



Centrifuges for Microgravity Simulation. The Reduced Gravity Paradigm

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Due to the cumbersome nature of performing real microgravity—spaceflight research scientists have been searching for alternatives to perform simulated microgravity or partial gravity experiments on Earth. For more than a century one uses the slow rotating clinostat as developed by von Sachs at the end of the nineteenth century. Since then, the fast rotating clinostat, the 3D clinostat or the random positioning machine, the rotating wall vessels, tail suspension and bed rest head down tilt and lately the levitating magnets have been introduced. Several of these simulation systems provide some similarities of the responses and phenotypes as seen in real microgravity experiments. However, one should always realize that we cannot reduce gravity on Earth, other than the relative short duration free fall studies in e.g., drop towers or parabolic aircraft. In this paper we want to explore the possibility to apply centrifuges to simulate microgravity or maybe better to simulate hypo-gravity. This Reduced Gravity Paradigm, RGP is based on the premise that adaptations seen going from a hypergravity level to a lower gravity are similar as changes seen going from unit gravity to microgravity.

Keywords: microgravity simulation, centrifuges, hypergravity, partial-gravity, reduced gravity paradigm, space flight, ground based research

INTRODUCTION

When we want to explore the impact of a certain parameter it is quite useful when we are able to vary such a parameter either in time and / or magnitude. This is what we do with exploring gravity. We can bring its magnitude to a minimum by going into free fall or increase its magnitude using centrifuges. The latter is relatively simple, however, to go into extended duration free fall one needs to make use of orbital spacecraft like the International or Chinese Space Stations or un-manned platforms. Such in-flight experiments and studies are still rare, expensive and logistically unreliable. Scientists have been exploring alternatives for long duration free fall, microgravity or better near weightlessness studies. One of the earlier known publication on the subject of microgravity simulation was from Julius von Sachs (1879) who slowly rotated flax (*Linum usitatissimum*) and cress (*Lepidium sativum*) around their horizontal axes. Von Sachs coined the term “*klinostat*” for such a device. Later other models such as regular (Newcombe, 1904; Dedolph et al., 1967) and fast rotating clinostats (Briegleb, 1967), 3D clinostats (Hoson et al., 1997), the Random Positioning Machine (van Loon, 2007; Borst and van Loon, 2009) or the Rotating Wall Vessel (Schwarz et al., 1992) were developed in order to improve on the initial design. Another microgravity simulation is by making use of the magnetic properties of atoms via magnetic levitation (Beaugnon and Tournier, 1991; Berry and Geim, 1997). For

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rodent studies the tail suspension model is often used while the space analog for humans would be the 6 degree head down tilt bed rest (See also Beysens and van Loon, 2015a).

Although the devices were and still are widely used one should also understand the limitations and artifacts associated with their applications (e.g., Brown et al., 1976; Laurinavicius et al., 1998; Leguy et al., 2011; Moes et al., 2011).

Gravity is generated by mass as defined by Sir Newton's 2nd law. However, gravity can also be generated by an accelerating object such as a rocket. It was Einstein who showed, for the first time, that there is no physical demonstrable difference between acceleration generated by mass or generated by linear accelerations, the equivalence principle. The fact that for a sample in a centrifuge we speak of acceleration while we deal with a centrifuge running at a constant speed has to do with the fact that the sample is constantly changing direction, forced to do so being attached or fixed somehow to a center of rotation. If the sample would detach from the centrifuge it will, because of its inertia, fly off in a straight line. It would not be accelerated anymore and "loses" its weight. The latter applies when such a centrifuge would be running in space in a place where there is hardly any gravity field from a nearby moon, planet or any other celestial body. One should note, however, that absolute zero gravity does not exist (Beysens and van Loon, 2015b). So it is because of the samples' inertia and the rotation of the centrifuge that one generates gravity. The magnitude of this gravity depends on the radius of the centrifuge and the angular velocity or speed of rotation. The sample experiences a centripetal acceleration $a_c = \omega^2 r$, where ω is angular velocity (rad s^{-1}) and r the centrifuge arm radius (in m).

In life sciences plant research, it was Sir Knight who exposed garden beans (*Phaseolus vulgaris*) to centrifugal forces and showed the direction of growth of both shoots and roots was the resultant of both Earth 1g and the artificial gravitation as generated by his water wheel centrifuge (Knight, 1806).

Centrifuges are applied in various disciplines mainly in material and particle technology like for separation (Sahuquillo et al., 1999; Kuang et al., 2015), for nanomaterials classification (Wohlleben, 2012), casting (Huisman et al., 1994; Liu and Barnett, 2002; Dikel et al., 2005; Velhinho and Rocha, 2011), combustion (Merzhanov, 1995), but also in areas like geology (Gopal Madabhushi, 2004; Costa et al., 2016), life sciences in plants (Musgrave et al., 2009; Herranz et al., 2013; Hoson and Wakabayashi, 2015), single cells (Loesberg et al., 2006; Tavakolinejad et al., 2015), animals (van Loon, 2001; van Loon et al., 2005; Tateishi et al., 2015) and humans (Akima et al., 2005; van Loon et al., 2012; Goswami et al., 2015).

EXAMPLES OF THE RGP HYPOTHESIS

But how can we use centrifuges for microgravity simulation? This hypothesis is based on the principle of the "Reduced Gravity Paradigm," RGP (Figure 1). With this paradigm we are not focusing on the absolute acceleration values but on the responses generated because of the delta between two accelerations levels.

In space and gravity related research the focus has been to study near weightlessness (microgravity). Samples are brought

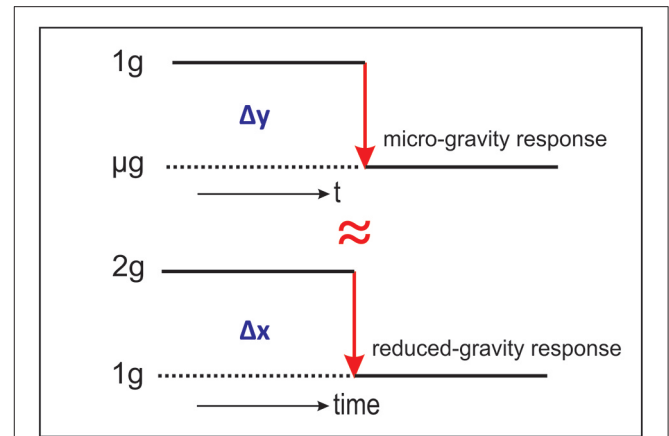


FIGURE 1 | Graphic representation of the Reduced Gravity Paradigm, RGP. Delta between μg and 1g is (nearly) the same as between 2 and 1g.

into near weightlessness or free fall either in drop towers, parabolic and suborbital flights, sounding rockets or with Earth orbiting missions (see Figure 1 Δy). The delta acceleration in such cases is unit gravity, 1g, 9.81 ms^{-2} (excluding the launch acceleration). But what would the response of these same samples be if we go from a steady 2g to a 1g level (see Figure 1 Δx)? Could we expect the same or a similar response?

It is not improbable to presume that such a reduction in gravitational load from 2 to 1g would display comparable responses as going into free fall coming from a 1g state. However, since free fall is such an exceptional environment, where phenomena as convection, buoyancy, sedimentation or hydrostatic pressure are no longer present, we could expect a different response in long duration experiments.

The RGP is best applied for a stable and steady system at a certain higher g level combined with measuring a, relatively, fast responding phenomenon when reducing the acceleration load. In the next part we will attend to some experimental configurations addressing the boundary conditions for a RGP set-up across various disciplines ranging from plant biology to human physiology. These past studies will provide insight how to best configure a RGP experiment.

Figure 2A is an example of hyper-g adaptation of mice from a study by Oshima et al. (2007). Mice were subjected to 2g in a 7.25 m radius centrifuge. Apparently 4 h exposure to 2g is not yet sufficient to reach a steady state for COX-1, COX-2, and VEGF expression in these animals. The mice need to adapt 24 h in order to stabilize these protein levels. Figure 2B shows that steady states might be different for different organs within an animal as is shown by the level of the stress protein HSP70 in rats exposed to $2.5 \times g$ for either 24 h (acute) or 7 months (chronic) acceleration (van Loon et al., 2005).

Figure 3A is an example of how a RGP experiment set-up could look like. In this study by Kozeko and Kordyum (2009) pea seedling (*Pisum sativum*) are exposed to various levels of hypergravity (HG) for 1 h each. After the HG exposure the samples are brought back to 1g for either one or 24 h and the

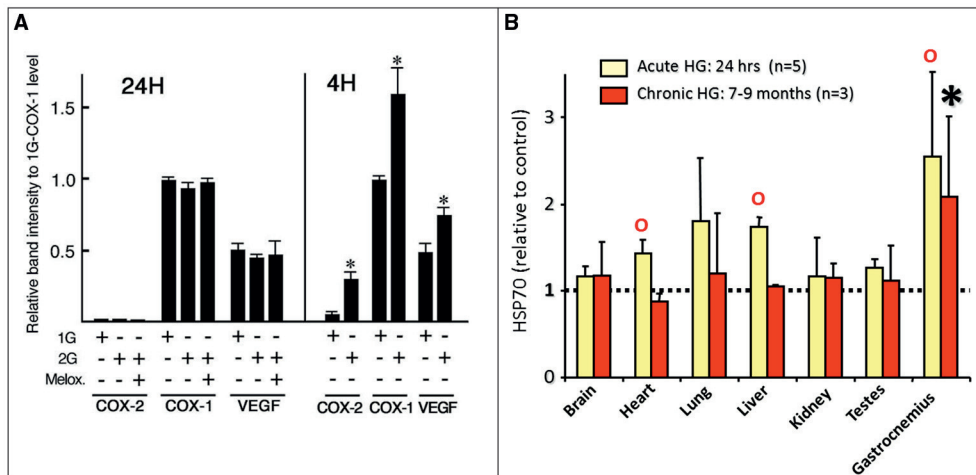


FIGURE 2 | Examples of reaching steady states. (A) Mice exposed to 2 g for 4 or 24 h. Stable levels of COX-1, COX-2, and VEGF in heart tissue seems to be reached at 24 h (from Oshima et al., 2007, with permission). **(B)** Rats exposed for either 24 h or 7 months to 2.5 g (van Loon et al., 2005). In both figures data are means \pm S.D., *and \circ identifies significance of <0.05 with respect to 1 g controls.

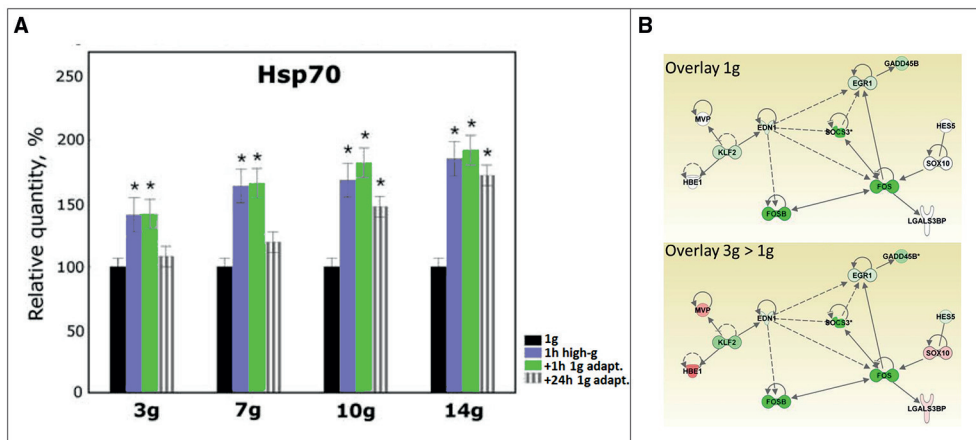


FIGURE 3 | (A) Example Hsp70 content of pea seedlings exposed to 1 h hyper-gravity levels ranging from 3 to 14 g. After HG exposure the seedlings were left for either 1 or 24 h to re-adapt to 1 g. Data are means \pm SD. * indicate values that are statistically different from control ($p \leq 0.05$). (Reproduced from Kozeko and Kordyum, 2009, with permission). **(B)** Example of a Reduced Gravity Paradigm experiment execution. A specific gene network was identified that specifically responds to a 1 day reduced gravity, i.e., going from 5 days at 3 g to 1 day at 1 g (from Aceto et al., 2015).

content of Hsp70 was measured. However, it appears that a 1 h exposure to hyper-g is not sufficient to reach a steady state regarding Hsp70 in these seedlings as can be seen especially in the 10 and 14 g groups (Figure 3A).

Figure 3B shows part of the results of a study where zebra fish (*Danio rerio*) were exposed for 6 days at control conditions at 1 g (Figure 3B, top panel) or one group exposed to 3 g for 5 days and then exposed to a relative reduced gravity of 1 g for 1 day (Figure 3B, lower panel). From this study a network composed of 7 genes (FOS, FOSB, EGR1, EDN1, SOCS3, GADD45B, KLF2) was identified which is affected by the altered gravity conditions, indicating that they represent a central network that is affected by gravitational conditions and are rapidly (within 24 h) adapted to the reduced gravity conditions. Five additional genes (MVP,

HBE1, HES5, SOX10, LGALS3BP) were only affected after 1 day at lower gravity ($3 > 1$ g), indicating that they may be actually involved in the mechanism for rapid adaptation to lower gravity and might be expected to change when exposed to real free fall conditions.

There are also very clear indications of the RGP emerging from studies on the vestibular system. European scientist astronauts of the D1 Spacelab mission identified that an exposure for 60–90 min at 3 g_x and then reduce it to 1 g elicited the same effects as a transition from 1 g to real free fall μ g (Nooij et al., 2007). It is remarkable to note that the post-centrifuge phenomenon remains for several hours after high g exposure, especially since the vestibular system is one of the fastest responding sensor systems with respect to gravity.

A similar phenomenon was described for fish. The vestibular related looping behavior often seen in fish when entering free fall was also reported for animals that were shifted from a high to a lower g-level (Von Baumgarten et al., 1972; Rahmann et al., 1996).

The application of the RGP has its restrictions and limitations. An important prerequisite for a successful RGP experiment is that the system needs to be in a stable / steady state condition at the high g level before lowering the acceleration. Also, the best results are to be expected from fast responding systems. Such systems will react due to the reduced gravity load while not yet responding, too much, to the actual g level the sample is exposed to and, in due time, completely adapted to.

Figure 4 displays some scenarios in gravity related research. A regular scenario in, for example, a sounding rocket experiment would be going from A1 to A2 for fast responding system. When using on-board 1 g centrifuge it would follow the red lines A1–A3. At T₀ the experiment is launched. At T₁ and T₂ the rocket

motor is stopped and the samples are in 1 g for the on-board 1 g centrifuge (T₁) or in free fall (T₂), respectively.

Figure 5A displays the possible responses in a RGP protocol. At T₀ we intend to bring the system in a steady state condition in order to later perform the microgravity simulation. We would have a successful measurement when we would stop the centrifuge at T₁ and measure the fast responding system (red line, A1–A3) measured at T₂. However, when the system has not reached a steady state yet (orange B1 and green C1 lines) a measurement at T₂ does not reflect a full reduced gravity response. (In order not to make the graph too complex, I left out the impact of 1 g. In such a case we need to adapt the system longer at the higher g level). A comparable limitation might arise in parabolic flight experiments (Figure 5B). The rapid cycling between 1 g, 1.8 g, and μg might for especially slow responding systems (orange and green lines) “contaminate” the actual microgravity measurements. A parabola length from T₁ to T₂ is sufficient for fast changes (red line, A3) but for an intermediated responding system one would need a parabola length of T₁–T₃. Even more time is required for slow responding systems (green line) although part of the slow responding system might have contributed to the fast and intermediate signals.

THE GRAVITY CONTINUUM

Finally it is interesting and necessary to note the phenomenon of the “Gravity Continuum” where we also make use of centrifuges in order to predict the value or behavior of a system at a partial- or micro-gravity condition (Figure 6). The basis for this paradigm is the extrapolation of the data of a series of hypergravity experiments as represented as red dots in Figure 6. The theory is that a system would continue to behave similarly as it does coming from higher g levels going toward 1 g. The results could have a linear (orange line Figure 6) or some exponential relationship (green and black lines). Examples of the gravity continuum can be found in humans (Bergstedt, 1961), animal research (Ronca, 2003; Wade, 2005) but also in combustion sciences (Lewis, 1973; Merzhanov, 1995) or geological studies (de Vet, 2010). Comparable results from applying both the gravity

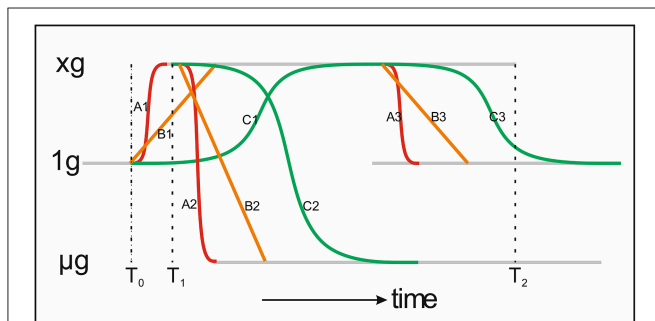


FIGURE 4 | This is a cartoon illustrating gravity levels (gray lines) and (biological) systems response times. Fast responding systems (red line, A1–A3), linearly somewhat intermediate responding systems (orange line, B1–B3) and slow responding systems (green line, C1–C3). When a sample is launched is goes from 1 g to x g, delta y, for a fast responding systems going through A1. When going into free fall after launch the fast system would follow A2, delta y + delta x. In this representation the times for both fast and slow responding systems regarding a Reduced Gravity Paradigm are set properly.

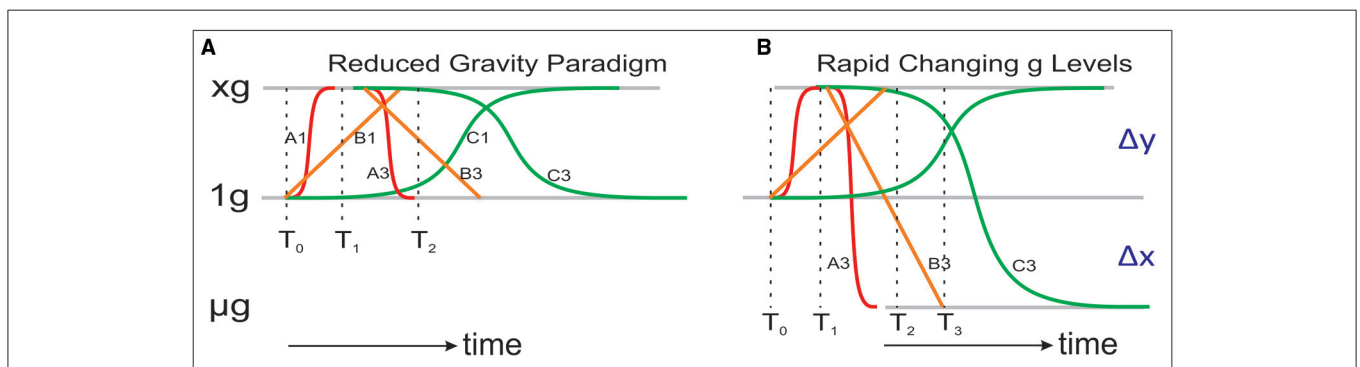
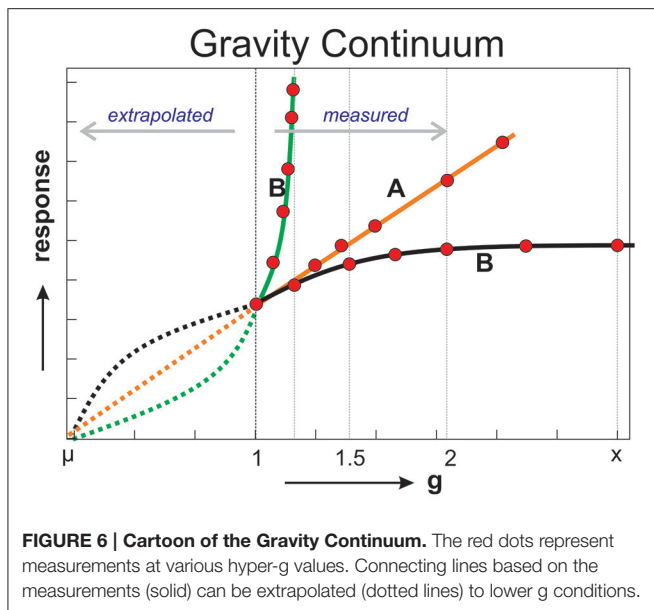


FIGURE 5 | This is a cartoon illustrating in more detail the (A) Reduced Gravity Paradigm (RGP) or (B) a real free fall experiment with rapidly changing g levels (e.g., a parabolic flight experiment). Fast responding systems (red line, A1–A3), linearly somewhat intermediate responding systems (orange line, B1–B3) and slow responding systems (green line, C1–C3).



continuum and RGP in the same study strengthen the final results.

CONCLUSION

Application of the Reduced Gravity Paradigm, RGP, within a study provides, in the first place, a basic knowledge and understanding of how a system responds to altered gravity conditions. In hypergravity studies often the more classical end point measurements at a certain g-level are applied. However,

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preferably on-line measurements of dynamic changes going from a lower to a hyper-gravity level provides a wealth of knowledge on gravity adaptations. On the other hand, with the RGP one focuses more on the changes going into lower g levels. The RGP should be further explored on cell, tissue and system level both in plants, animal and humans.

Although the concept of RGP has to some extent been used in previous studies a systematic approach would provide insight on whether the RGP is a valid concept or if other responses are more pertinent. Such an approach requires the execution of protocols in real free fall conditions to verify their validity. When the RGP hypothesis is proven to be valid it will provide a cheap and reliable way to generate simulated microgravity and thus provide insight in the effect of real microgravity onto a system. It provides an additional simulated microgravity paradigm complementing systems like clinostats, RPMs, tail suspension, tilt tables etcetera.

As with other ground-based simulation paradigms the real response to reduced gravity always need to be verified in actual spaceflight free fall conditions.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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