G. Gauquelin-Koch, Life Sciences Program Manager
CNES, 2 place Maurice Quentin, 75039 Paris, France.

Life Sciences in Space

Technological advances have always exposed humans to extreme situations and hostile environments. And our species has always explored, exploited and colonized its environment while seeking to extend its horizons. Today, space is our new frontier. The microgravity of space is an unfamiliar environment to all living things - be it a cell, animal or human - and at the same time a fertile ground for life sciences research. The scientific method—tracing its roots back to French physiologist Claude Bernard—that consists in studying the effects of removing or changing the scale of gravity to gain greater knowledge of a system amply justifies the efforts expended on research into humans and animals in space. The microgravity or minigravity environment found on the Moon or Mars is therefore vital for fundamental sciences to better understand how life first appeared and developed.

The motivations and challenges of an exploration program are many. They may be:

- scientific, aimed at increasing our knowledge and understanding of the Universe and studying the human organism's behavior;
- technological and industrial, with a view to nurturing innovation;
- political, seeking to develop international cooperation through a joint effort or to demonstrate the nation's technological capability;
- social, to encourage science education and engage the public's interest;
- or economic.

At the present time, crewed missions to Mars in the short or medium term are not possible, given the obstacles to overcome, which include the length of the trip, launch window constraints, radiation to which the crew would be exposed and life support (food and water).

Life support is essential to mission success. Throughout evolution, one of the main processes of selection has been and still is the ability of living organisms to match their calorie intake to the energy they expend finding food. This key concept of evolutionary biology obviously applies equally well to humans and can be encapsulated in the notion of energy balance. The most patent examples we know of the effects of a long-term imbalance are on the one hand malnutrition and cachexia, and on the other hand overweight and obesity. History abounds with examples of exploratory expeditions where poor knowledge of nutrition proved fatal.

At the start of the 21st century, we find ourselves facing the same dilemma. Focusing our space exploration policy on the Moon and Mars obliges us to carry out a precise and exhaustive assessment of the long-term macro- and micro-nutritional effects of the space environment on astronauts. Indeed, failing to define these effects in optimal fashion can have a serious physiological and psychological impact on the crew, potentially compromising not only their health (muscular atrophy, exercise capacity, bone loss, immunodeficiency disorders and cardiovascular problems) but also the proper conduct of the mission (anorexia, nausea, stress, relational problems, etc.).

We therefore need to proceed in a stepwise manner by first sending shorter crewed missions to the Moon or asteroids, and for that we need the International Space Station (ISS) and ground simulation models. CNES is working on international programs with a range of French laboratories, with its partners in Europe, Russia, the United States and China. These scientific efforts must focus on space medicine, with experiments on the cardiovascular system, bones and muscles (see the article on bears), immunology (see the article on this topic), neurosciences and psychology.

In the field of space medicine, CNES has developed a tele-operated system for performing remote ultrasound scans. Such scans are frequently conducted in hospitals here on Earth and will be vital on future long-duration spaceflights to gauge the effects of microgravity. They will also be needed to diagnose any of the numerous "terrestrial" pathologies that astronauts are likely to carry with them into space.

An ultrasound scan is one of the non-invasive imaging examinations used to investigate abdominal, pelvic and cardiovascular organs in an emergency or for clinical monitoring. But it is not possible to guide an untrained operator with vocal or video cues to obtain a scan of an organ, because rotating or tilting the probe just a few degrees either way can make all the difference between a clear image and one that is unusable for diagnostic purposes. Teletransmission systems must therefore be capable of "teleporting" the hand movements of an echography expert to the site where the patient is being examined (telemanipulation). They must also allow rapid transfer of ultrasound imagery from the site back to the expert center for processing and diagnosis off line. As a result, two modes of remote ultrasound scanning have been

Fig. 1: Zinnia flowers are starting to grow in the International Space Station's Veggie facility as part of the VEG-01 investigation. Veggie provides lighting and nutrient supply for plants in the form of a low-cost growth chamber and planting "pillows" to provide nutrients for the root system. *© NASA*

Fig. 2: ESA (European Space Agency) astronaut Samantha Cristoforetti prepares the TripleLux-A experiment for return on SpaceX's Dragon cargo craft. TripleLux-A is investigating immune suppression in space as understanding such risks is essential in maintaining the health and performance of crew members during long-duration missions. *© NASA*

Fig. 3: NASA astronaut Reid Wiseman, Expedition 40 flight engineer, installs Capillary Channel Flow (CCF) experiment hardware in the Microgravity Science Glovebox (MSG) located in the Destiny laboratory of the International Space Station. CCF is a versatile experiment for studying a critical variety of inertial-capillary dominated flows key to spacecraft systems that cannot be studied on the ground. *© NASA*

developed, one in real time using telemanipulation of the probe by a robot, and the other in near-real time based on 3D image capture and later processing.

Other ground-based experiments simulating weightlessness, based for example on prolonged bedrest of up to three months, are being performed in various countries and are vital for studying the effects of sedentary conditions and confinement. Some are being conducted in France at the MEDES space clinic and others in Cologne and Beijing. But as the current limiting factors are psychological problems and radiation exposure, new programs have had to be developed.

CNES is working with IMPB, the Russian Institute of Biomedical Problems in Moscow, and the Astronaut Chinese Center (ACC) in Beijing on confinement experiments that will be of prime importance in determining the psychological impact on humans of a trip to Mars. Experiments are also being performed at Dome C by the Concordia research base in Antarctica, which is able to accommodate a team of 15 people in winter as opposed to 40 in summer. In addition to glaciology, climatology, astronomy and geophysics experiments, psychology and medical research is also being conducted. Long-term confinement of a small group of humans is ideal for defining typical profiles for Mars exploration missions. Medical research is also looking at altitude hypoxia, dehydration and permanent night from May to August, which alters circadian rhythms through continuous exposure to artificial light.

Few programs on the ground enable radiation effects to be studied. That is why CNES is giving scientists opportunities to fly experiments on balloons for different durations at a range of altitudes.

We therefore firmly believe that the exploration program must be envisioned as a balanced international collaboration in which each contributor has a key role to play.

G. Tascher[1] - S. Blanc[2] - E. Lefai[3] - F. Bertile[2]
[1] University of Strasbourg.
[2] Institut pluridisciplinaire Hubert Curien, CNRS.
[3] Laboratoire CarMeN, Inserm, Inra.

Life Sciences in Space

Muscle atrophy mechanisms: a case study of hibernating brown bears

Muscle wasting in humans is usually associated with ageing, physical inactivity, certain diseases (cachexia) and microgravity conditions. While we are starting to gain a better understanding of the underlying molecular mechanisms [4], there is still no effective solution for preventing or treating it. Investigating the biodiversity of how animals adapt to environmental constraints, notably in hibernating brown bears, could help to develop new therapeutic approaches. Our results suggest that regulation of metabolic networks, reduction of oxidative stress, recycling of nitrogen and anti-atrophy factors are all key elements in preserving muscle in inactive bears that could possibly be transposed to humans.

Fig.1

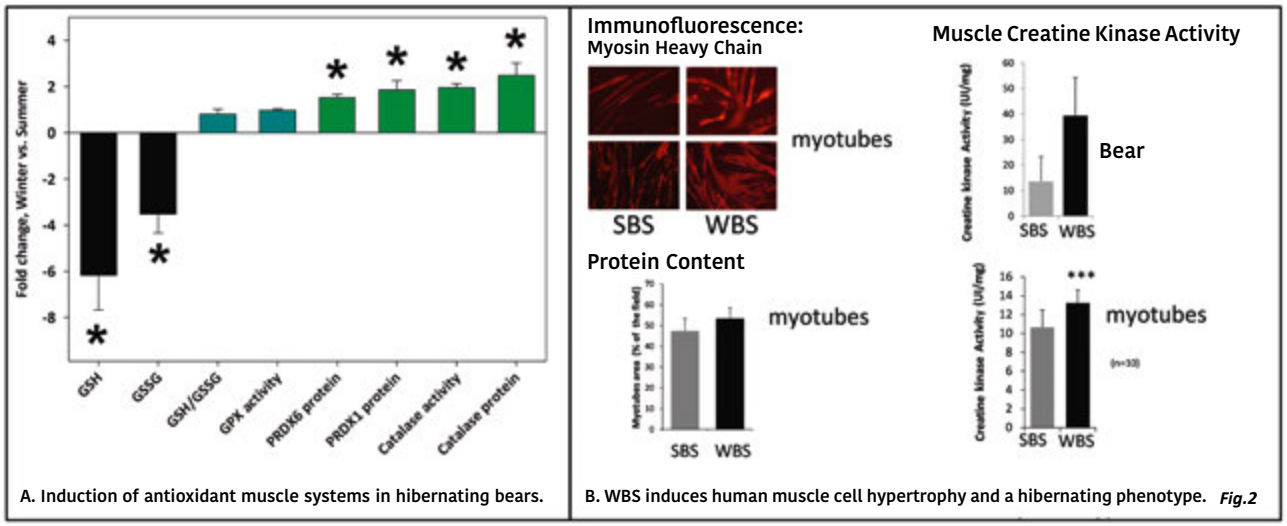
We examine protein-sparing strategies in the Scandinavian brown bear [1, 2], which is a unique counter-model able to maintain muscle mass and strength [3] during a hibernation period of five months, while remaining totally inactive and without food or water. Rates of protein synthesis and loss are reduced by 60-70% during hibernation [4], but the molecular mechanisms controlling protein sparing are unknown. Working with biopsies and blood samples, and using a multidisciplinary approach at the interface between physiology, cellular biology and analytical chemistry, we compare active and inactive states in bears.

We first developed overall omic analysis methods [5] and showed that the processes governing muscle development and strengthening of muscle fibers are activated and that muscle function (e.g. mitochondrial metabolism and biogenesis) remains stable during hibernation. Regulation of lipid metabolism (eicosanoids, glycerophospholipids and sphingolipids) could also be a contributor to protein sparing. To complement these data, we shall evaluate the role of metabolic sensors (e.g. mTOR, ChREBP, PPARs, AMPK, SIRT) and miRNAs.

A reduction in oxidative stress in inactive bears could also contribute to protein sparing [6]. We show that levels of antioxidant enzymes in muscles are increased during hibernation (Fig. 2A), resulting in lower levels of lipid peroxidation and protein carbonylation.

We also seek to determine whether nitrogen recycling mechanisms exist during hibernation. Ursodeoxycholic acid (UDCA) has cytoprotective and anti-apoptosis effects, and appears to limit the tissue loss associated with cancer cachexia [7, 8]. Our measurements show that levels of all bile acids except UDCA are reduced in inactive bears. The exact origin of UDCA (produced by the liver or from the intestinal microbiome) is under investigation, as well as its possible role in sparing muscle protein.

An isolated study performed some ten years ago showed that levels of proteolysis and messenger RNA encoding proteolytic factors were reduced in the muscles of rats incubated ex-vivo with plasma from hibernating bears [9].



A. Induction of antioxidant muscle systems in hibernating bears.

B. WBS induces human muscle cell hypertrophy and a hibernating phenotype. *Fig.2*

Fig.3

Fig. 1: Brown bear *© Thinkstock*

Fig. 2: Mechanisms governing protein sparing in hibernating brown bears. *© Fabrice Bertile, CNRS, Strasbourg, France*

Fig. 3: Captured brown bear in its den. *© Andrea Friebe, the Scandinavian Brown Bear Research Project.*

By exposing human myotubes to serum from hibernating and non-hibernating bears, we demonstrate the existence of anti-atrophy factors circulating in the serum of hibernating bears, capable of producing powerful cross-species effects (Fig. 2B). We are now working to identify this compound/these compounds, which represents a major analytical challenge but the expected benefits if we are successful are simply huge. We will then need to verify if the anti-atrophy factor(s) are capable of limiting, preventing or even reversing induced muscle wasting.

We therefore identified mechanisms likely to contribute to protein sparing in hibernating brown bears. In particular, the demonstration that there are circulating factors capable of inducing a hibernating phenotype in human muscle cells holds promise for devising possible new preventive or therapeutic solutions to combat human muscle wasting, notably in microgravity conditions.

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C. Fonte, J. Jeandel, S. Kaminski, S. Ghislin and J.-P. Frippiat
EA7300, Faculté de Médecine, Université de Lorraine, 9 Avenue de la Forêt de Haye, 54500 Vandoeuvre-lès-Nancy, France.

Life Sciences in Space

Gravity changes and immune cell development

While the effects of spaceflight on innate immunity and cell mediation are starting to be better understood, its impacts on humoral immunity and immune cell development are much less well known. We therefore studied these impacts on the amphibian *Pleurodeles waltl* and the mouse. We highlighted qualitative and quantitative changes in the production of antibodies in response to antigen stimulation in flight, a reduction in B lymphopoiesis in real and simulated microgravity conditions, and a significant change in the repertoire of antigen receptors of T lymphocytes when mice develop in hypergravity.

Spaceflight entails a combination of mechanical and socio-environmental stresses (accelerations on liftoff and landing, microgravity, vibrations, confinement, social isolation, radiation and altered circadian rhythms) that weaken the immune system in humans and animals [1].

These effects induce a reduction in phagocytary and oxidative function in neutrophils and monocytes, in production of Th1 cytokines, and in activity of natural killer (NK) cells and T lymphocytes. Moreover, the distribution of certain leucocytes also appears to be affected. This weakening of immunity — combined with reduced activity of certain antibiotics and increased aggressiveness of pathogens in space conditions — may increase susceptibility to infection, a key concern for future long-duration space missions (Fig. 2).

With regard to humoral immunity, we showed that spaceflight has a qualitative and quantitative effect on the production of antibodies in response to antigen stimulation. Significant changes in the expression of the gene segments required to build a functional antibody gene were observed. Moreover, we noted two times fewer somatic mutations in the antibodies of animals immunized in flight; such mutations are important, as they increase the affinity of the antibody's antigen-binding site to the antigen.

Interestingly, it has been shown that spaceflight reduces granulocyte and monocyte progenitor cells in rodents that have flown and been subjected to anti-orthostatic suspension. More recently, Ortega et al. [2] have shown changes in maturation of granulocyte cells in mice after spaceflight. It would appear that T lymphopoiesis is sensitive to microgravity, but nothing is known about B lymphopoiesis. We therefore studied this question using two models: the amphibian *P. waltl*, well suited to space experiments and possessing all the key elements of the mammal immune system, and the mouse.

Gravity and lymphopoiesis

To ascertain if a change in gravity affects specific immunity, we left embryos of *P. waltl* to develop for 10 days on the International Space Station (ISS). The study of the larvae thus obtained after they returned to Earth revealed a significant change in the amount of mRNA in IgM heavy chains. To establish the cause, we reproduced in the laboratory the main stresses to which the embryos were subjected during their development (radiation, altered circadian rhythms, hypergravity, microgravity, thermal shock during atmospheric re-entry). This study showed that it is the perturbing effects of gravity, and not the other stresses encountered during the mission, that alter the expression of mRNA in IgM heavy chains. A perturbation of the Ikaros expression encoding the transcription factors required for lymphopoiesis is associated with this change in the expression of IgMs, which suggests that B lymphopoiesis could be affected in space conditions [3].

We then verified this hypothesis on mice aged four months suspended for 21 days with their hind limbs unloaded. It was shown that this model induces gradual changes in bone structure similar to those observed in older mice (Fig. 1A), as well as a reduction in B lymphopoiesis. We recorded fewer common lymphoid progenitors (CLPs) and pro-B, pre-B and immature B cells in the femoral bone marrow of suspended mice and older mice with respect to the four-month-old control group (Fig. 1B). Interleukin-7 plays an important role in differentiation of B lymphocytes. We therefore studied its receptor signaling and recorded a significant decrease in pro-B cells of suspended and older

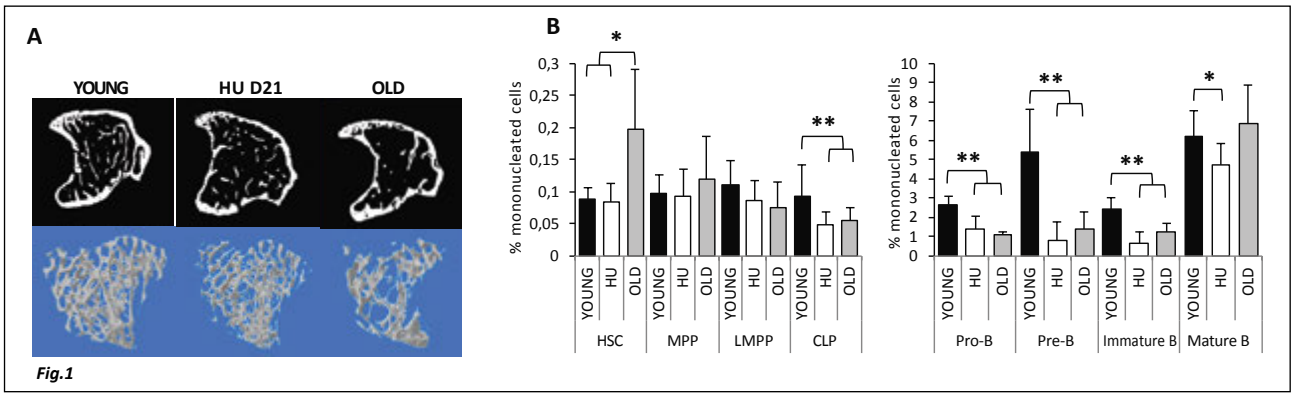

Fig.1

Fig. 1: Effects of simulated microgravity by anti-orthostatic suspension (HU) on murine bone structure and B lymphopoiesis23. **(A)** Segmented cross-sectional images of the proximal tibia (top) and 3D reconstruction of metaphyseal trabecular bone separated from the cortex (bottom) for four-month-old control (YOUNG), 21-day-HU (HU) and 19-month-old (OLD) mice. **(B)** Frequencies of early hematopoietic progenitors and B lineage cells in the femurs of young, HU and old mice. *© J. P. Frippiat University Nancy (France).*

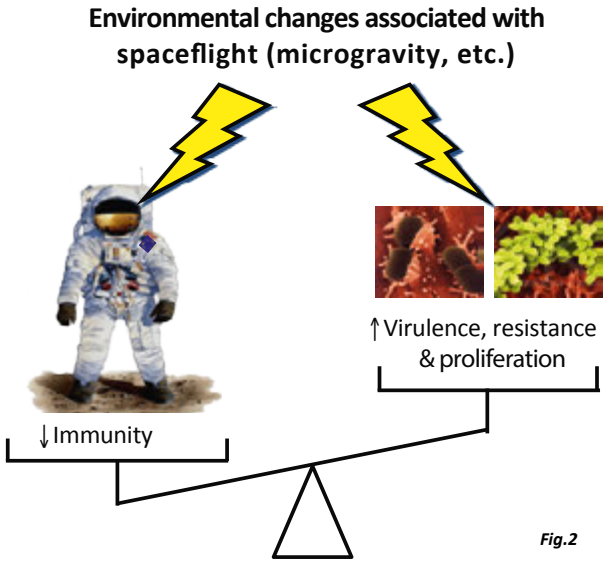

Fig.2

Fig. 2: Spaceflight-associated environmental modifications weaken the immune system but have a positive impact on the virulence, resistance and proliferation of pathogens. This imbalance may increase susceptibility to infections. *© J. P. Frippiat University Nancy (France).*

mice. This study confirms the decrease in B lymphopoiesis in flight and shows that hind limb unloading induces changes similar to those observed in older mice [4].

As previous studies suggest that T lymphopoiesis could be sensitive to microgravity, we got mice to mate at 2g so that gestation and birth took place in hypergravity. Analysis of the thymus of mice subjected to 2g revealed an alteration in signaling by the T cell antigen receptor (TCR). We then studied the TCR's β-chain repertoire. Our work revealed that hypergravity induces changes in the frequencies of Vβ and Jβ gene segments used to build these chains. The basic result is that approximately 85% of the TCR's β-chains in the mice subjected to 2g are different to those in control mice born at 1g. Lastly, the Vβ gene segments whose expressions are modified in the thymus of the 2g mice would appear to be clustered in the TCRβ βlocus on chromosome 6. This suggests local changes in the chromatin structure. This hypothesis matches a study that has shown that simulated microgravity induces epigenetic changes in human lymphocytes [5]. All of these data show that development in hypergravity modifies T lymphopoiesis and the repertoire of T lymphocyte antigen receptors, which could affect the host's immune defenses [6].

It therefore appears that as well as affecting the functions of mature immune cells, space conditions and variations in gravity affect B and T lymphopoiesis, which certainly contributes to a weakening of the immune system in spaceflight.

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