions and one control condition during 5 HDBR campaigns of 4 days each. The treatment conditions included passive (standing still) or active (walking at 3 mph on a treadmill) $+G_z$ stimulation for 8 times 15 min or 16 times 15 min. The interval between two successive sessions was 1 h. When comparing these four treatment conditions and the control condition for which no intervention was used, the investigators showed that periodic +1 $G_z$ as low as 2 h per day was effective in mitigating cardiovascular deconditioning during HDBR.

However, 1 $G_z$ standing was found to be more effective for protecting against orthostatic intolerance and decrease in plasma volume, whereas 1 $G_z$ walking was found to better mitigate the decreased peak $VO_2$ and the increased urinary calcium excretion during bed rest. The investigators suggest "that passive upright standing imposes a greater orthostatic challenge to maintenance of cardiac output and cerebral perfusion than walking since the contraction of leg muscles during walking, in combination with competent venous valves, contributes to venous return via the skeletal muscle pump"[10]. They further recommend that a combination of standing and walking should prove the most effective $G_z$ prescription. Also supporting this

**Table 2.** Summary of the results of the ESA First Bed Rest and Artificial Gravity (BR-AG1) study performed at MEDES in Toulouse in 2010. BR-AG1 consisted in a series of three 5-d HDBR campaigns in which 10 male subjects were not centrifuged (CON), or were exposed to short-radius centrifugation (SRC) generating 1 $G_z$ at the heart for one single session of 30 min (1 x 30 min) per day, or for 6 sessions of 5 min (6 x 5 min) per day. Data from Kos et al.[52]; Clément et al.[53]; Linnarsson et al.[36]; and Rittweger et al.[49].

| Measures | CON | 1 x 30 min SRC | 6 x 5 min SRC |
|---|---|---|---|
| Metabolism | | | |
| - Nitrogen balance | Decreased | Same as CON | No changes |
| - Urinary adrenaline | No changes | Same as CON | Increased |
| Cardiovascular | | | |
| - Plasma volume | Decreased | Same as CON | Same as CON |
| - Exercise capacity | Decreased | Same as CON | Same as CON |
| - Orthostatic tolerance | Decreased | Same as CON | Decreased less |
| - Heart rate | Increased | Same as CON | Same as CON |
| Sensorimotor | | | |
| - Postural instability | No changes | Same as CON | Same as CON |
| - Gait | No changes | Same as CON | Same as CON |
| - Vestibular symptoms | | Moderate | None |
| - Subjective efficiency | | Yes | Yes |
| Muscle | | | |
| - Knee extensor MVC | No changes | No changes | Increased |
| - Knee flexor MVC | No changes | Increased | Increased |
| - Plantar extensor MVC | No changes | Same as CON | Same as CON |
| - Plantar flexor MVC | No changes | Same as CON | Increased |
| - Elbow extensor MVC | No changes | Same as CON | Same as CON |
| - Elbow flexor MVC | No changes | Same as CON | Same as CON |
| - Maximum jump height | Decreased | No changes | No changes |
| Bone | | | |
| - Bone resorption | Increased | Increased less | Increased less |
| - Bone formation | Decreased | No changes | Decreased less |
| - Calcium level | Increased | Same as CON | Same as CON |

view are the results of studies by Yajima et al.[56] and Iwase[32], which demonstrated that daily SRC combined with light leg exercise prevented most of the plasma volume loss during 4- and 14-day HDBR, respectively. Therefore, it appears that exercise is a more effective method of preserving plasma volume during HDBR than centrifugation without exercise.

The above results were recently confirmed in another series of 5-d HDBR during the ESA Simulated Artificial Gravity (SAG) study[35]. This crossover design study was performed under the following conditions with 10 male subjects during 6° HDBR: (a) with no countermeasure; (b) while standing upright for 25 min per day (STA); and (c) during a locomotion-replacement training (LRT) including a combination of heel raising, squatting, and hopping exercise for 25 min per day. The results of the investigations utilizing this protocol are summarized in Table 3. The LRT treatment was found to be more effective than STA for maintaining knee extensor and plantar flexor muscles' integrity. LRT also increased the maximal voluntary contraction capability for the knee extensor muscles after HDBR[57]. STA and LRT $G_z$ treatments were equally useful for preserving postural stability after HDBR[58]. However, neither countermeasure protected against metabolic[59], cardiovascular[35], or bone[49] deconditioning induced by HDBR. The differences between this study and Vernikos' study[10] suggest that $+G_z$ stimulation for 25 min per day applied continuously may be too short to be effective as a countermeasure.

## Limitations and lessons learned

Centrifugation along the $G_z$ axis in supine subjects not only restores the reduced orthostatic intolerance that occurs after HDBR or DI deconditioning, but also redistributes and retains blood in the venous system of the lower extremities similar to the effect of standing. In fact, most subjects perceive themselves to be standing upright when they are exposed to 1 $G_z$ at heart level, i.e. close to their body's center of mass[60]. As discussed above, significant benefits of a 1-$G_z$ stimulation at the heart for as little as 30 min per day were observed for muscle maximum contraction, jump performance, and changes in levels of markers for bone homeostasis during HDBR and DI. A repeated, shorter exposure (6 x 5 min) was more effective than a continuous, longer exposure (1 x 30 min) and was also better tolerated by the subjects. With the shorter exposure, subjects complained of less discomfort due to the prolonged straining caused by high $+G_z$ at the feet. The severity of the neurovestibular symptoms reported by the subjects during SRC was relatively low, with the highest score (13 on a scale from 0 to 45, with 45 being the most severe) reached during the first SRC session[53]. In addition, subjects reported that their perceived rate of recovery after HDBR was faster with SRC than without it.

As pointed out by Kaderka[19] "an important consideration that must be realized when comparing different countermeasure groups is the variation in intent of treatment protocol." Some AG protocols in Table 1 were created specifically to counteract a particular physiological deconditioning, e.g. muscle atrophy or bone loss. Only recently, starting with Young & Paloski[33], HDBR studies have investigated the mitigating effects of AG across several physiological systems. The aim of the protocol is not to benefit any specific physiological system, but rather to evaluate the efficiency of a particular AG prescription across a large range of physiological and psychological responses.

Because both the SAG and the BR-AG1 studies used the same

**Table 3.** Summary of the results of the ESA Simulated Artificial Gravity (SAG) study performed at DLR in Cologne in 2010-2011. SAG consisted of a series of three 5-d HDBR campaigns in which 10 male subjects stayed supine (CON), stood upright by the bed (STA) for 25 min per day, or performed an upright locomotion replacement training (LRT). Data from Mulder et al.[35,57-58].

| Measures | CON | 25 min STA | 25 min LRT |
|---|---|---|---|
| Metabolism | | | |
| - Body mass | Decreased | Same as CON | Same as CON |
| - 24-h urine volume | Increased | Same as CON | Same as CON |
| - Nitrogen balance | Decreased | Same as CON | Same as CON |
| Cardiovascular | | | |
| - Plasma volume | Decreased | Same as CON | Same as CON |
| - Exercise capacity | Decreased | Same as CON | Same as CON |
| - Orthostatic tolerance | Decreased | Same as CON | Same as CON |
| - Heart rate | | Increased | Increased |
| Sensorimotor | | | |
| - Postural instability | Increased | No changes | No changes |
| - Gait | No changes | Same as CON | Same as CON |
| Muscle | | | |
| - Knee extensor CSA | Decreased | Decreased | No changes |
| - Plantar flexor CSA | Decreased | Decreased | No changes |
| - Knee extensor MVC | Decreased | No changes | Increased |
| - Plantar flexor MVC | No changes | Same as CON | Same as CON |
| - Maximum jump height | Decreased | Same as CON | Same as CON |
| - Neural activation | No changes | Same as CON | Same as CON |
| - Fatigability | No changes | Same as CON | Same as CON |
| Bone | | | |
| - Bone resorption | Increased | Same as CON | Same as CON |
| - Bone formation | Increased | Same as CON | Same as CON |

HDBR duration (5 d), +$G_z$ stimulus duration (25-30 min), standardized bed rest core data measures, and a crossover study design, a direct comparison could be made between the effectiveness of intermittent standing, walking-like, and SRC. A qualitative comparison between the changes reported in Tables 2 and 3 indicates that SRC has a better protective effect than standing or walking in terms of metabolism, cardiovascular performance and bone marker changes after HDBR.

The challenge of a crossover study design is to determine the period of time needed between two consecutive HDBR campaigns, so that the effects of the first HDBR have completely washed out before the second HDBR begins. The longer this interval, the better; however, it is difficult to find volunteers who are available for very long periods. Both the SAG and BR-AG1 studies used a crossover design. During the SAG study the interval between the first and second HDBR campaigns was 65 days and the interval between the second and third HDBR campaigns was 114 days. During the BR-AG1 study, the interval between the three HDBR campaigns was 32 days. This 32-d interval was too short, as some of the sensorimotor and musculoskeletal responses had not completely returned to baseline between HDBRs[35,49,52]. For example, bone loss tends to continue for about 30 days after bed rest lasting 35-90 days[61-63], and the exact nature of the bone loss during this recovery period is unclear.

A decrease in serum levels of markers for bone formation (CD200) and an increase in serum levels of markers for bone resorption (CD200R1) were observed after a few days of HDBR, and these changes were attenuated by SRC[52]. Nevertheless, a few days is too short for actually assessing structural changes in bone[64]. Smith et al.[65] did not find significant differences in bone mineral density during SRC compared to controls after a 21-d bed rest. Bed rest studies that have shown a protective effect of exercise on bone were of much longer duration; e.g., 56 days[63] to 117 days[66].

No bed rest studies combined with intermittent centrifugation have examined the structural integrity of muscle fibers (i.e., cross-sectional area and distribution by fiber type) after deconditioning, although this test has been performed in many of the traditional countermeasure studies[7,67]. Future artificial gravity studies on skeletal muscle deconditioning should therefore focus on the analysis of global muscle parameters, such as muscle volume and endurance, but also on individual muscle fibers by fiber type.

Testing the effectiveness of centrifugation as a countermeasure for sensorimotor deconditioning is rendered difficult by the fact that small changes in sensorimotor functions are generally observed after HDBR. A recent systematic study of sensorimotor behavior after long-duration (42-63 days) HDBR demonstrated changes in postural reflexes and functional mobility, but no changes in balance control[68]. The investigators suggested that changes in postural reflexes and functional mobility result from ascending somatosensory changes caused by postural muscle and plantar surface unloading during HDBR. By contrast, postural equilibrium would not be affected by HDBR because the vestibular system is still receiving normal graviceptive inputs even when one is recumbent. When testing postural equilibrium during dynamic head movements, though, Mulder et al.[58] found larger postural instability after a 5-d HDBR, which was mitigated by daily 25-min sessions of standing or locomotion-like exercise. In addition, Moore et al.[69] found that the error in the subjective visual vertical was significantly different from zero in a centrifuged group of subjects and not different in a control group after a 21-day HDBR. The ability to perceive verticality depends on input from visual, vestibular, and somatosensory systems. The abnormal subjective tilt after HDBR may therefore be caused by ascending somatosensory changes through prolonged unloading. Also, because abnormal subjective tilt and postural instability during dynamic head movements are commonly observed in astronauts returning from space[70], we recommend that these two measurements be included in the battery of standardized sensorimotor tests after HDBR or DI.

## Recommendations for future studies

### *Protocol duration*

The European Space Agency (ESA) funded the 5-d HDBR SAG and BR-AG1 studies for a first screening of the potential benefits of intermittent SRC as a countermeasure for mitigating the physiological deconditioning induced by (simulated) weightlessness. As discussed above, these short-duration studies have demonstrated that intermittent SRC was more effective than intermittent standing or walking for mitigating orthostatic intolerance, but longer durations studies are needed to determine the actual effects of SRC on muscle and bone strength. One option is to repeat medium-duration (e.g. 21-d) campaigns, possibly with crossover design to minimize inter-subject variability, for determining the optimal AG prescription. Once the initial beneficial effects are verified during these medium-duration studies, then the duration of the studies would be extended.

Another option is to proceed with 60-d campaigns. For all intents and purposes, the effort and cost of performing a 60-d parallel group study is about the same as for three 21-d crossover design studies. A 60-d intervention also induces larger deconditioning effects, making it easier to characterize the efficiency of the countermeasure on muscle and bone. These long-duration studies would allow a better comparison of the effects of SRC combined or not with exercise, since pilot studies have clearly shown that exercise can complement SRC for mitigating plasma volume loss, as well as muscle and bone loss[71-75]. For exercise during centrifugation, Kaderka[19] suggests adopting the protocol used by many traditional countermeasure studies for preserving leg muscle and bone. This protocol includes a combination of squat/calf presses and cycling in a two-day cycle alternating aerobic and resistive exercise.

Another argument in favor of testing AG during long-duration studies as soon as possible is related to the time limitations of the space program. Indeed, the ultimate goal of these studies is to determine whether AG delivered by SRC can effectively protect crew health and performance during long-duration missions. For a human Mars mission scheduled to launch in 2030, the mission vehicle and habitat designers will need the AG requirements in terms of gravity level and rotation rate several years before, i.e. presumably around 2022. Consequently, there is barely enough time between now and then to conduct at least five long-duration campaigns.

### *AG prescription*

The primary objective of the recommended long-duration studies is to determine the optimal countermeasure prescription in terms of +$G_z$ stimulation amplitude, duration, and frequency on the physiological functions that are affected by exposure to weightlessness. A +$G_z$ acceleration increases the weight of blood and thus the hydrostatic pressure gradient from head to foot. Although the hydrostatic effects on the arterial side of the circulation become important only at high acceleration, even moderate acceleration has relatively large effects on the low-pressure side of blood circulation, i.e. the venous circulation. Venous return is compromised and cardiac output to regions above the heart is reduced. Healthy subjects can tolerate 3-4 $G_z$ at the feet for 90 min[76]. However, deconditioned space travelers and bed rest volunteers may not be able to tolerate these levels of acceleration. In fact, in previous studies using SRC during HDBR or DI, the acceleration at the feet did not exceed 3 $G_z$. Given this limitation and the gravity gradient, the range of $G_z$ stimulus that can be applied at the heart in supine subjects is constrained to 0.38-1 $G_z$. Only a small protective effect of 0.38 $G_z$ at the heart was observed when intensive cycling exercise was used[13,16]. Therefore, a logical path forward is to use HDBR and DI studies to determine the effects of duration and frequency of SRC while keeping a 1 $G_z$ stimulus at the heart.

Another path forward might be the following: rather than imposing a level, duration, and frequency for +$G_z$ stimulation using SRC, each subject will decide what +$G_z$ stimulus they can tolerate on any particular day. By analogy with the individualized prescription used in sports medicine[77], in the proposed studies the individualized $G_z$ prescription will be tailored to a subject's (or crewmember's) specific goals, needs, and abilities. During each daily session, the subject will decide on the duration and frequency of $G_z$ stimulation, as well as its intensity (by adjusting the rotation rate of the centrifuge), within some guidelines specified by a fitness or rehabilitation specialist. A similar approach is currently used for the exercise regime of the astronauts on board the ISS. An individualized prescription motivates the participant to comply with it, thus better achieving the goal of the countermeasure. Unlike athletes, who receive feedback frequently during training and competition, bed rest subjects and space flight crewmembers do not have the benefit of feedback until the HDBR study or flight is completed. However, the assumption is that the more intense the $G_z$ stimulus, the more efficient the countermeasure. The rationale for allowing centrifuged subjects to set the $G_z$ stimulus is based on the desire to achieve optimal $G_z$ loads. When HDBR subjects are exposed to a tilt test or LBNP, they often detect the onset of syncope more quickly than the medical monitor. The same is expected during centrifuge runs. Allowing subjects to set the $G_z$ stimulus will likely bring them closer to their tolerance limit.

Another advantage of an individualization of the AG protocol is that one AG protocol may not work for all, as shown by recent findings of gender differences in response to AG training[78]. One drawback of the personalization of $G_z$ level though, is that comparison with fixed protocols with fixed gravitational force where subjects stand could introduce bias in interpretation of the results.

### *Subjects*

All the 18 studies listed in Table 1 were conducted on male subjects. Despite the fact that female crewmembers comprise only 11% of the individuals who have flown in space[79], and that only two female crewmembers will visit the ISS between May 2015 and May 2018 (vs. 32 male crewmembers, i.e. 6.3%), it is likely that the crew of the human Mars mission will be a mixed gender crew. A recent study indicates that men and women demonstrate different mechanisms for regulating their cardiovascular responses to orthostatic tolerance limit tests following 90 min of AG and 90 min of HDBR. Women appeared to regulate blood pressure while men did not[80]. It is therefore important that AG protocols examine the effectiveness of protocols across gender.

An emphasis should also be placed on documenting the user's point of view in a more systematic manner. In addition to the standardized questionnaire on neurovestibular symptoms, the individuals should provide subjective rating of comfort/discomfort, perceived exhaustion, perceived benefits, and any other physiological or psychological issues associated with the $G_z$ prescription[81].

Finally, the goal of an operational countermeasure is not only to maintain physiological functions within reasonable limits, but also to ensure that individuals can perform nominally after flight[82]. For testing the effectiveness of the $G_z$ prescription, it is necessary to also include some tests of individual functional performance before and after the HDBR. These tests could be based on NASA's Functional Task Test (FTT) or Field Test (FT), which are performed on astronauts immediately after they return from the International Space Station. These simple tests evaluate the crewmembers' ability to stand up from a seated position, recover from falling, walk and step over obstacles without assistance, and see clearly while moving[83-84].

### *Study design*

Although the crossover study design for a 5-d HDBR was time and cost-effective for a quick-look assessment, a longer duration HDBR is more suitable to test countermeasure efficacy. However, a longer HDBR would require a longer washout period, which makes crossover study design impractical from both a time and cost perspective. Also, it is more difficult to recruit volunteers for long-duration HDBR with a crossover design. Therefore the recommendation is to use long-duration HDBR in a randomized, controlled parallel group design. A potential design could be the following: (a) one group of subjects is exposed to HDBR with daily SRC exposure combined with exercise (e.g., squatting, hopping, cycling) on the centrifuge; (b) a second group of subjects serves as a control for the combined effects of HDBR and the superimposed countermeasure. Subjects in this group are exposed to HDBR and perform the same daily exercise in a supine position; (c) a third group of subjects could also be exposed to HDBR except when they stand up and perform the same daily exercise as the other subject groups on the centrifuge. The difference between this third group and the group of subjects who exercise while on the centrifuge should allow a direct comparison of the effects of a $G_z$ stimulus provided by gravitational force and the effects of a stimulus provided by centrifugal force.

With a parallel group design it is imperative that both groups are as homogeneous as possible. For example, in addition to the standardized selection criteria used for previous studies, subjects should be screened for motion sickness susceptibility[85-86] before they are included in a study. Indeed, the single subject who had to withdraw during the BR-AG1 study had a history of high susceptibility to motion sickness. It is also recommended to expose subjects to several SRC sessions with progressively increasing rotation rates during an ambulatory period prior to the HDBR study to ensure they all have a similar tolerance to centrifugation.

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## References

1. Buckey JC, Lane LD, Levine BD, et al. Orthostatic intolerance after spaceflight. J Appl Physiol 1996;81:7-18.
2. Levine BD, Lane LD, Watenpaugh DE, Gaffney FA, Buckey JC, Blomqvist CG. Maximal exercise performance after adaptation to microgravity. J Appl Physiol 1996;81:686-694.
3. LeBlanc A, Schneider V, Shackelford L, et al. Bone mineral and lean tissue loss after long duration space flight. J Musculoskelet Neuronal Interact 2002;1:157-160.
4. Adams GR, Caiozzo VJ, Baldwin KM. Skeletal muscle unweighting: Spaceflight and ground-based studies. J Appl Physiol 2003;95:2185-2201.
5. Norsk P. Blood pressure regulation IV: Adaptive responses to weightlessness. Eur J Appl Physiol 2014;114:481-497.
6. Pavy-Le Traon A, Heer M, Narici M, Rittweger J, Vernikos J. From space to Earth: Advances in human physiology from 20 years of bed rest studies (1986-2006). Eur J Appl Physiol 2007;101:143-194.
7. LeBlanc AD, Spector ER, Evans HJ, Sibonga JD. Skeletal responses to space flight and the bed rest analog: A review. J Musculoskelet Neuronal Interact 2007;7:33-47.
8. Spector E, Smith S, Sibonga J. Skeletal effects of long-duration head-down bed rest. Aviat Space Environ Med 2009; 80 Suppl 5:A23-28.
9. Navasiolava NM, Custaud MA, Tomilovskaya ES, et al. Long-term dry immersion: review and prospects. Eur J Appl Physiol 2011; 11:1235-1260.
10. Vernikos J, Pharm B, Ludwig DA, et al. Effect of standing or walking on physiological changes induced by head down bed rest: implications for space flight. Aviat Space Environ Med 1996;67:1069-1079.
11. Shulzhenko EB, Vil-Viliams IF. Short radius centrifuge as a method in long-term space flights. Physiologist 1992; 35:S122-125.
12. Vernikos J. Artificial gravity intermittent centrifugation as a space flight countermeasure. J Gravit Physiol 1997; 4:13-16.
13. Clément G, Bukley A (eds). Artificial Gravity. Springer, New York, USA, 1997.
14. Benson AG, Guedry FE, Parker DE, Reschke MF. Microgravity vestibular investigations: perception of self-orientation and self-motion. J Vestib Res 1997;7:453-457.
15. Clément G, Moore S, Raphan T, Cohen B. Perception of tilt (somatogravic illusion) in response to sustained linear acceleration during space flight. Exp Brain Res 2001;138:410-418.
16. Moore S, Diedrich A, Biaggioni I, Kaufmann H, Raphan T, Cohen B. Artificial gravity: A possible countermeasure for post-flight orthostatic intolerance. Acta Astronaut 2005;56:867-876.
17. Convertino VA. Exercise as a countermeasure for physiological adaptation to prolonged spaceflight. Med Sci Sports Exerc 1996;28:999-1014.
18. Clément G, Pavy-Le Traon A. Centrifugation as a countermeasure during actual and simulated microgravity: a review. Eur J Appl Physiol 2004;92:235-248.
19. Kaderka J. A Critical Benefit Analysis of Artificial Gravity as a Microgravity Countermeasure. Dissertation for the MSc in Aeronautics & Astronautics, Massachussetts Institute of Technology: Cambridge, MA, USA, 2010. http://dspace.mit.edu/handle/1721.1/59561
20. White WJ, Nyberg W, White P, Grimes R, Finney L. Biomedical Potential of a Centrifuge in an Orbiting Laboratory. Douglas Report SM-48703 and SSD-TDR-64-209-Supplement. Douglas Aircraft Co, Santa Monica, CA, USA, 1965.
21. Nyberg JW, Grimes RH, White WJ. Consequence of heart-to-foot acceleration gradient for tolerance to positive acceleration ($+G_z$). Aerospace Med 1966;37:665-668.
22. Kamenskiy YN, Shulzhenko YB, Andreyeva VG. Effects of systematic exposures to gravity on the physiological function during prolonged immersion. Kosm Biol Aviakosm Med 1976;10:40-45.
23. Gale RR, Usachev VV, Gavrilova LN, et al. Some human reactions to prolonged centripetal accelerations ($+G_z$) of low intensities. Kosm Biol Aviakosm Med 1976;10:35-40.
24. Grigoriev KI, Shulzhenko EB. Effects of minimal gravitational loads on fluid electrolyte metabolism and renal function of man during prolonged immersion. Kosm Biol Aviakosm Med 1979;13:27-31.
25. Vil-Viliams IF, Shulzhenko EB. State of cardiovascular function after immersion for three days and prophylactic spinning on a short-radius centrifuge. Human Physiol 1980a;6:150-154.
26. Vil-Viliams IF, Shulzhenko EB. Functional state of the cardiovascular system system during combined exposure to 28-day immersion, rotation in a short radius centrifuge, and physical loading on a bicycle ergometer. Kosm Biol Aviakosm Med 1980b;14:42-45.
27. Yajima K, Miyamoto A, Ito M, et al. Human cardiovascular and vestibular responses in long minutes and low $+G_z$ loading by a short arm centrifuge. Acta Astronaut 1994;33:239-252.
28. Vil-Viliams IF. Principle approaches to selection of the short-arm centrifuge regimens for extended space flight. Acta Astronaut 1994;33:221-229.
29. Lee S, Bennett B, Hargens A, et al. Upright exercise or supine lower body negative pressure exercise maintains exercise responses after bed rest. Med Sci Sports Exerc 1997; 29:892-900.
30. Iwasaki K, Hirayanagi K, Sasaki T, et al. Effects of repeated long duration $+2G_z$ load on man's cardiovascular function. Acta Astronaut 1998;42:175-183.
31. Katayama K, Sato K, Akima H, et al. Acceleration with exercise during head down bed rest preserves upright exercise responses. Aviat Space Environ Med 2004;75:1029-1035.
32. Iwase S. Effectiveness of centrifuge-induced artificial gravity with ergometric exercise as a countermeasure during simulated microgravity exposure in humans. Acta Astronaut 2005;57:75-80.
33. Young LR, Paloski WH. Short radius intermittent centrifugation as a countermeasure to bed-rest and 0-G deconditioning: IMAG pilot study summary and recommendations for research. J Gravit Physiol 2007;14:P31-33.
34. Yang CB, Wang YC, Gao Y, Geng J, Wu YH, Zhang Y, Shi F, Sun XQ. Artificial gravity with ergometric exercise preserves the cardiac, but not cerebrovascular, functions during 4 days of head-down bed rest. Cytokine 2011;56:648-655.
35. Mulder E, Frings-Meuthen P, von der Wiesche M, Clément G, et al. Study protocol, implementation, and verification of a short versatile upright exercise regime during 5 days of bed rest. J Musculoskelet Neuronal Interact 2014;14:111-123.
36. Linnarsson D, Hughson RL, Fraser K, Clément G, Karlsson LL, Mulder E et al. Effects of an artificial gravity countermeasure on orthostatic tolerance, blood volumes and aerobic capacity after short-term bed rest (BR-AG1). J Appl Physiol 2015;118:29-35.
37. Butler GC, Xing H, Northey DR, Hughson RL. Reduced orthostatic tolerance following 4 h head-down tilt. Eur J Appl Physiol 1991;62:26-30.
38. Baecker N, Tomic A, Mika C, Gotzmann A, Platen P, Gerzer R, Heer M. Bone resorption is induced on the second day of bed rest: results of a controlled crossover trial. J Appl Physiol 2003;95:977-982.

39. Nixon JV, Murray RG, Bryant C, et al. Early cardiovascular adaptation to simulated zero gravity. J Appl Physiol 1979; 71:596-600.
40. Volicer L, Jean-Charles R, Chobanian AV. Effects of head-down tilt on fluid and electrolyte balance. Aviat Space Environ Med 1976;47:1065-1068.
41. Vernikos J, Dallman MF, Keil LC, O'Hara D, Convertino VA. Gender differences in endocrine responses to posture and 7 days of -6° head-down bed rest. Am J Physiol Endocrinol Metab 1993;28:E153-161.
42. Heer M, Drummer C, Baisch F, et al. Effects of head-down tilt and saline loading on body weight, fluid, and electrolyte homeostasis in man. Acta Physiol Scand Suppl 1992;604:13-22.
43. Trappe S, Costill D, Gallagher P, et al. Exercise in space: Human skeletal muscle after 6 months aboard the International Space Station. J Appl Physiol 2009;106:1159-1168.
44. Cavanagh PR, Genc KO, Gopalakrishnan R, Kuklis MM, Maender CC, Rice AJ. Foot forces during typical days on the international space station. J Biomech 2010;43:2182-2188.
45. Sasaki T, Iwasaki K, Hirayanagi K, Yamaguchi N, Miyamoto A, Yajima K. Effects of daily 2-$G_z$ load on human cardiovascular function during weightlessness simulation using 4-day head-down bed rest. Jap J Aerosp Environ Med 1999;36:113-123.
46. Stenger M, Evans J, Patwardhan A, et al. Artificial gravity training improves orthostatic tolerance in ambulatory men and women Acta Astronaut 2007;60:267-272.
47. Stenger M, Evans J, Knapp C, et al. Artificial gravity training reduces bed rest-induced cardiovascular deconditioning. Eur J Appl Physiol 2012;112:605-616.
48. Iwasaki K, Shiozawa T, Kamiya K, et al. Hypergravity exercise against bed rest induced changes in cardiac autonomic control. Eur J Appl Physiol 2005;94:285-291.
49. Rittweger J, Bareille MP, Clément G, et al. Short arm centrifugation as a partially effective musculoskeletal countermeasure during 5-day head-down tilt bed rest – Results from the BRAG1 study. Eur J Appl Physiol 2015;115:1233-1244.
50. Iwasaki K, Sasaki T, Hirayanagi K, Yajima K. Usefulness of daily +2$G_z$ load as a countermeasure against physiological problems during weightlessness. Acta Astronaut 2001; 49:227-235.
51. Akima H, Katayama K, Sato K, et al. Intensive cycle training with artificial gravity maintains muscle size during bed rest. Aviat Space Environ Med 2005;76:923-929.
52. Kos O, Hughson RL, Hart DA, Clément G, et al. Elevated serum soluble CD200 and CD200R as surrogate markers of bone loss under bed rest conditions. Bone 2013;60:33-40.
53. Clément G, Bareille MP, Goel R, et al. Effects of five days of bed rest with intermittent centrifugation on neurovestibular function. J Musculoskelet Neuronal Interact 2015;15:60-68.
54. Caiani EG, Massabuau P, Weinert L, Vaida P, Lang RM. Effects of 5 days head-down bed rest, with and without short-arm centrifugation as countermeasure, on cardiac function in males (BR-AG1 study). J Appl Physiol 2014;117:624-632.
55. Choukèr A, Feuerecker B, Matzel S, et al. Psychoneuroendocrine alterations during 5 days of head-down tilt bed rest and artificial gravity interventions. Eur J Appl Physiol 2013; 113:2057-2065.
56. Yajima K, Iwasaki K, Sasaki T, Miyamoto A, Hirayanagi K. Can daily centrifugation prevent the haematocrit increase elicited by 6-degree, head-down tilt? Pflugers Arch 2000;441:R95-97.
57. Mulder E, Clément G, Linnarsson D, et al. Musculoskeletal effects of 5 days of bed rest with and without locomotion replacement training. Eur J Appl Physiol 2015;115:727-738.
58. Mulder E, Linnarsson D, Paloski WH, et al. Effects of five days of bed rest with and without exercise countermeasure on postural stability and gait. J Musculoskelet Neuronal Interact 2014;14:359-366.
59. Feuerecker M, Feuerecker B, Matzel S, et al. Five days of head-down tilt bed rest induces noninflammatory shedding of L-selectin. J Appl Physiol 2013;115:235-242.
60. Clément G, Deliere Q, Migeotte PF. Perception of verticality and cardiovascular responses during short-radius centrifugation. J Vestib Res 2014;24:1-8.
61. Rittweger J, Frost HM, Schiessl H, et al. Muscle atrophy and bone loss after 90 days of bed rest and the effects of flywheel resistive exercise and pamidronate: results from the LTBR study. Bone 2005;36:1019-1029.
62. Rittweger J, Simunic B, Bilancio G, et al. Bone loss in the lower leg during 35 days of bed rest is predominantly from the cortical compartment. Bone 2009;44:612-618.
63. Rittweger J, Beller G, Armbrecht G, et al. Prevention of bone loss during 56 days of strict bed rest by side-alternating resistive vibration exercise. Bone 2010;46:137-147.
64. Sun B, Cao XS, Zhang LF, et al. Daily 4-h head-up tilt is effective in preventing muscle but not bone atrophy due to simulated microgravity. J Gravit Physiol 2003;10:29-38.
65. Smith SM, Zwart SR, Heer MA, et al. Effects of artificial gravity during bed rest on bone metabolism in humans. J Appl Physiol 2009;107:47-53.
66. Shackelford LC, LeBlanc AD, Driscoll TB, Evans HJ, Rianon NJ, Smith SM, et al. Resistance exercise as a countermeasure to disuse-induced bone loss. J Appl Physiol 2004;97:119-129.
67. Trappe S, Creer A, Minchev K, Slivka D, Louis E, Luden N, et al. Human soleus single muscle fiber function with exercise or nutrition countermeasures during 60 days of bed rest. Am J Physiol Regul Integr Comp Physiol 2008; 294:R939-947.
68. Reschke MF, Bloomberg JJ, Paloski WH, et al. Postural reflexes, balance control and functional mobility with long-duration head-down bed rest. Aviat Space Environ Med 2009;80:A45-54.
69. Moore S, MacDougall HG, Paloski WH. Effects of head-down bed rest and artificial gravity on spatial orientation. Exp Brain Res 2010;204:617-622.
70. Jain VJ, Wood SJ, Feiveson AH, Black FO, Paloski WH. Diagnostic accuracy of dynamic posturography testing after short-duration spaceflight. Aviat Space Environ Med 2010;81:625-631.
71. D'Aunno D, Robinson R, Smith G, et al. Intermittent acceleration as a countermeasure to soleus muscle atrophy. J Appl Physiol 1992;72:428-433.
72. Suzuki Y, Kashihara H, Takenaka K, et al. Effects of daily mild supine exercise on physical performance after 20 days bed rest in young persons. Acta Astronaut 1994;33:101-111.
73. Greenleaf JE, Gundo DP, Watenpaugh DE, et al. Cycle-powered short radius (1.9m) centrifuge: exercise vs. passive acceleration. J Gravit Physiol 1996;3:61-62.
74. Chou JL, Leftheriotis GPN, Stad NJ, et al. Human physiological responses to cycle ergometer leg exercise during +$G_z$ acceleration. NASA TM-112237, 1998.
75. Caiozzo VJ, Haddad F, Lee S, et al. Artificial gravity as a countermeasure to microgravity: A pilot study examining the effects on knee extensor and plantar flexor muscle groups. J Appl Physiol 2009; 107:39-46.
76. Piemme TE, Hyde AS, McCally M, Poror G. Human tolerance to $G_z$ hundred per cent gradient spin. Aerospace Med 1966;37:16-21.
77. American College of Sports Medicine. Guidelines for exercise testing and prescription, 8th edn. Lippincott Williams & Wilkins, Baltimore, MD, USA, 2010.
78. Goswami N, Evans J, Schneider S, et al. Effects of individualized centrifugation training on orthostatic tolerance in men and women. PLoS ONE 10(5): e0125780. doi:10.1371/journal.pone.0125780.
79. Clément G, Bukley AP. Human space exploration – From surviving to performing. Acta Astronaut 2014;100:101-106.
80. Evans JM, Ribeiro LC, Moore FB, et al. Hypovolemic men and women regulate blood pressure differently following exposure to artificial gravity. Eur J Appl Physiol 2015; 115:2631-2640.
81. Bacal K, Billica R, Bishop S. Neurovestibular symptoms following space flight. J Vestib Res 2003;13:93-102.
82. Paloski WH, Oman CM, Bloomberg JJ, et al. Risk of sensory-motor performance failures affecting vehicle control during space missions: a review of the evidence. J Gravit Physiol 2008;15:1-29.
83. Arzeno NM, Stenger MB, Bloomberg JJ, Platts SH. Spaceflight-induced cardiovascular changes and recovery during NASA's Functional Task Test. Acta Astronaut 2013;92:1014.
84. Reschke MF, Kozlovskaya IB, Kofman IS, et al. Initial sensorimotor and cardiovascular data acquired from Soyuz landings: Establishing functional performance recovery time constant. Proceedings of the 20th IAA Humans in Space Symposium, Prague, IAA-HIS-15-1A1-1, 2015.
85. Antonutto G, Linnarsson D, di Prampero PE. On-Earth evaluation of neurovestibular tolerance to centrifuge simulated artificial gravity in humans. Physiologist 1993; 36:S85-S87.
86. Jarchow T, Young LR. Neurovestibular effects of bed rest and centrifugation. J Vestib Res 2010;20:45-51.