11

Centrifuges

Jack J. W. A. van Loon

VU Medical Center, Amsterdam, The Netherlands

11.1 Introduction

Research in orbital space flight is using to a large extent the fact that gravity is compensated by the spacecraft's free fall around the globe. Reducing gravity to a minimum (weightlessness) has great advantages with respect to the decrease in mechanical forces, decreased convection, buoyancy, hydrostatic pressure, etc. Minimizing these phenomena also provides opportunities to study processes otherwise obscured at terrestrial gravity acceleration levels ($1g$) such as Marangoni convection or studies involving materials phase shift phenomena.

The use of centrifuges in this field of research is motivated by interest in the impact of weight on systems. One thus needs to explore the full range of the gravity spectrum, from the theoretical zero g up until a certain maximum, whatever that maximum is for the system under study.

When we consider physical parameters acting upon a system, the factor weight is basically not any different from, for example, temperature or pressure, and in order to understand how the systems respond to environmental variables, we need to modulate them. For many systems, it is therefore as relevant to look at hypergravity (any value above Earth $1g$) as well as hypogravity (between 1 and μg) or even near weightlessness.

Gravity generated in a centrifuge is caused by inertia where an object in motion will move at the same speed in the same direction unless forced to change its direction. This is exactly what a centrifuge does; it forces the object to constantly change its direction. This causes acceleration or when compared to acceleration brought about by, for example, the mass of Earth, artificial gravity.

In a constantly rotating centrifuge, the object moves with a constant velocity. However, since the orientation is constantly changed, the object is submitted to the centripetal acceleration $a_c = \omega^2 r$, where ω is the angular velocity (rad s^{-1}) and r the centrifuge arm radius. An object thus experiences the centripetal force $F_c = ma_c = m\omega^2 r$.

Centrifuges/hypergravity is used in both life and physical sciences. Surely, in the process industry and laboratories, centrifuges are widely applied to separate substances from different specific densities being it particles in liquids, liquids with different specific densities [1] or radioisotopes [2]. Sometimes, centrifuges are even helpful to shed light on geological historical events with biblical significance [3]. In diamond synthesis research, both microgravity [4] and hypergravity [5] are used and both environments have their specific benefits.

Over the years, operating the large-diameter centrifuge (LDC) (Figure 11.1 right [6]) at ESA-ESTEC (Noordwijk, The Netherlands), we have performed experiments in both life and physical sciences' domains. These range from, for example, cell biology [7], plant [8] and animal physiology [9, 10] to granular matter [11], geology/planetary sciences [12], and fluid [13] or plasma physics [14]. In all these studies, the impact of weight was explored from 1 up to 20g. Doing so, one could also try to explore the influence of gravity at lower g levels by extrapolating the hypergravity data. Such extrapolation has been proposed earlier via the so-called gravity

Figure 11.1 Two examples of research centrifuges. *Left* The medium-diameter centrifuge for artificial gravity research (MidiCAR) is a 40-cm-radius system for (mainly) cell biology research [16]. *Right* The large-diameter centrifuge (LDC) [6] is an 8-meter-diameter system used for life and physical sciences and technological studies. Both centrifuges are located at the TEC-MMG Lab at ESA-ESTEC, Noordwijk, The Netherlands.

continuum [15]. See for a great publication on artificial gravity in human research the book by Clément and Bukley [16].

11.2 Artifacts

11.2.1 Coriolis

One of the side effects, or artifacts, of rotating systems, like gravity generated in a centrifuge, is the Coriolis effect. Some call it the Coriolis force, but actually it is not a force.

These Coriolis accelerations are experienced by objects which move within a rotating system. It is an effect of rotation that contributes to the impurity of gravity generated by the rotating system.

$$a_{\text{Corliolis}} = (2\upsilon \times \omega)g$$

where a_{Coriolis} is the Coriolis acceleration expressed in units of g, υ = velocity of the moving object, and ω = angular velocity of the rotating system. When the angular velocity is expressed in revolutions per minute (rpm),

$$a_{\text{Corliolis}} = (2\pi\upsilon \times \omega)g.$$

The extend of the Coriolis effect on a sample depends on the axes along which the object moves with respect to the rotation axes. The largest impact is when the motion of the sample occurs in a plane at a 90° angle with relation to the rotation axes. The impact of the effect is relatively larger in fast rotating systems. Therefore, one likes to work with large-diameter centrifuges in order to keep this variable as small as possible. Two rotating systems with different radii but spinning at similar speeds would generate the same Coriolis accelerations on an object that moved with the same speed within such rotating systems. For more details regarding life sciences, see, for example, van Loon [18], and for physical sciences, see, for example, Battaile et al. [19].

Particularly, in vestibular research, the phenomena of cross-coupled angular acceleration is apparent. This occurs while a person rotates the head in another plain than that of the rotating device. This results in an apparent rotation in a direction unrelated to what is actually happening and is perceived as tumbling, rolling, or yawing. The phenomenon results from simultaneous rotation about two perpendicular axes, and the magnitude of this type of acceleration is the product of the two. Cross-coupled acceleration is one of the main courses of motion sickness in rotating systems. See also Antonutto et al. [20] and Elias et al. [21].

11.2.2 Inertial Shear Force

Another quite common artifact in rotating systems is inertial shear. This effect is mainly experienced by samples that are attached to a flat surface fixed in a centrifuge. Since a centrifuge, clinostat, or RPM is describing curved or round trajectories, also the surfaces used, for example to culture cells, need to be shaped to the same curvature as the rotating device. This means cells on a flat surface in a large-radius rotating system experience less inertial shear as the same cells on the same surface in a smaller-diameter system both generating the same *g*-level. The laterally directed inertial shear confounds the actual centrifugal force which acts perpendicular to a sample. This phenomenon and artifact was identified in 2003 [22]. Although we predicted an impact of this artifact for biological systems, no in situ evidence had been shown until recently. It became clear that using large flat surfaces in a fast rotating system (a clinostat) generated inertial shear forces within the cell layers studied [23].

For completeness, we need to mention that inertial shear force is, however, not restricted to rotating system but should also be taken into account with fast accelerating or decelerating linear systems or in vibration studies.

11.2.3 Gravity Gradient

Besides the inertial shear due to lateral forces, one also has to take into account an artifact in the axial direction: the gravity gradient. A volume exposed to gravity within a centrifuge experiences more acceleration further from the center of rotation. Also here, the larger the centrifuge, the smaller the impact of this artifact. Centrifuges are not only used on-ground but also in-flight. In-flight systems are either used to generate an in-flight 1*g* control in order to discriminate between, for example, launch and operations effects and cosmic radiation [24]. In a facility such as the European Modular Cultivation System (EMCS) tailored for plant studied, full-grown plants will have a huge *g*-gradient of +40 and –40 % around the 1*g* center due to its limited radius [24]. This also applies for the application of small-radii human centrifuges that are currently explored as a possible in-flight countermeasure [25]. The gravity gradient in such centrifuges can be as much as over 300 %. Again, large radii would be the credo. A novel and challenging project explores the possible applications for a large ground-based human hypergravity habitat (H^3) that focuses on a centrifuge diameter of some 150–200 m. In this H^3 facility, where humans can live in for periods of weeks or month at constant hypergravity, both the Coriolis and body *g*-gradient are reduced to a minimum [26, 27].

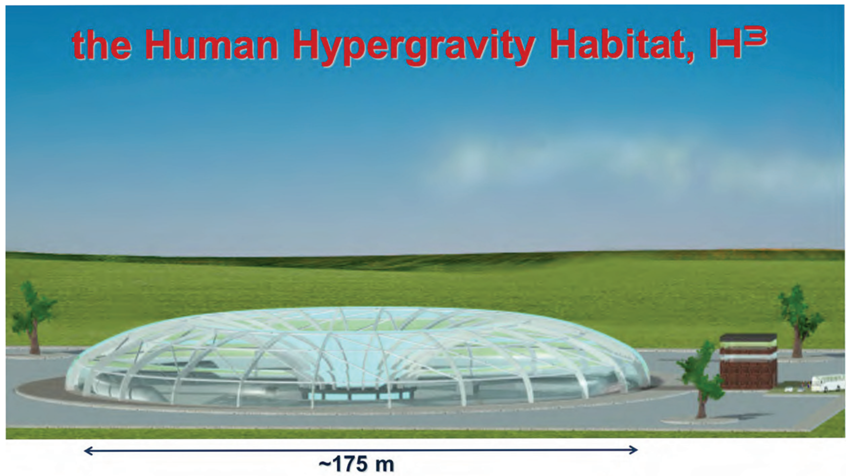

Figure 11.2 View of the outside structure that accommodates the envisaged human hypergravity habitat (H^3). The H^3 is a large-diameter (~175 m) ground-based centrifuge where subjects can be exposed to higher g-levels for periods up to weeks or months. The H^3 can be used in preparation for future human exploration programs as well as for regular human physiology research and applications [26, 27]. (*Image* © BERTE bvba/van Loon et al. 2012).

11.3 The Reduced Gravity Paradigm (RGP)

Particularly, in life sciences cells, tissues or whole organisms respond to gravity based on, most likely, their mass and mechanical properties by changes in, for example, morphology. We see this in cells which change their growth, morphology, and extracellular matrix when cultured at high g loads (e.g. [28]). Also, the cytoskeleton protein actin is increased at increased g-level [29], while actin fiber levels are decreased in real microgravity [30]. In human being, we see an increase in vertebral disk height with decreased gravity loads [31], while disk height decreases with an increased mechanical load [32]. But also in plants, we see an increase in the structural component lignin at higher g-levels compared to $1g$ controls [33].

Based on these observations, I would like to introduce the "reduced gravity paradigm (RGP)". The paradigm would provide insight on the changes seen while going into space (from $1g$ to μg) based on the observations while going from hyper-g to $1g$ condition.

The premise for this paradigm is that the system under study should have reached some sort of steady state at a higher g-level before transferring to a lower g-level. In practice, this would imply starting the experiment in a centrifuge and after some time stop centrifugation, or reducing the centrifugal force to some level. The sample will adapt to the reduced g force. The presumption is that the adaptations during this period are the same, or at least similar, to changes seen while going into real microgravity coming from *terra firma*. Basically, we use centrifuges for microgravity simulation.

A first indication for this paradigm was from a very simple cell biological experiment we did in 2002 where we exposed bone cells to high g forces in the MidiCAR centrifuge [34]. After some time, the centrifuge was stopped, and the cells retrieved and fixed. They were analyzed for cell height. From these experiments, we initially concluded that cell heights increased with increasing g-levels. However, we expected cell heights to decrease with increasing g. To verify this hypothesis, we also conducted a more sophisticated experiment where we measured cell height by means of an atomic force microscope (AFM) accommodated inside a centrifuge. Cell heights were measured while going through three g-levels, 1, 2, and $3g$ [35]. The AFM data showed that the cells were indeed flattened at higher g-levels, verifying our initial hypothesis. However, this did not explain the initial increase in cell heights from the first experiments. What might have happened is that the cells in the initial study were indeed flattened at high g but displayed a kind of overshoot recovery after stopping the centrifuge and before fixation. This response to a reduced gravity might indicate what might happen when cells are exposed to real microgravity.

Such experimental designs could be applied in all fields of research and especially in life sciences where cells and tissues respond, in general, in a more gradual manner than, for example, in purely physical sciences phenomena. Prerequisite for such a "microgravity simulation"—"reduced gravity paradigm" experiment would be that one starts to enter the reduced gravity phase coming from a steady state at a higher g-level. The applicability of this RGP is especially useful for relatively rapid processes. In such processes, we can study the response to a lower g load, while on slower processes, the signals are fainted by the present $1g$ environment. Many, if not all, hypergravity studies are terminated by quickly stopping the centrifuge after which samples are fixed and processed. One might consider to add some additional groups to hyper-g studies and carefully study the readaptation process after the hypergravity loads. The paradigm was introduced at the COSPAR meeting in 2010 [36].

Besides the indication of the RGP by the cell biological experiment mentioned earlier, there has also been a very nice indication of this paradigm from a human physiology study. Quite some astronauts experience motion sickness symptoms referred to as space adaptation syndrome (SAS). Spacelab-D1 crew members together with researchers from TNO (Soesterberg, The Netherlands) were able to "reproduce" these specific in-flight phenomena by exposing astronauts to a 1.5-h +3*g* supine centrifuge run which evoked a sickness induced by centrifugation (SIC) [37, 38]. It appeared that this SIC protocol very closely resembles the in-flight experience by the crew members. So also here the immediate post-centrifuge period displays phenomena similar or even the same as seen in real microgravity, supporting this reduced gravity paradigm.

This RGP might also shed some light on experiments conducted in parabolic flights where we have a series of cyclings from 1*g* to hyper-*g*, to micro-*g* to hyper-*g* and back to 1*g* again. The data gathered in the various gravity stages might be "contaminated" by the previous *g* history. So measuring an effect in micro-*g* might be the actual effect at micro-*g*, but it there might also include some contribution from the transition from $\sim$1.8*g* to a lower *g* period.

There is quite some debate on the reliability of ground-based facilities to simulate a real microgravity environment. Discussions are focused on the one hand on fluid motions and shear stress within clinostats and RPMs and rotating wall vessels (e.g., [39, 40]) or on the other hand on the impact of the magnetic field in levitation studies (e.g., [41]). The advantage of the reduced gravity paradigm is that such artifacts are not present in such studies, other than possible post-rotation effects. The first indication of the applicability of the RGP or "relative microgravity" in a biological study is shown in a zebra fish study by Aceto et al. [42].

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