HYPERGRAVITY STUDIES IN THE NETHERLANDS

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ABSTRACT

It looks like that with the utilization phase of the International Space Station (ISS) scientists will have the possibility to perform long duration and more sophisticated microgravity experiments than could be performed previously. In preparation for these spaceflight studies, ground based experiment tools for simulated (or real) microgravity and hypergravity are important.

To provide the infrastructure and user support necessary to perform these ground based studies we have setup the Dutch Experiment Support Center, DESC (1).

This paper will focus on the three centrifuge facilities. It is shown that these hypergravity facilities can be used to show sounding rocket launch effects, identify alterations in body mass, bone parameters and matrix composition in rodents as well as to derive a test protocol for the Space Adaptation Syndrome in humans.

DESC coordinates the use of these centrifuge facilities.

INTRODUCTION

In general it might be stated that ground based hypergravity (HG) research is important in gravitational biology. Facilities for ground based research can be used to identify possible effects of gravity before performing costly and time consuming real microgravity experiments for shuttle or space station. The facilities available within the Netherlands for ground based research are the *Random Positioning Machine (RPM)*, the *Free Fall Machine (FFM)* and the *2D fast rotating clinostat* for microgravity simulations and the *MidiCAR centrifuge* for cell / tissue culture studies, the *large animal centrifuge* and finally the *human rated centrifuge*.

Ground based research covers all the activities that are performed on Earth as ongoing research, in preparation of real microgravity experiments or as post-flight control studies. Why would one perform ground based research studies?

Various reasons come to mind when thinking of ground experiments within the field of acceleration research (2), some of which are interesting, while others are even necessary. Some of these incentives are:

? to perform basic scientific studies on the effects of accelerations (weight) on living systems

? in preparation of real microweight experiments on board orbiting spacecraft, sounding rockets, parabolic flight aircraft

? define, more specifically, the parameters which might be changed in real microweight conditions to better define the 'flight-experiment' set-up

? test hardware performance under simulated or hypergravity (*e.g.* launch) conditions

? define the interaction of the system under study in relation to the hardware being used in a real microweight experiment

? investigate the effect of launch accelerations and vibrations on the system under study or in combination with the applied hardware.

And, most of all:

? since most ground based research facilities are readily available, studies may be performed on a day to day basis and generates sound scientific data publishable in applicable journals.

The importance of ground based research has been addressed before. In reports both from the European Science Foundation (ESF) (3) as well as from the American National Research Council (NRC) (4) this issue is clearly emphasized. The ESF paper states, "Adequate funding of ground-based research is of utmost importance for the utilization of the space station." To implement these recommendations, the ground-based facilities addressed in this paper are available for ESA and non-ESA scientists, engineers, students and others. (5)

NL HYPERGRAVITY RESEARCH FACILITIES

With respect to gravitational physiology gravity should be regarded as a continuum. Within the Netherlands the upper part of this continuum is covered by 3 types of centrifuges ranging for research on cells or other small systems (MidiCAR centrifuge), to the large radius animal centrifuge and even a human rated centrifuge.

The MidiCAR centrifuge

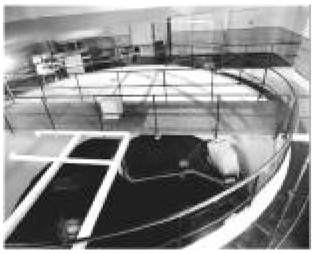
The Medium Sized Centrifuge for Acceleration Research (MidiCAR) is a dedicated centrifuge in which samples may be exposed to accelerations up to 100 × Earth gravity.(6). The facility is accommodated in a temperature-controlled incubator and driven by dedicated software. Gravity levels may be chosen according specific user requirements, either static or dynamic. The system may be applied for short term (seconds) or long term (weeks) studies. Dedicated experiment vessels are compatible with standard laboratory hardware but also flight specific modules (such as *e.g.* for Biopack, Biobox or Biolab) may be accommodated. Data and power lines are available on the rotating and static positions. Both rotating and static control samples are housed in the same environment. Electrical connections are integrated at static and rotating positions so experiments can be commanded or monitored. The centrifuges can be used for typical cell / tissue culture experiments or for studies using small animals or plants.

The large radius animal centrifuge

The large radius animal centrifuge may be used for applying access gravity to small laboratory animals (rat, mouse, hamster, rabbit, pig, fish), plants, 'non-living' samples, technology experiments or complete (small, locker/drawer sized) payloads for Shuttle or space station. Animals may be housed in the centrifuge for prolonged periods of time up to several life cycles. This large diameter system (up to nearly four meters) is powered by a 3.5 kW DC motor that drives two arms on which the swing-out gondolas are attached. Maximum acceleration of the system is dependent on the mass of the experiment but ranges from 1 to ~ 8×g for a payload mass of about 70 kg. The system is equipped with power and data lines. Animals can be monitored by video cameras while rotated. Dark / light regimes can be freely programmed. In addition to the presently 2 arms these is also the possibility to have a rotation control experiment in the central axes of the centrifuge.

The human rated centrifuge

The human rated centrifuge at the Netherlands Aeromedical Institute (NAI) is mainly used for g-load training of fighter pilots. In addition the 8-meter diameter is used in research projects. A 175 kg payload can be accelerated to a maximum of 23.5 Gz with an onset rate of 3.5 Gz per second. The facility provides ample means for measuring physiological parameters. The size of the swing out gondola is 0.5 meter wide and 2 meters long. The gondola also accommodates a virtual 'outside world' and voice / video communication.



RESULTS OBTAINED

MidiCAR Centrifuge

Real microgravity experiments are preceded by launch accelerations especially in sounding rockets. In an experiment performed by Guntermann and Jones (7) it was investigated whether hypergravitational loading leads to changes in the protein phosphorylation pattern of primary bovine osteoblasts as it does in mechanically loaded cells. The second aim was to evaluate whether reducing the ambient temperature can minimize these processes. The cells were exposed to 11×g for two min. at 37°C and 8°C respectively. Total cellular proteins were resolved by gradient SDS-PAGE and immunoblotted using anti-phospho amino acid specific monoclonal antibodies. Primary results showed that 11×g loading can induce phosphorylation / dephosphorylation and that keeping cells at 8°C can reduce these effects.

The large radius animal centrifuge

The present large radius centrifuge has been used for more than a decade now. Most of the previous experiments were performed to study the effect of increased acceleration on the vestibular system. More than a year ago a long duration, second generation rat study has been initiated. In this study rats were conceived, born and raised under 2.5×g. At termination of this experiment these rats were around 9 or more months of age. While the vestibular system is the major topic for this study, other research themes are total body composition, bone (composition and structure), tissue stress proteins, collagen degradation, matrix protein. Future experiments might include studies on brain development and general metabolism. Scientists interested in participating in these studies are invited to contact DESC.

Vestibular system: Effects on the vestibular system for the HG rats are reported by Wubbels *et al.* (in this issue)

Bone: The studies on bone and total body composition are performed by various research groups from the Netherlands, Belgium, France and Denmark.

Bravenboer *et al*. (8) looked into total body composition and bone mineral content of 2.5×g HG rats. In this mechanical loading model they wanted to investigate the effect on bonemass by DXA (VU-Amsterdam) and by histomorphometry, (Dr Zerath and Dr. Holy, France). In addition these bones were also tested for structure and biocompetence using microCT, which was performed in Nijmegen (Tanck *et al*. (9)) and by biomechanical bending tests, which were performed by Dr. Mosekilde. Furthermore, Bravenboer *et al*. measured the concentration of IGF-I and IGFBP3 in tibiae. DXA scans of extracted tibiae did not show any significant differences between hypergravity and control. At present measurements on total body DXA-scans of hypergravity and control animals are performed. Preliminary data of 10 rats show that BMC and BMD as well as bodyweight and lean body mass do not differ between the two groups in female rats. However fatmass measured in grams ($p<0.01$) and as percentage of bodyweight ($p<0.01$) is significantly lower in the hypergravity female rats compared to the control female rats. Most of these parameters were changed in male rats (See Table I).

In conclusion so far: Bonemass and bone composition (IGF-I and TGF-ß) are unchanged under hypergravity conditions. Hypergravity reduces fatmass but not total bodyweight in female rats.

	HG	Con	%change
BM MALES	441.3±31.9g	551.5±28.0g	-20%
BMC MALES	9.9±0.9g	12.5±0.7g	-21%
LBM males	371.5±20.4g	420.9±13.5g	-12%
Fat males	42.5±9.7g	91.8±14.4g	-54%
Fat females	18.3±5.1g	33.9±6.4g	-46%
% fat males	9.9±1.6%	17.4±1.9%	-43%
% fat females	6.4±1.9%	11.2±1.7%	-43%

Table I: DXA scan results of body weight (BM), bone mineral content (BMC) and lean body mass (LBM) of HG males were significantly reduced compared to control males although there was no difference in bone mineral density (BMD). No significant differences in these variables were seen between HG females and control females. Both in females and in males there was a significant reduction of fat mass in the HG rats compared to the control rats. This reduction was still significant as a percentage of body weight.

Preliminary data on bone histomorphometry showed a reduced ash weight and % cancellous bone volume in pelvis and humeri from HG rats, respectively. Humeri from HG rats showed an increase in cortical thickness of about 10-20% compared to 1×g controls.

Evaluation of bone architecture by 3D μCT revealed a decrease in the ratio for total bone volume over trabecular volume in HG rats. In addition there was a significant increase in degree of anisotrophy in the trabeculae of the femoral head. The latter was not seen in proximal tibia of HG rats (9). In another series of animals Schoutens (Univ. Libre, Brussels) looked into a xx kDa bone marrow protein, a protein reported to be associated with mechanical loading (10). There was no difference found in the inhibition of proliferation of osteoblast like cells in contact with bone marrow derived from HG rats. Bank *et al*. investigated the biochemical properties (amount of Hyl residues in the triple helix, cross-links hydroxylysylpyridinoline, and lysylpyridinoline) and the percentage denatured molecules of the collagen network in rat bone (tibia and femur) and tail tendon. Although large differences exist between the various parameters in the different tissues, no significant differences were found between the control group and the hypergravity group. Van Nieuwenhoven *et al*. measured the stress protein HSP70 both in rats exposed to HG for 7-9 months and in animals exposed to 24 hrs. acute HG (11). The HSP-70 content of heart, liver and gastrocnemicus muscle was increased significantly in acute HG rats. Chronic animals showed a significant increased HSP-70 content in only the gastrocnemicus muscle. There were no significant differences in brain, lung, kidney and testis.

The human rated centrifuge

One of the research projects using this facility was to study the effects of the Space Adaptation Syndrome (SAS). Groen *et al*. (12) exposed experienced astronauts to an access 3×g before challenging their vestibular and visual systems using rotating chairs, tilting rooms and simple head movements. Using this protocol the vary same astronauts (total n=8) that did or did not report SAS symptoms in flight stated similar sensations after exposure to the above protocol. Since in this pilot study there was a 100% correlation with in-flight and on-ground data the authors argue that this Sickness Induced by Centrifugation (SIC) protocol might be used to identify and maybe select persons that are susceptible to SAS.

DISCUSSION / CONCLUSION

Ground based research provides a way to test working hypotheses and generates more knowledge on the system under study. It should identify, more precisely, the parameters that might change under real microgravity conditions. However, as the results in Figure 1 clearly show, data generated in *e.g.* hypergravity experiments cannot be directly extrapolated towards microgravity values. In this figure the data of both real microgravity (IML-1 and IML-2 missions) (13) and ground based hypergravity (14) experiments on growth, mineralization and mineral resorption in fetal mouse metatarsal long bones are shown. And although for mineralization (and growth) there seems to be some linear relation with gravity this relation does not exist for mineral resorption, which seems to have an optimum at 1×g.

An active ground based research program will generate more data hence science papers then can be expected

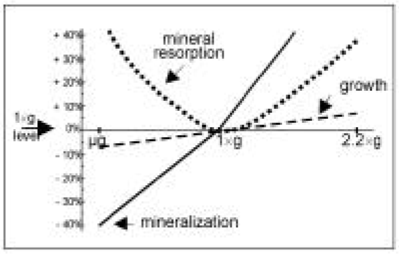


Figure 1: Fetal mouse long bones response to gravity ranging from micro-gravity to 2.2×g hyper-gravity after 4 to 5 days in tissue culture.

from the present situation. Ground based research could easily be incorporated in student programs. This will generate a young and active basis of future gravitational biologists and physiologists. However, ground based research should be conducted in concert with studies in facilities available for sounding rockets, shuttle and the International Space Station.

Spaceflight experiments are expensive and time consuming, while the flight opportunities are very limited. It could be considered to handle spaceflight experiment proposals in a two-step process. First a peer reviewed proposal has to be tested in microgravity simulators as well as in hypergravity facilities on ground to test the proposed hypothesis. If the system under study does not respond to any of the gravity regimes on ground it might be considered to 'disqualify' this study for a real microgravity flight experiment. This would be in favor for experiments that have been proven to be responsive to gravity.

The use of ground based microgravity simulators as well as the extension of the acceleration gamma from hypogravity to hypergravity greatly attributes to the understanding of the effects of weight on living systems and the mechanisms involved, as has been described above. More detailed hypothesis of the effects of (micro) gravity may be generated using ground based facilities. However, these hypotheses have to be verified under real microgravity conditions.

NOTES & REFERENCES

1: DESC. The Dutch Experiment Support Center is a new Dutch initiative which main objective is to increase the scientific output for acceleration research. To accomplish this, DESC supports, initiates and facilitates acceleration research by providing know-how and access to ground based research facilities and by offering services and (laboratory-) assistance for ground based and flight experiments. DESC is part of a broader user support entity named DUC, the Dutch Utilisation Center, which is part of a European wide network of user centers. Other entities within DUC are the Dutch Operations Center (DOC) mainly located at the NLR and the associated industrial partners for hardware and software development (Bradford Engineering, CCM, Fokker Space, Stork Engineering, Origin and others). The main location for DESC is the Vrije Universiteit in Amsterdam. DUC is a combined initiative for user support and is governed by the Netherlands Agency for Aerospace Programs (NIVR) and the Space Research Organisation of the Netherlands (SRON).

2: Acceleration research covers the total area of research from hypoweight ('hypogravity') and hyperweight ('hypergravity'). This includes real microweight aboard orbiting spacecraft, sounding rockets or parabolic flight aircraft. Hypoweight ranging from microweight up to normal weight conditions (='1×g') and hyperweight generated by centrifuges. Ground based acceleration studies apply to centrifuges and microweight simulators. Some also include parabolic flights and droptowers. The term weight is used in stead of, the more common term, gravity, since especially in orbiting spacecraft it is the weight, which is brought to a minimum, while the gravity levels are only slightly reduced.

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5: Web page information for all the mentioned ground based research facilities is: http://www.desc.med.vu.nl. The go to 'Ground Facilities'.

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