ERISTO: European Research In Space and Terrestrial Osteoporosis:

principales réalisations et perspectives

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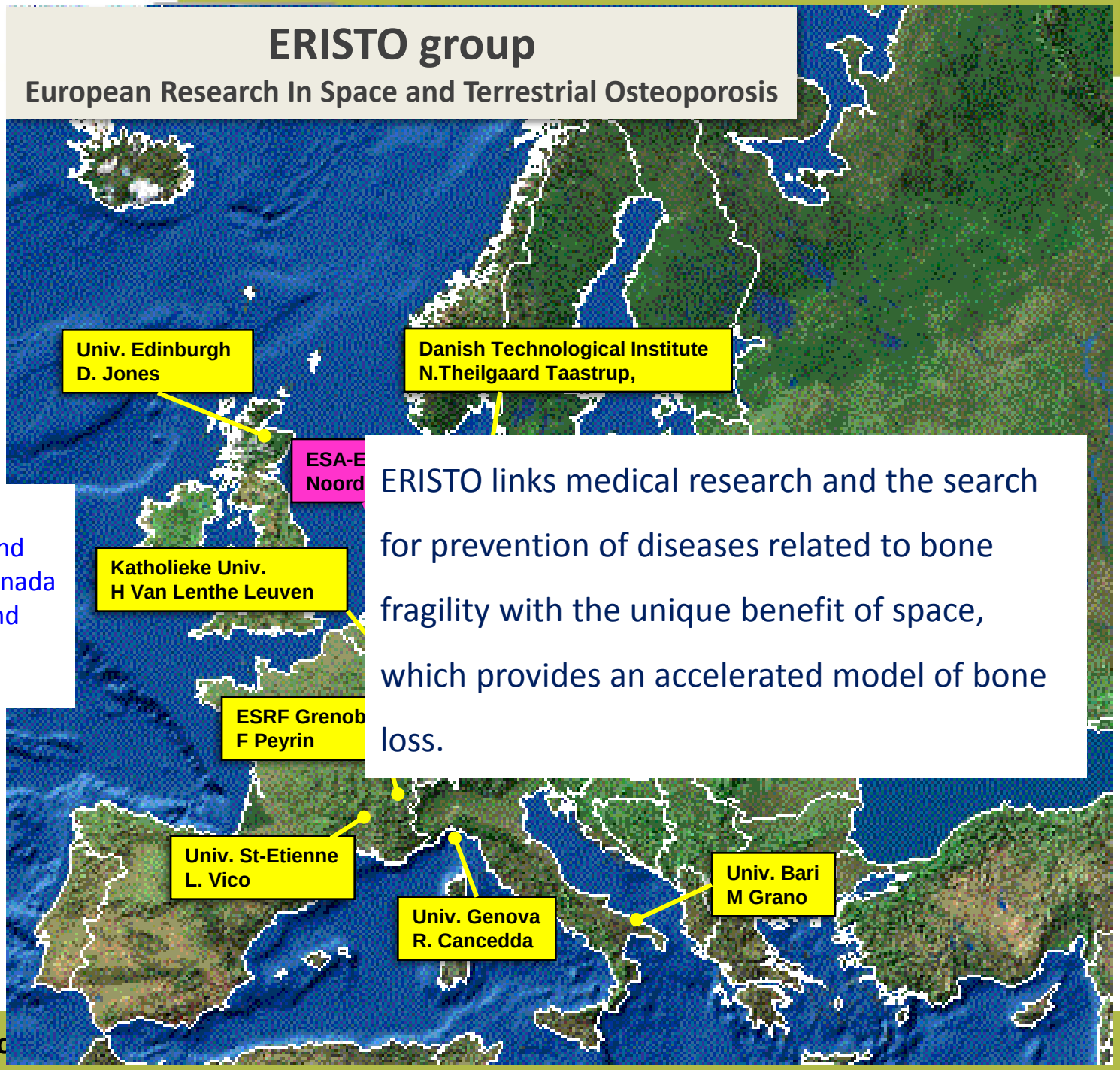
ERISTO group

European Research In Space and Terrestrial Osteoporosis



ERISTO links medical research and the search for prevention of diseases related to bone fragility with the unique benefit of space, which provides an accelerated model of bone loss.

Industrial partners

Scanco Medical, Switzerland
Octane Orthobiologics, Canada
Cytoscience SA, Switzerland
Amgen Inc., USA
Servier, France

HAZARDS OF SPACEFLIGHTS

Altered gravity

Lack of:

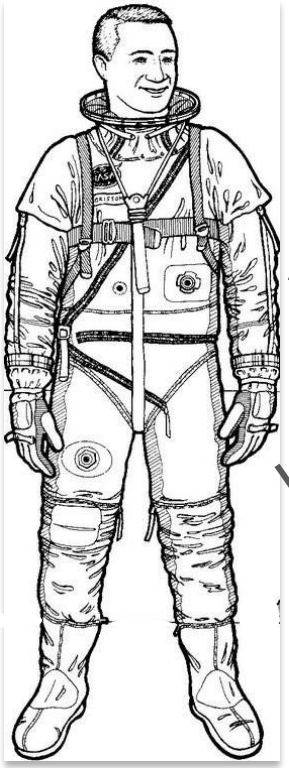
- Gravitational loading
- Hydrostatic pressure
- Convection
- Buoyancy
- Sedimentation

Ultraviolet and ionizing radiations, increased risk in deep space

- Acute in-flight effects
- Long-term cancer risk

Extravehicular activity

- High vacuum
- Extremes of temperature
- Meteoroids
- Space debris
- Ionospheric plasma
- Hand and shoulder injuries



Habitat

- Distance from Earth
- 90 min. day and night cycles
- Acoustic noise (60 dBA)
- Reduced and closed space
- Isolation
- Lack of natural light and surrounding
- Modest elevated ambient PCO_2

Conditions of life

- Limited privacy
- Floating
- Disturbances in sleep
- Hygiene
- Performance pressures

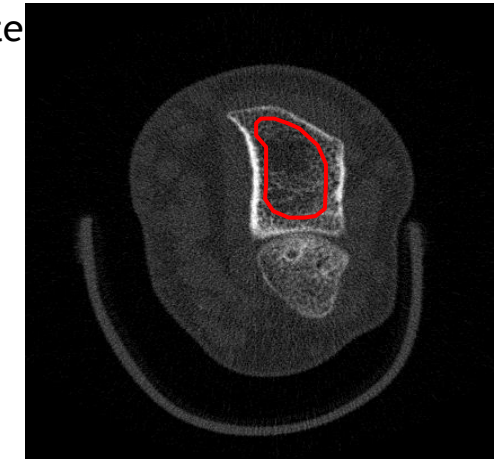
Reduced ground force reaction
Physiological changes
Behavioral changes
Psychological changes

BONE LOSS IN COSMONAUTS/ASTRONAUTS

- ✓ DXA: spine and hip aBMD losses (1 - 1.5%/**month**) greater than typical age-related loss rate (0.5 - 1%/**year**) in older individuals. Leblanc A et al., *J Musculoskelet Neuronal Interact*, 2000 ; Orwoll ES et al., *JBMR*, 2013

- ✓ QCT : vBMD decrease in trabecular and cortical proximal femur Lang T et al., *JBMR*, 2004

- ✓ pQCT, Densiscan (Scanco Medical) greate distal tibia, no loss at



Two hours of physical activities / day

http://spaceflight.nasa.gov/gallery/images/shuttle/sts-133/html/s133e008323.html

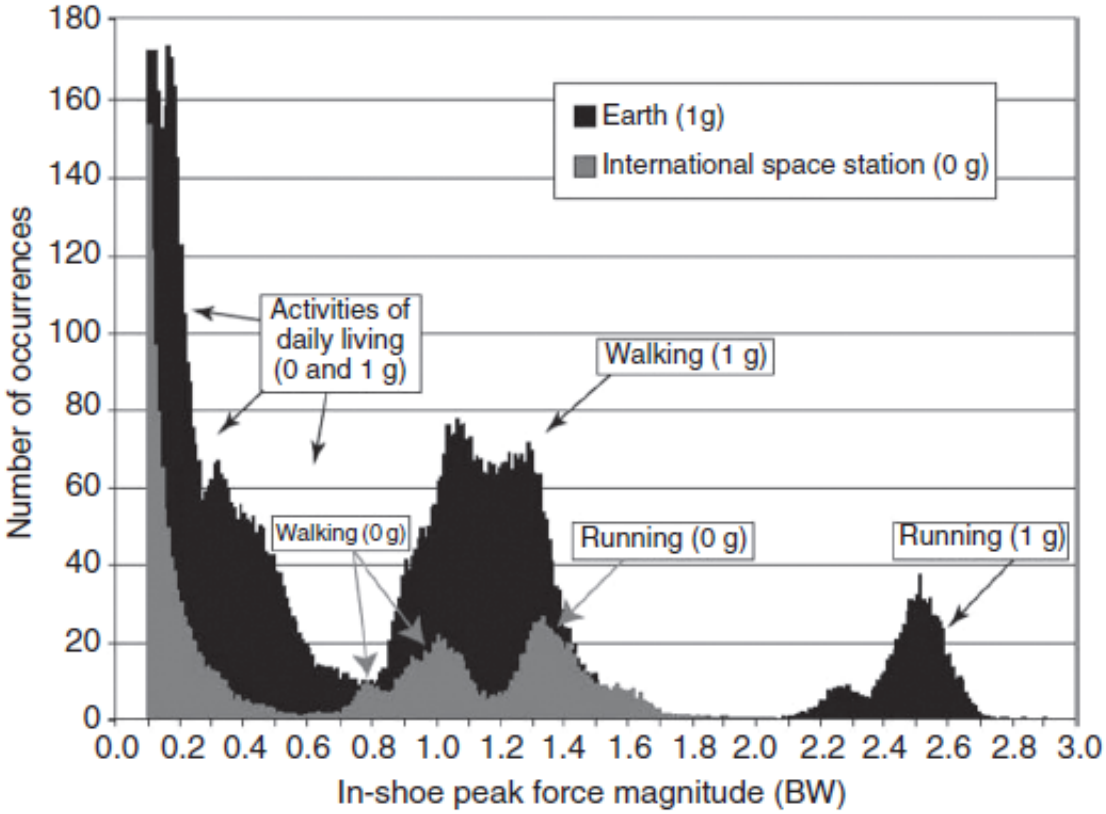
Astronaut Edward T. Lu,
Expedition 7 NASA ISS
Interim Resistive Exercise Device (IRED)
Max load (1337 N)

Steve Lindsey, STS-133, 2011
aRED, Advanced Resistance Exercise Device
Max load (2675 N),

http://www.agences-spatiales.fr/thomas-pesquet-salle-de-sport-de-liss/

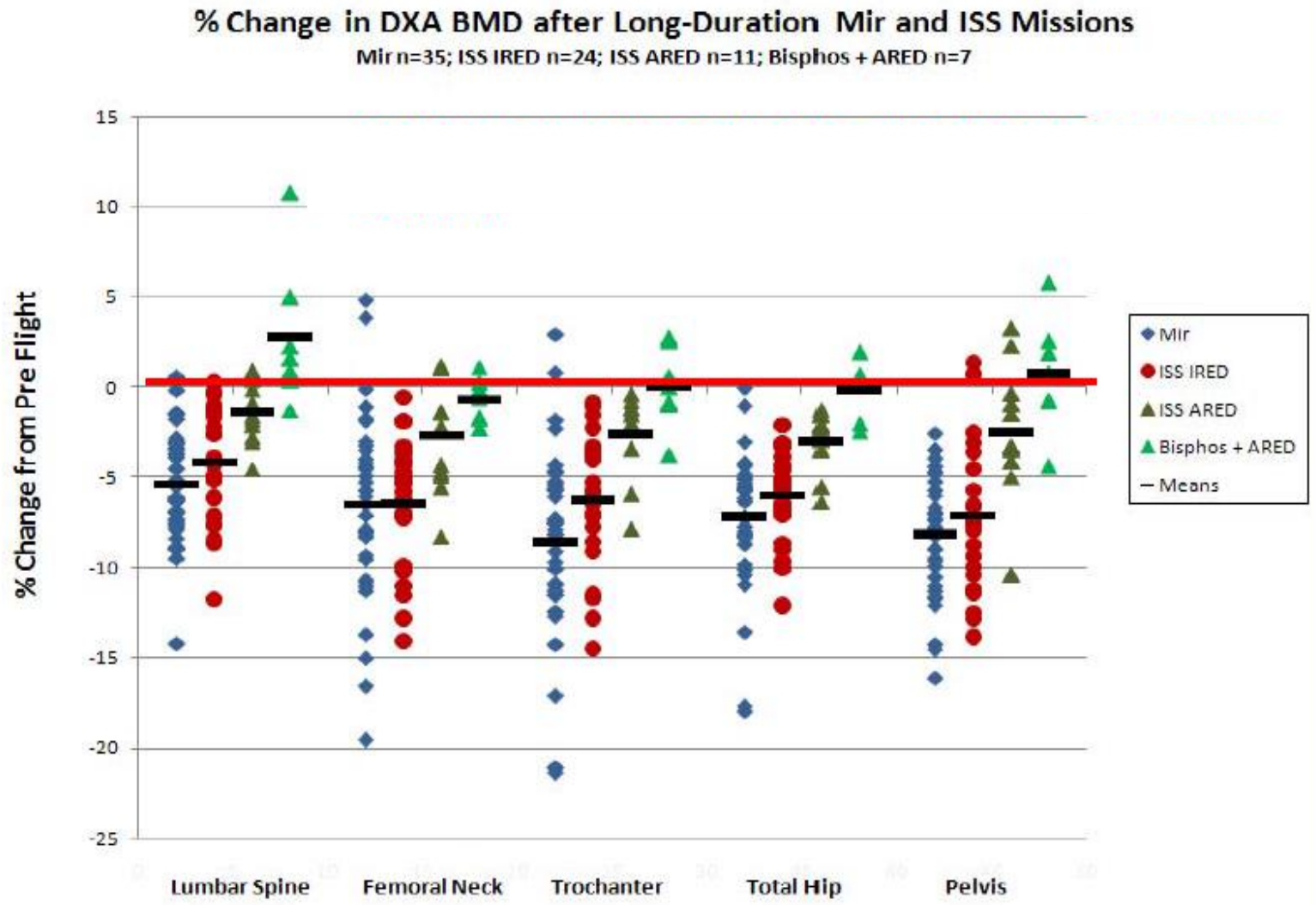
Failed to affect bone

FOOT FORCES ABOARD ISS ARE MUCH REDUCED COMPARED TO 1G



In-shoes peak force magnitudes recorded during activities of daily living on Earth and aboard ISS. Both magnitude and frequency of the GRFs observed in 1g are reduced onboard ISS. No GRF's> 2xBW, from Cavanagh et al., 2010

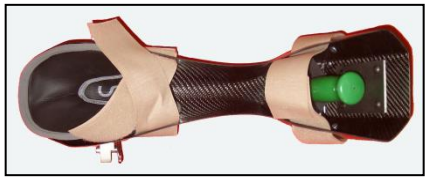
EFFECTS OF COUNTERMEASURES USING DXA aBMD



Bone summit 2010, NASA Sibonga J

The XtremeCT is an *In Vivo* system for measurements on human extremities on the radius and the tibia

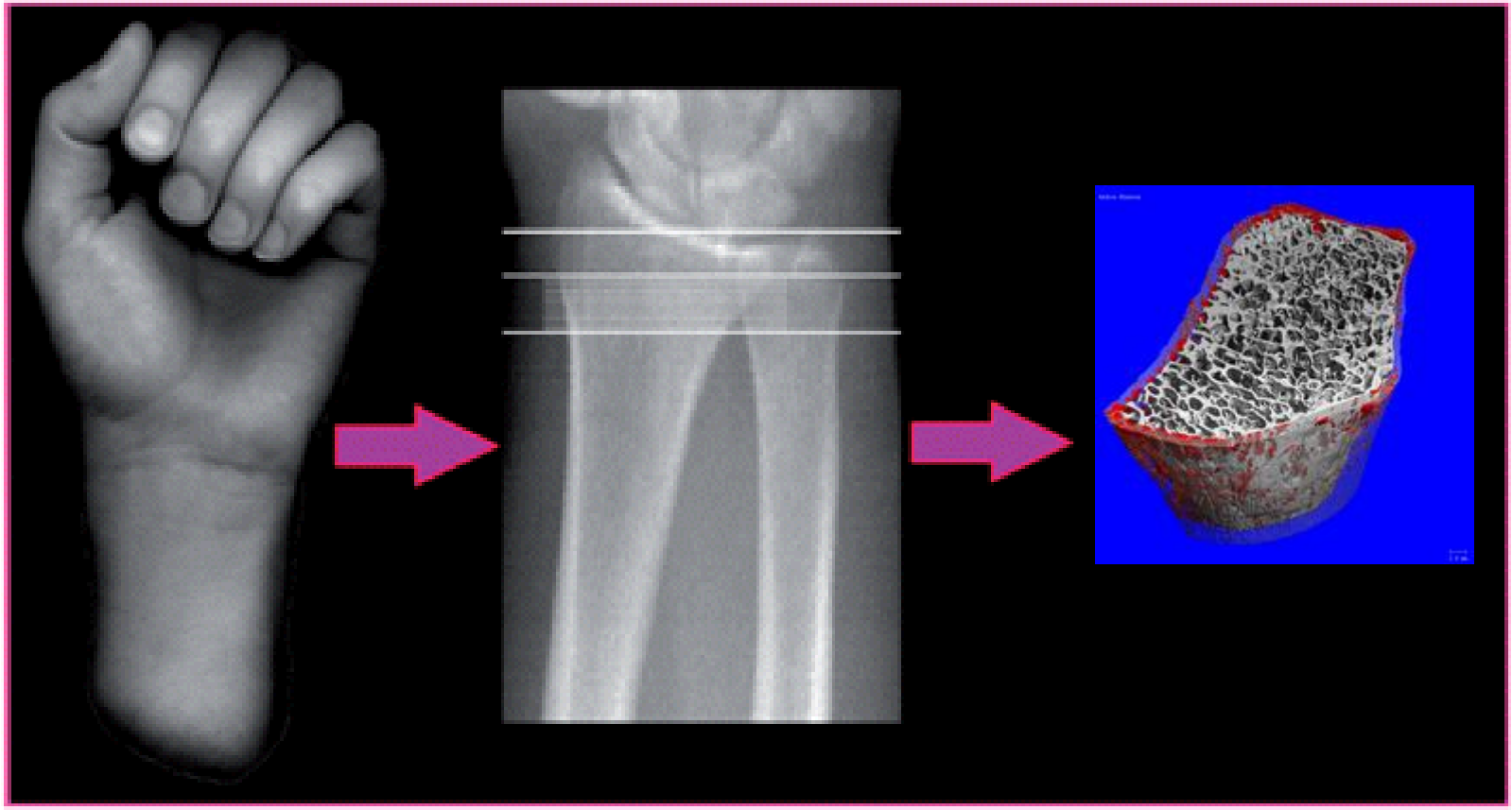
- **Hardware description**



Size (WxDxH): 1.35 m x 0.9 m x 1.35 m
(without Computer)
Weight: 450 kg
Voxel size 82 μm^3

The effective dose (ICRP91) of the 3D-pQCT examination is inferior to **3 µSv**.

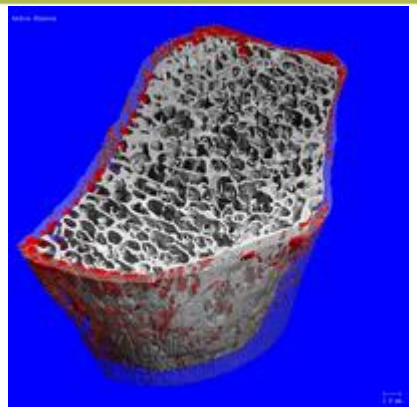
Region of interest

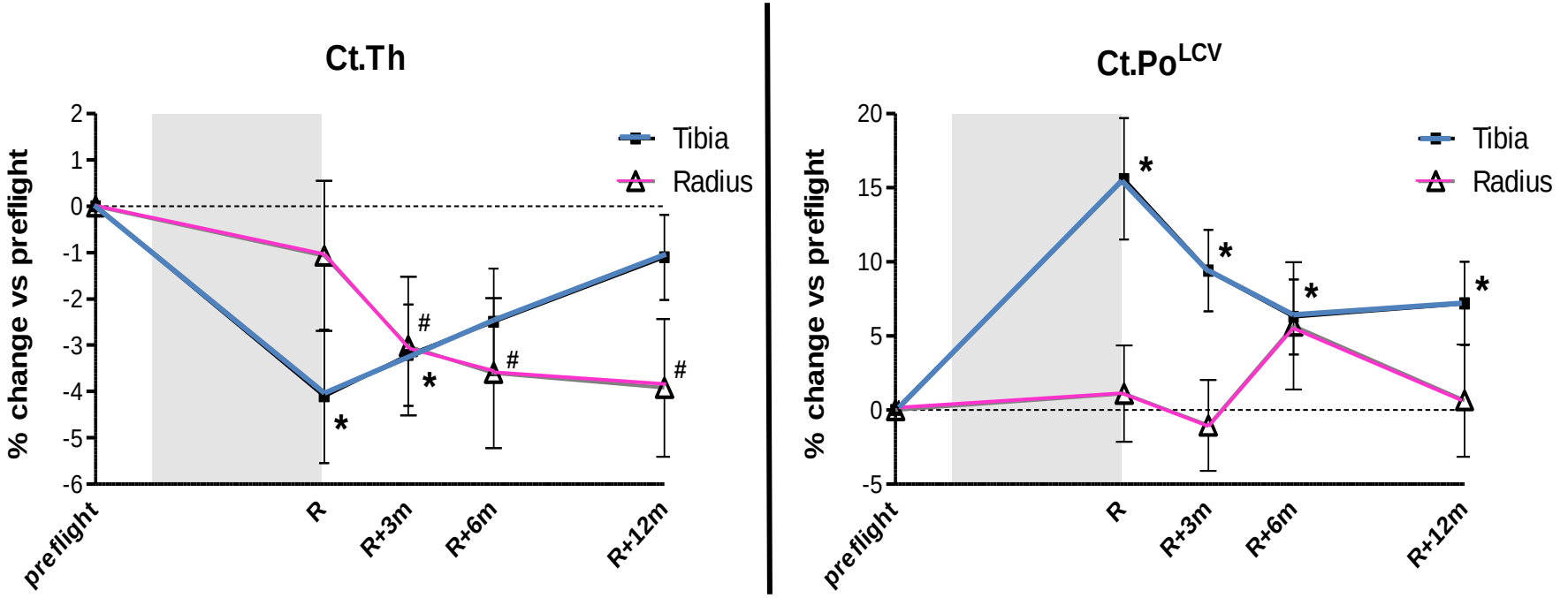


In vivo µCT of the distal radius using the XtremeCT system, with images showing the region of the distal radius imaged (centre) and the resulting 3D trabecular and cortical envelopes (right).

Courtesy Bruno Koller.

RADIUS and TIBIA: cortical bone

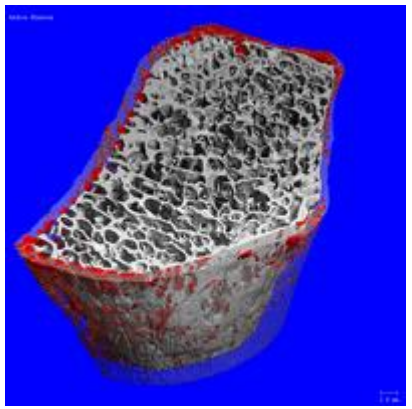


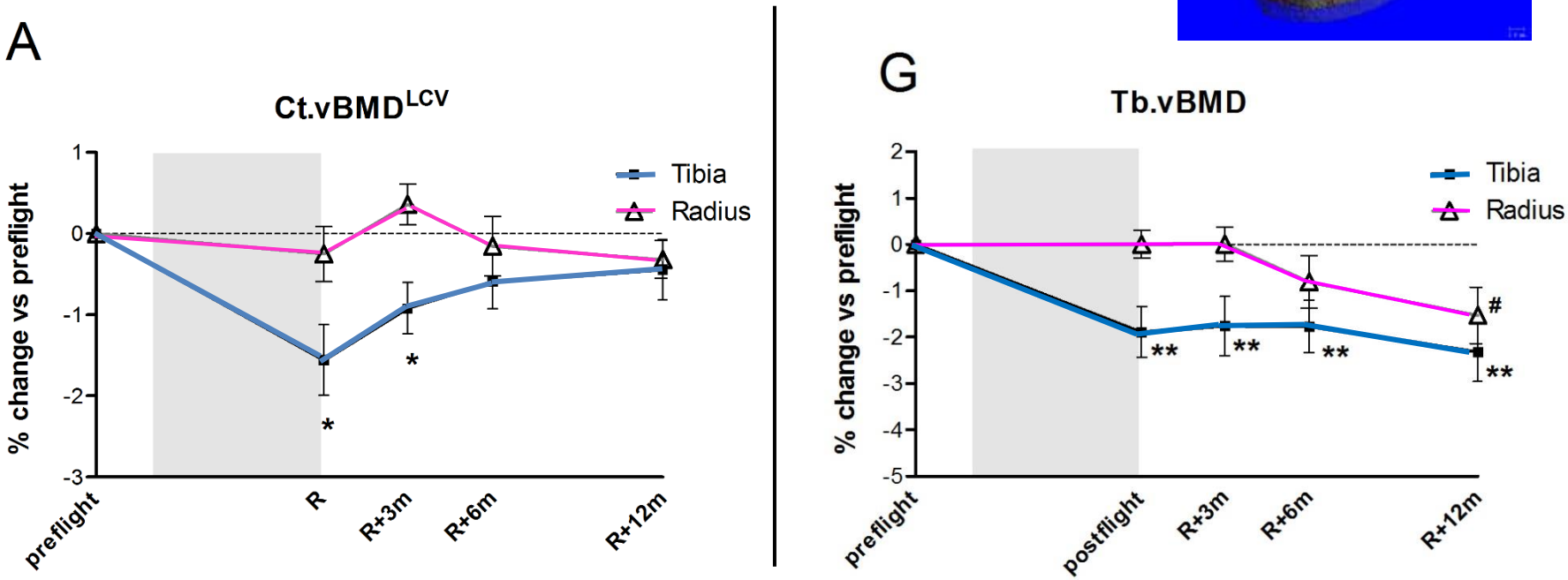


Differences in % vs preflight (mean±SE), tibia *p<0.05; radius # p<0.05

Vico et al., *JBMR*, 201

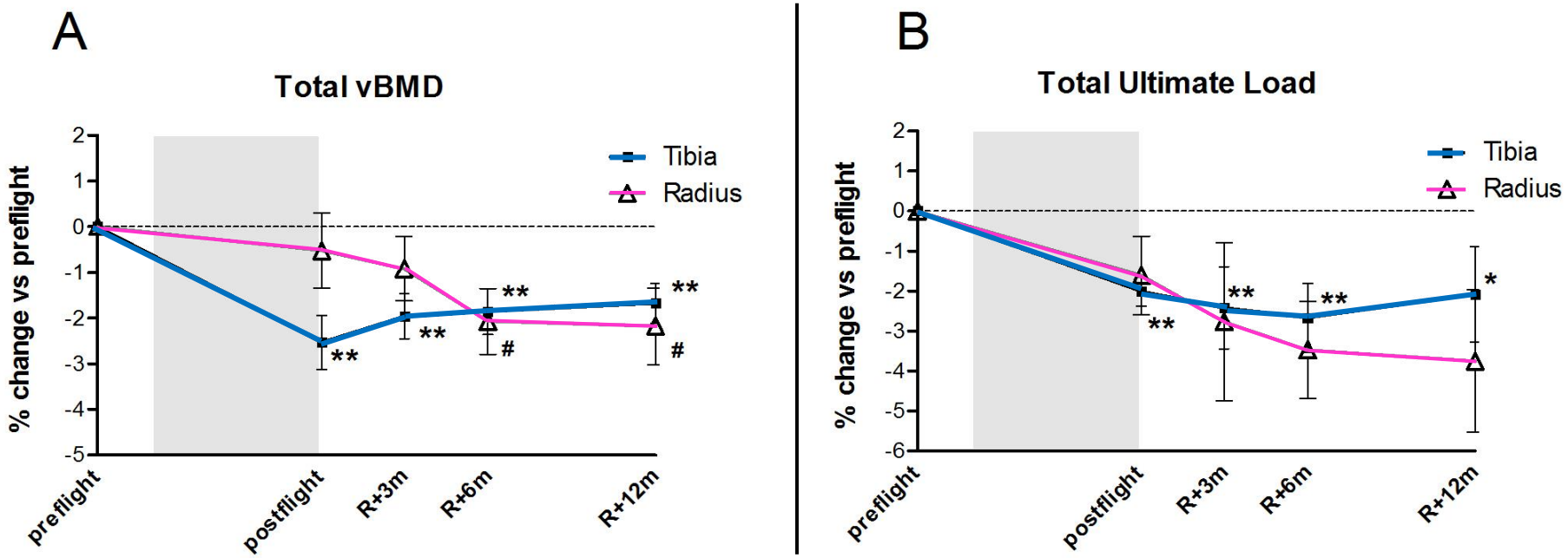
RADIUS and TIBIA: trabecular bone





Differences in % vs preflight (mean±SE), tibia *p<0.05; **p<0.01, radius # p<0.05

RADIUS and TIBIA : total bone (cortical +trabecular)



Differences in % vs preflight (mean±SE), tibia *p<0.05; **p<0.01, radius # p<0.05

SUMMARY: new findings

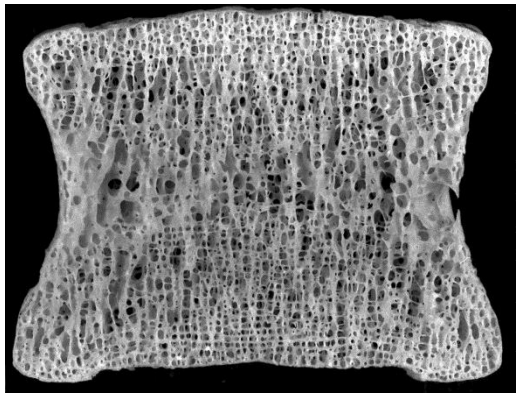
- **TIBIA**
  - 6-m post flight, cortical bone recovers its size, the porosity remains elevated
  - 12-m post flight, trabecular bone is as low as after landing
- **RADIUS**
  - No alteration at immediate return
  - Then a progressive fragility develops, becoming significant at 12-m
- **ULTIMATE LOAD** is compromised at both sites 1 year after reentry
- **BONE MARKERS**
  - Resorption is elevated at postflight, P1CP rebound 2 weeks after return
  - Between 6 and 12-month post flight, bone markers declined below preflight values.

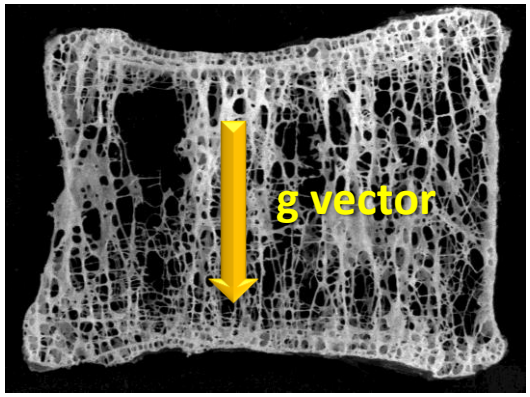
Still to investigate…

CORTICAL BONE

http://www.vet.uga.edu/ivcvm/courses

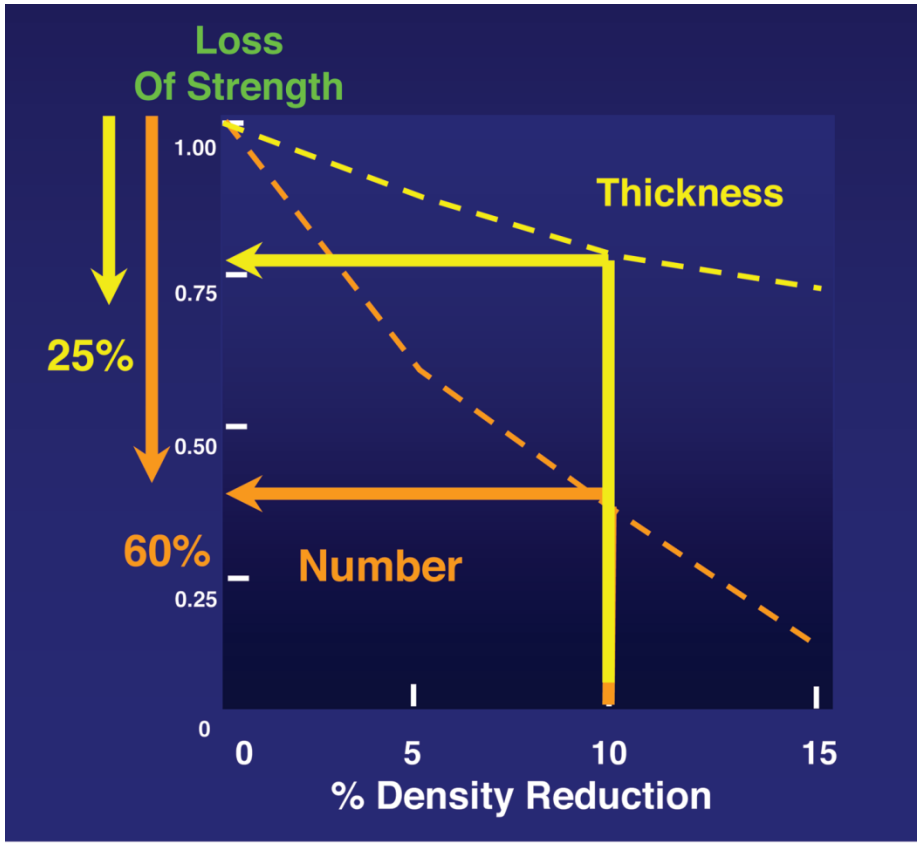
TRABECULAR BONE





http://en.wikipedia.org

Trabecular compartment

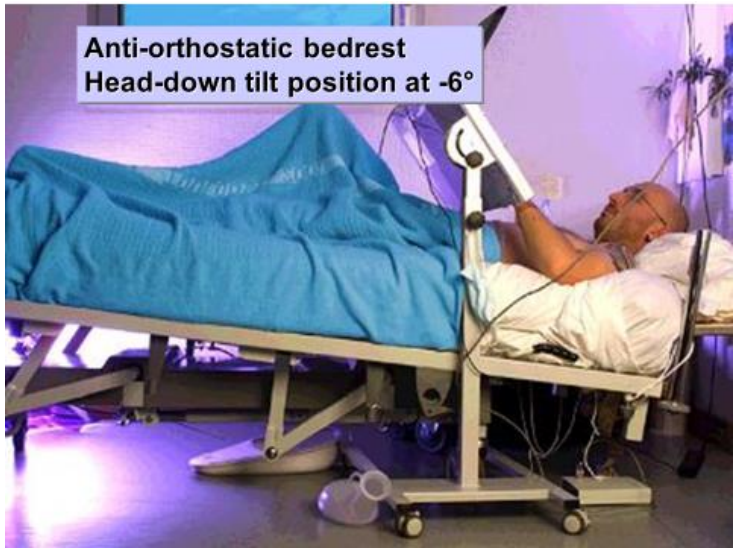


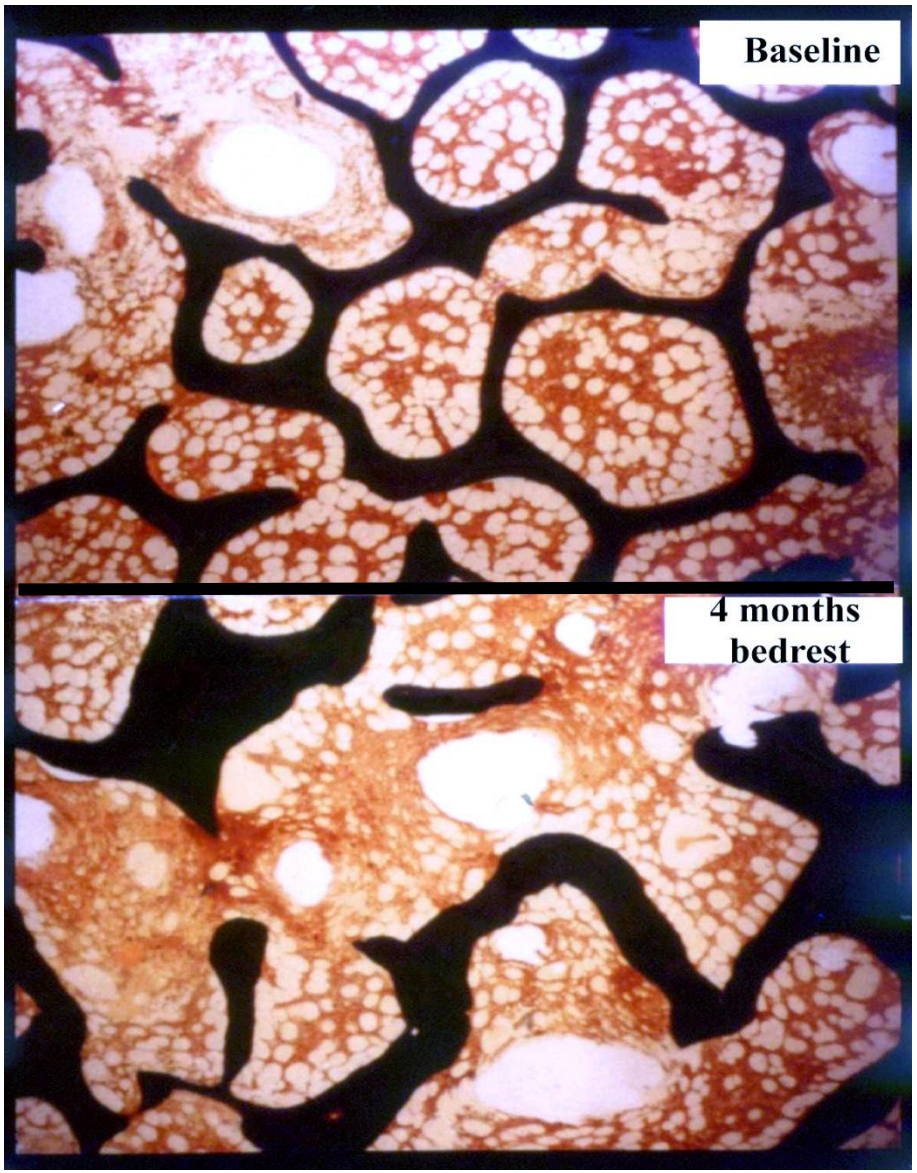
Bjørnerem Å et al., *Bone* 2011

van der Linden JC et al., *JBMR*, 2001

Trabeculae are rapidly lost because their large surface area facilitates remodeling ⇨ loss of connectedness

Bone iliac crest 4-month antiorthostatic bedrest





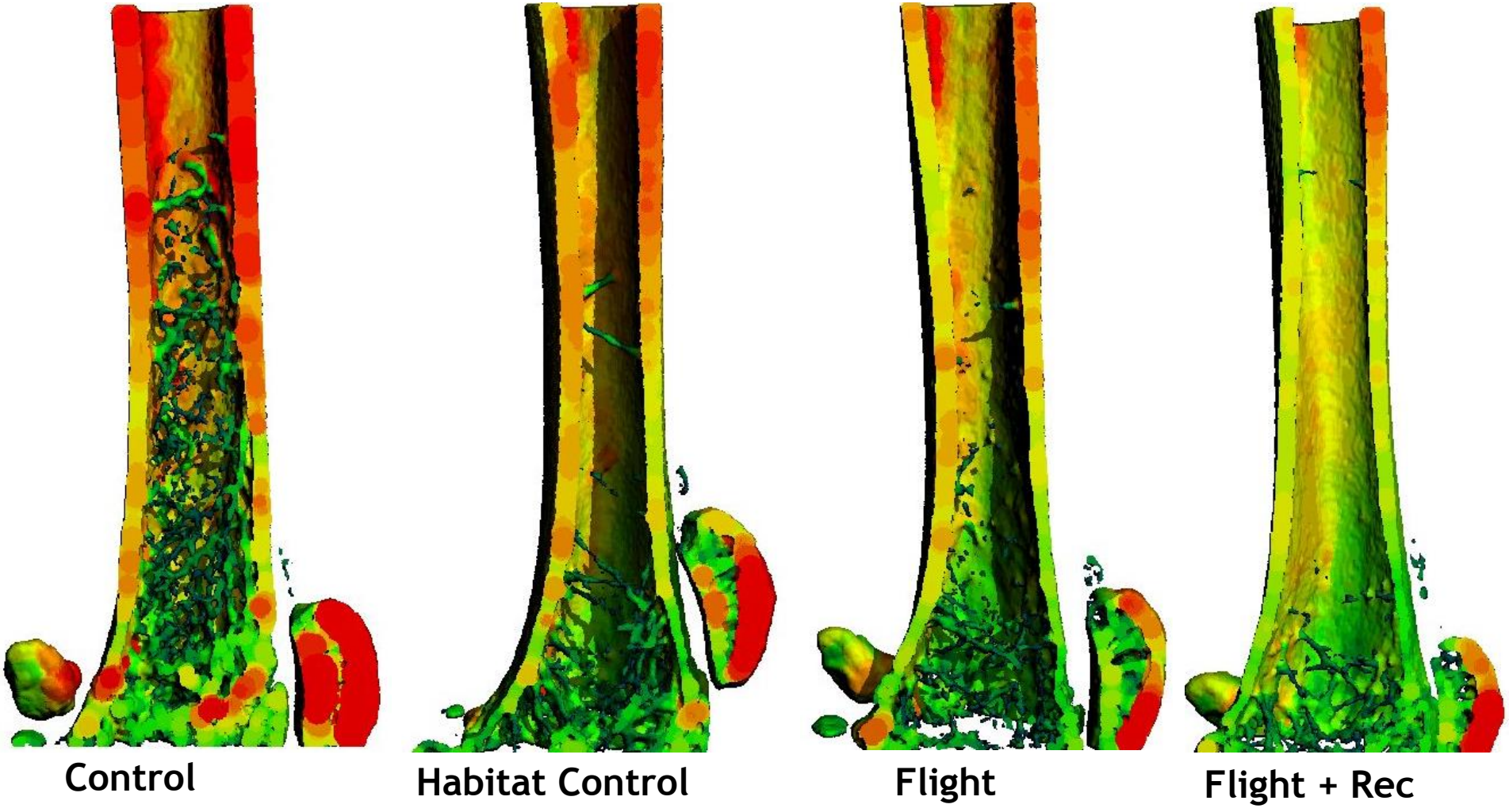
Palle S, Calcif Tissue Int. 1992

Bion-M1 biosatellite (April 19–May 19, 2013)

male mice C57BL6/J
3-month old

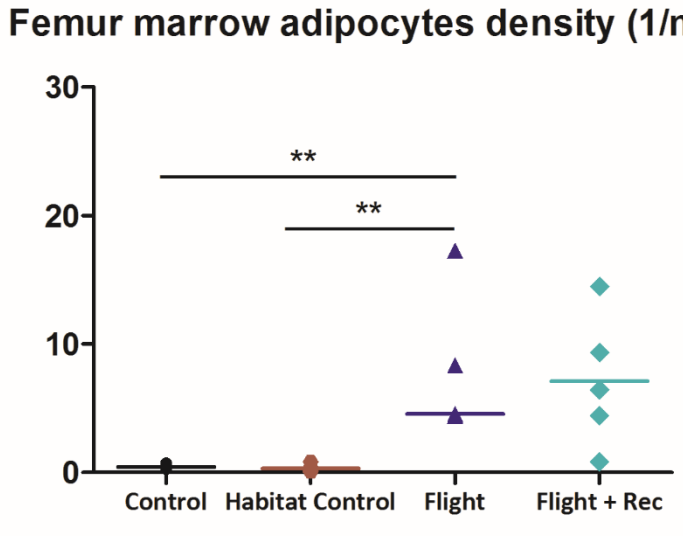
1. Flight, n=5
2. Flight + 8-d.recov, n=5
3. Habitat Ctr, n=6
4. Ctr, n=15

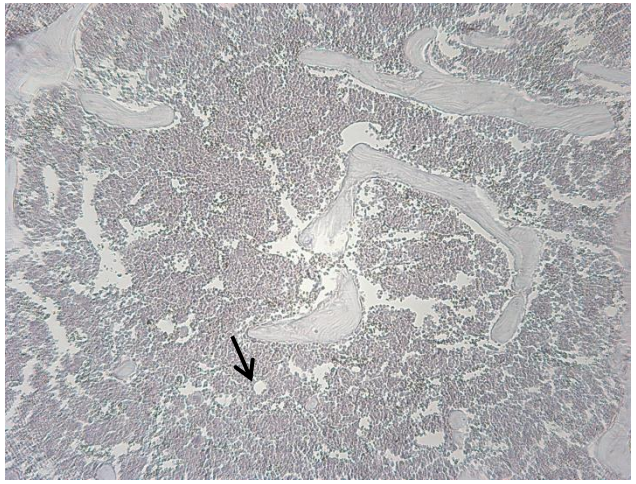
Bion-M1 : femoral bone loss worsens in flight+recovery



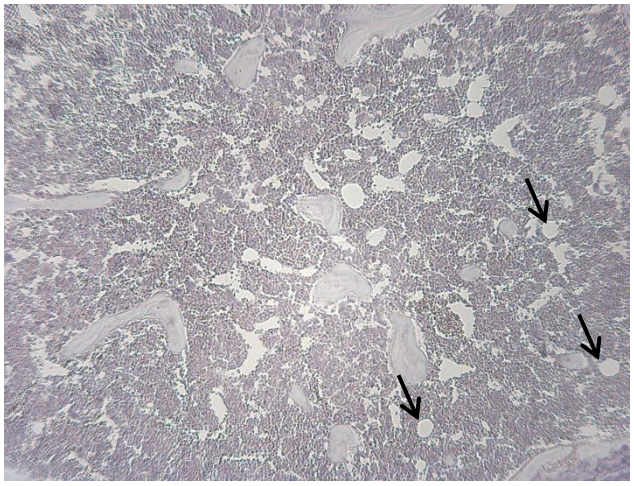
Gerbaix M et al., *Sci Re*

Bion-M1 : lipid droplets increase in the bone marrow

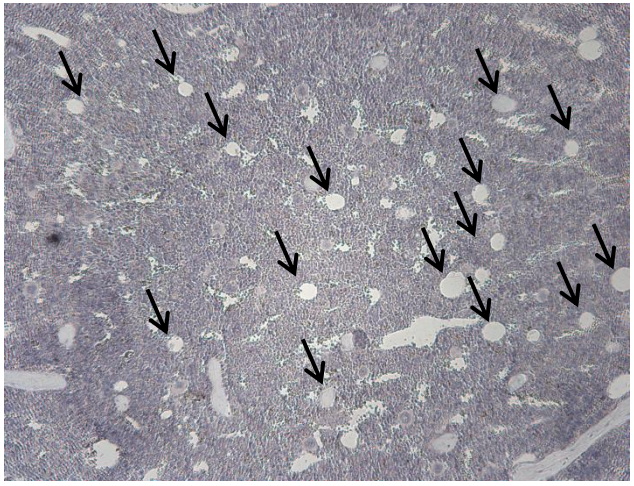




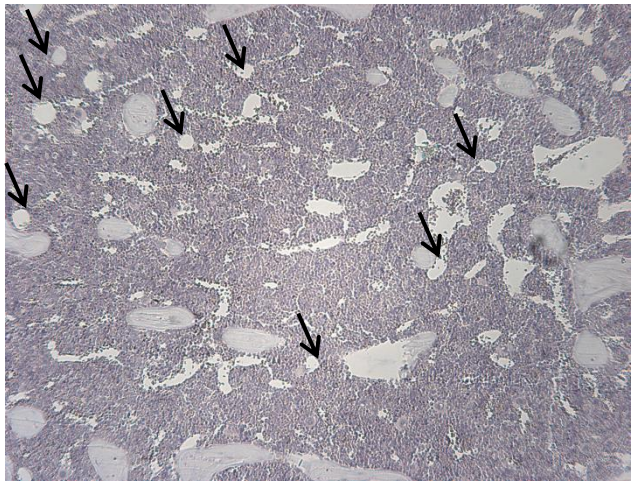
Control



Habitat Control



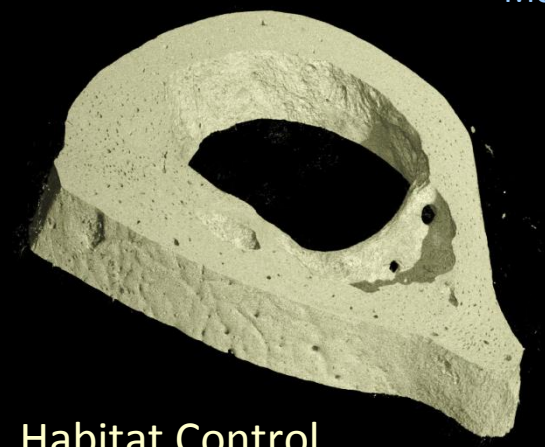
Flight



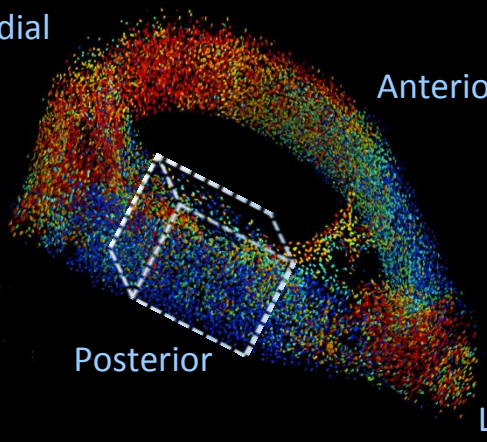
Flight + Rec

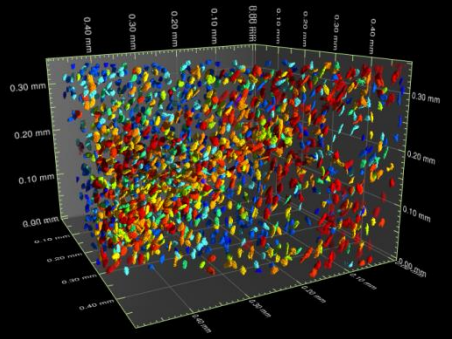
Gerbaix M et al., *Sci Re*

BION-M1 : synchrotron radiation osteocyte lacunae

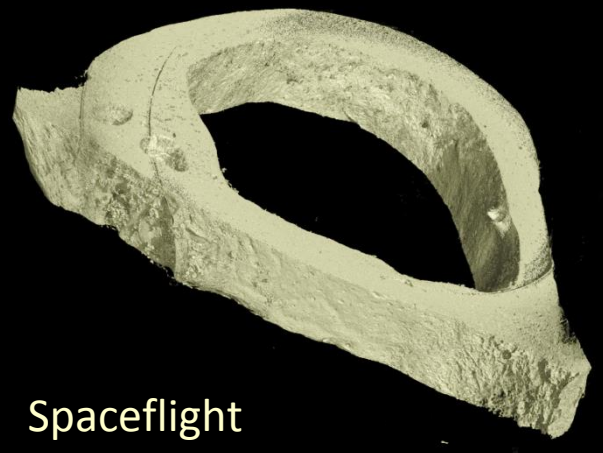


Habitat Control

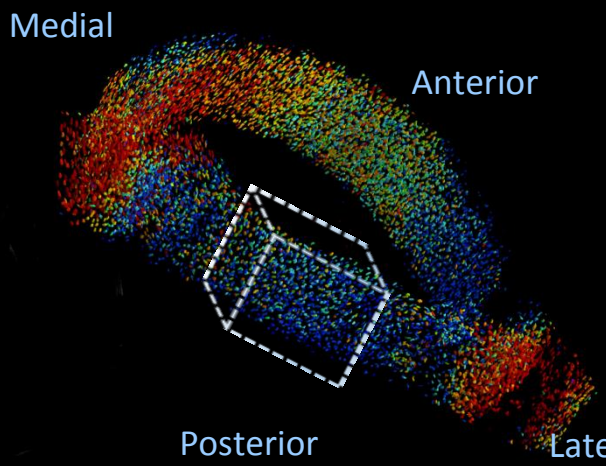


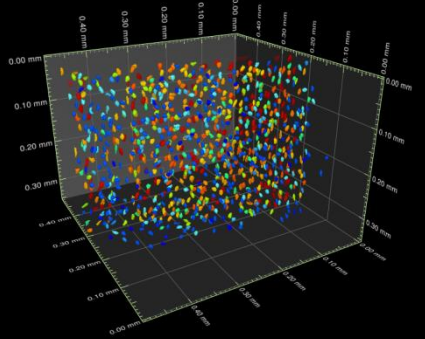


Posterior

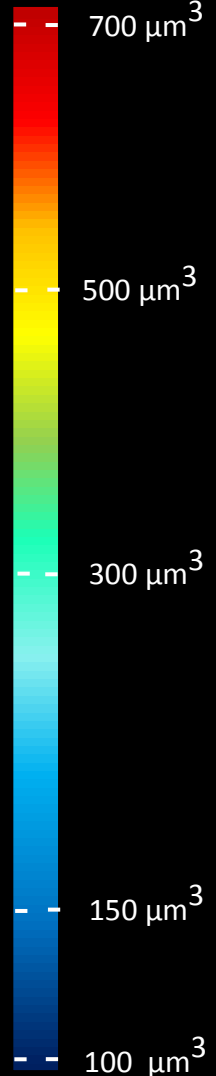


Spaceflight



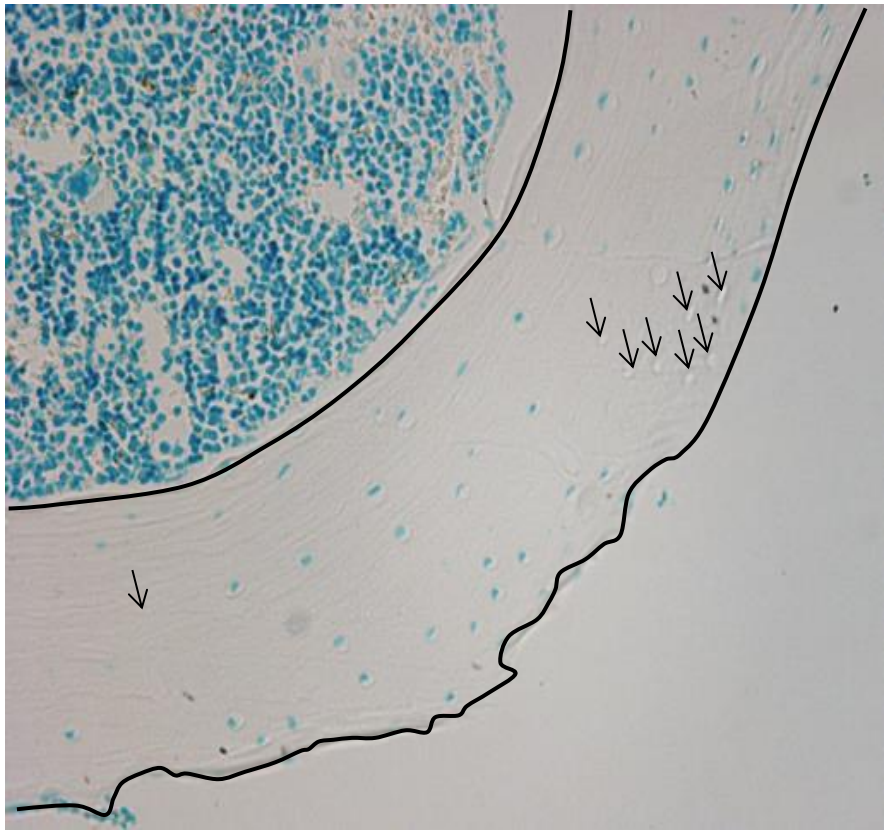


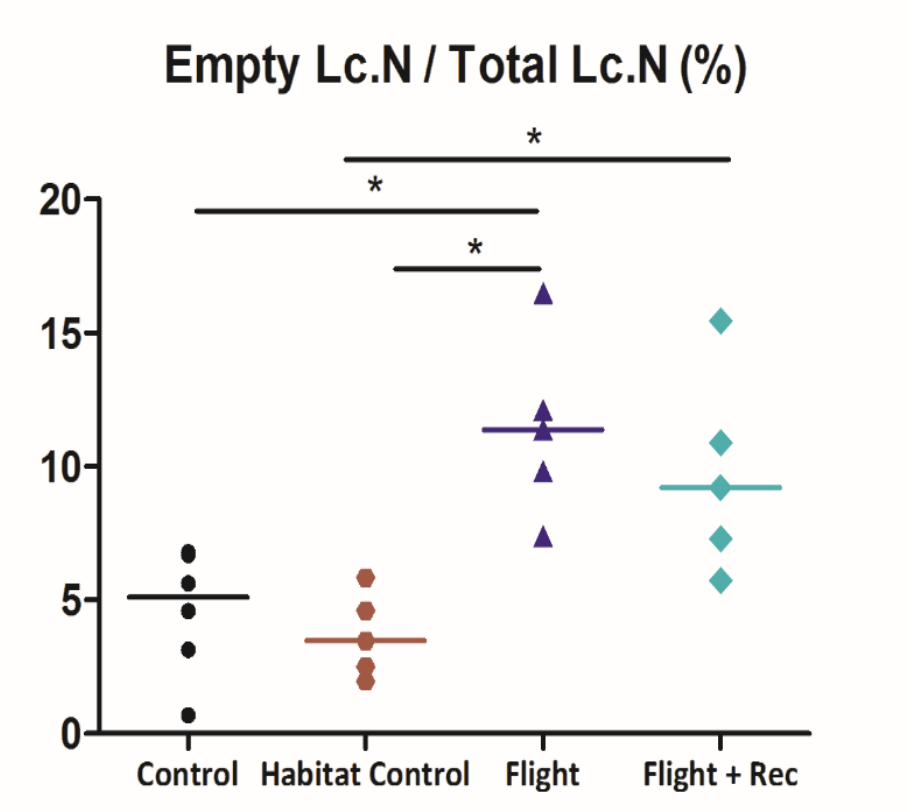
Posterior



Gerbaix M et al., *Sci Rep.* 2017

Bion-M1 : osteocytes





Gerbaix M et al., *Sci Rep,* 2

Bion-M1 : SUMMARY

Space habitat conditions decreased trabecular BV/TV and increased trabecular and endosteal resorption.

VERTEBRA: Trabecular bone volume decreased (-26%, vs Ctr) +++ in lumbar site, osteoclast surface increased (X6, vs Ctr) and osteoblast surface was not significantly modified.

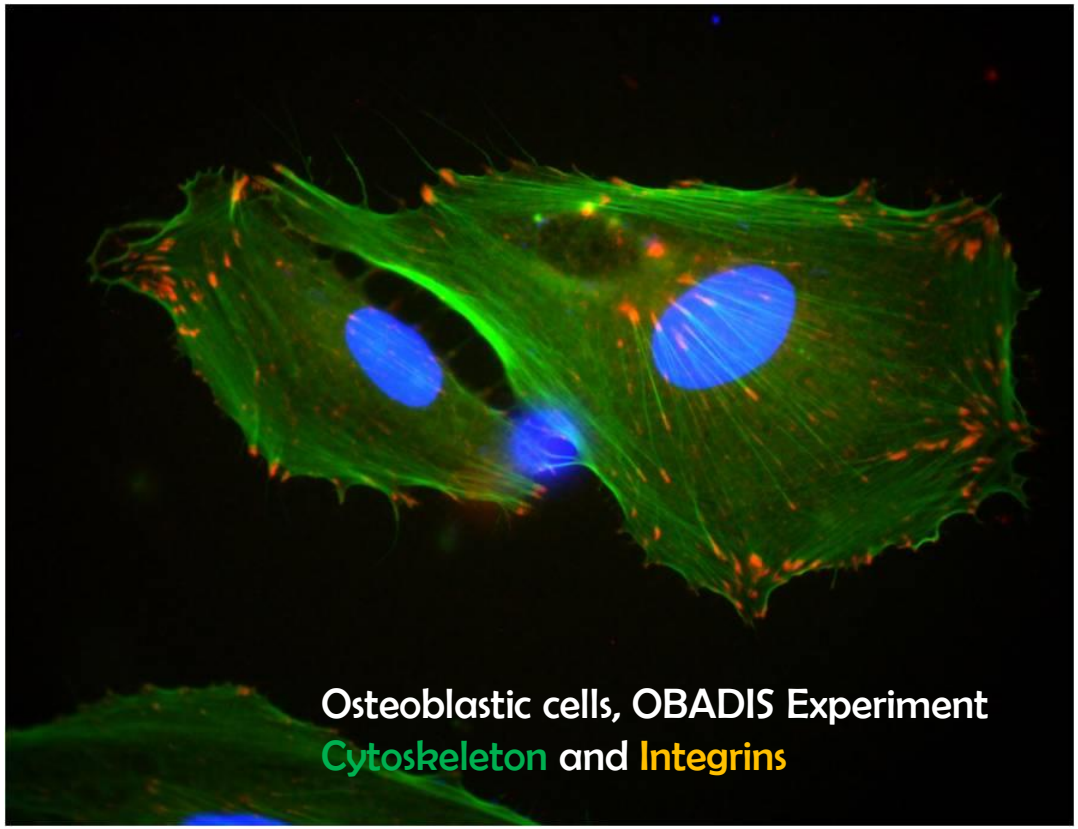
FEMUR: Trabecular bone volume decreased (-65%, vs Ctr), resorption activity (X3 vs Ctr) and marrow adiposity (X22 vs Ctr) increased.

Cortical thickness decreased (-20% vs Ctr) + periosteal resorption.

Osteocyte Lacunae mean volume was smaller in posterior zone and the number of empty lacunae increased ▸ osteocyte death

At cellular level, mechanical coupling: interactions matrix/cells?

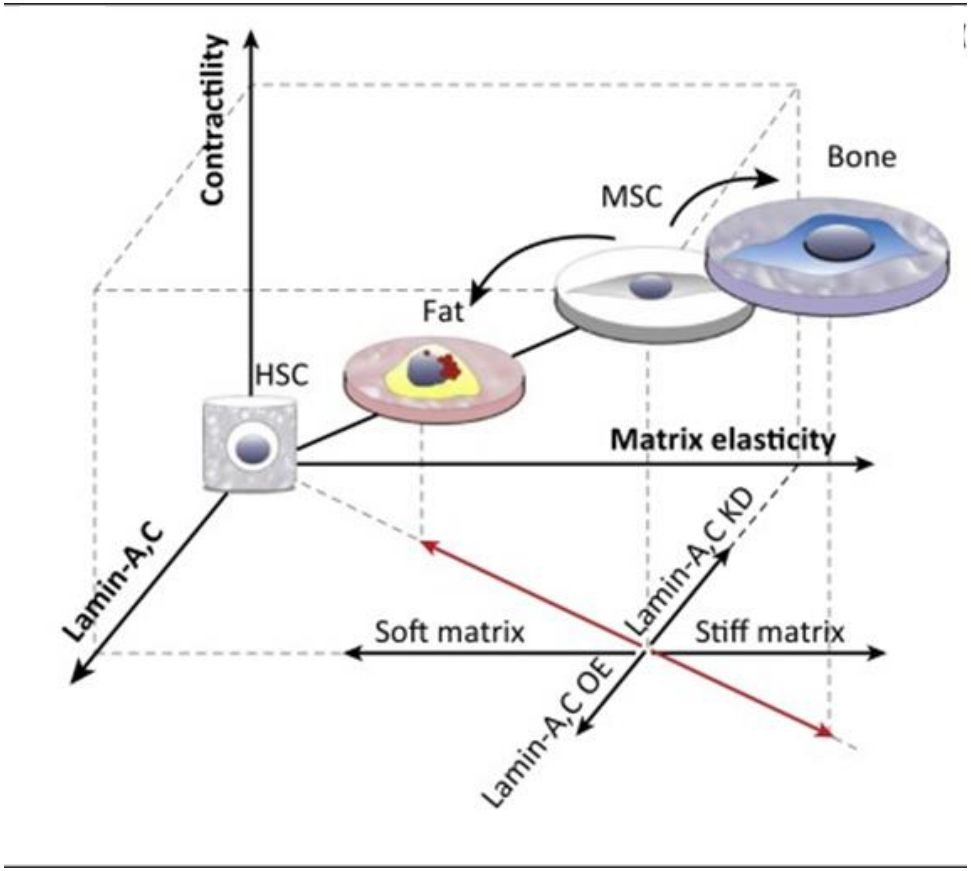
- loss of gravity-dependent convection
- negligible hydrodynamic shear
- lack of sedimentation

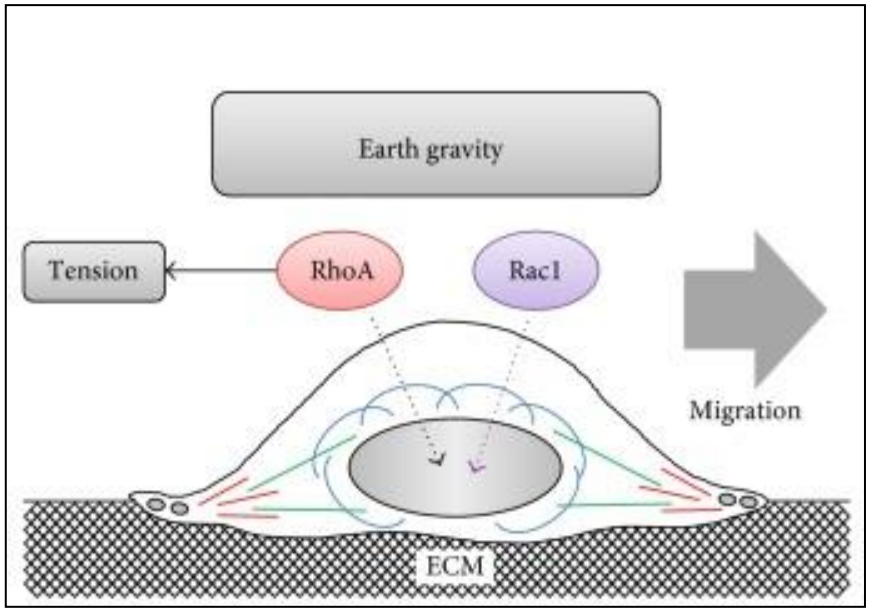


Disorganization of the cytoskeleton associated with disassembling of vinculin spots and phosphorylated proteins within focal contacts (post-mitotic cells).

Guignandon et al., Faseb, 2001

Cellular mechanotransduction

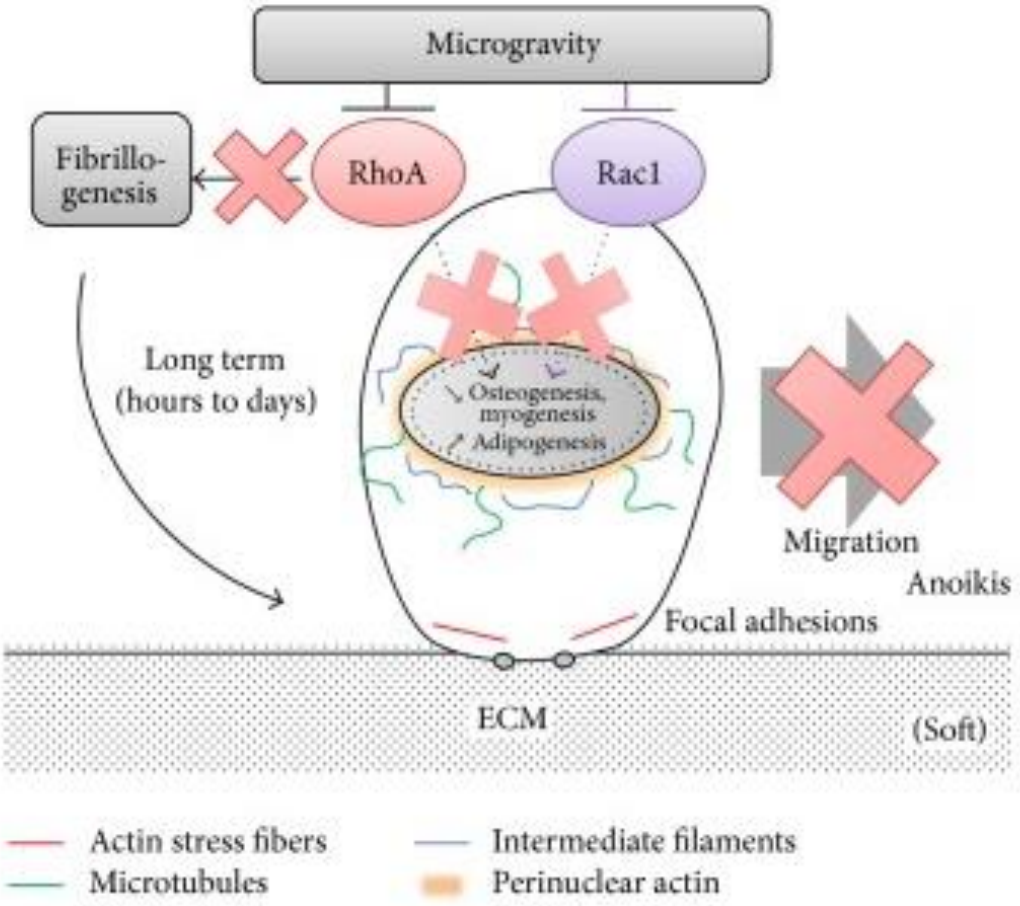




Mesenchymal stem cells are well spread and exhibit a tensed cytoskeleton in particular of microtubules, intermediate filaments, and actin stress fibers associated with stable focal adhesions within the extracellular matrix. These elements are controlled by GTPases RhoA and Rac1.

Guignandon et al., *Faseb* 2014
Louis et al., *BioMed*

Regulation of RhoA and Rac1 activities in space-related conditions

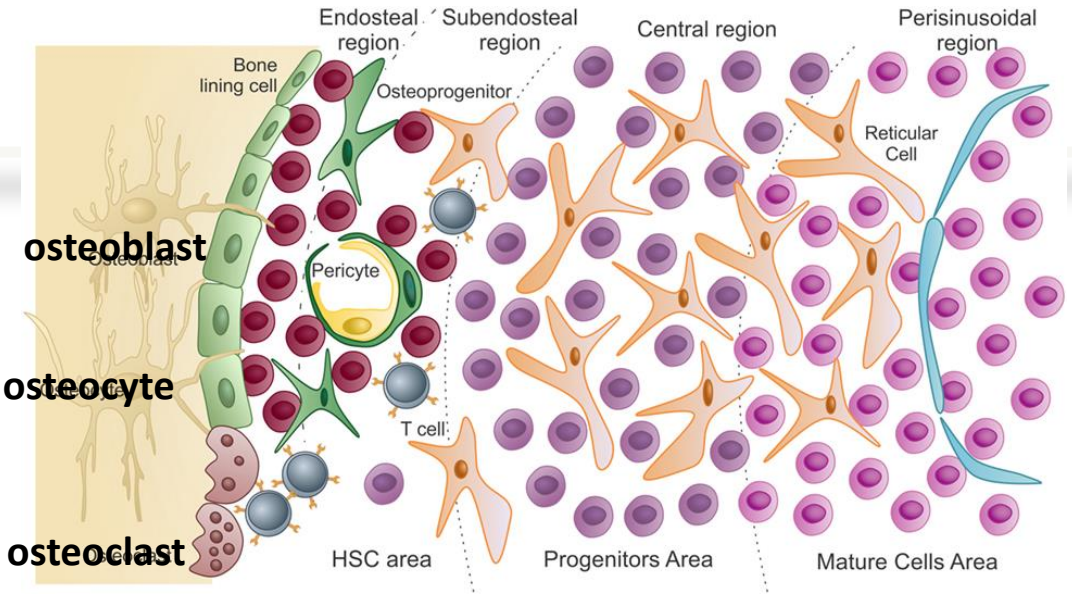


Inhibition of RhoA and Rac1 : ↘ osteogenesis and myogenesis and ↗ adipogenesis of mesenchymal stem cells.

- rounder cell shape with disorganization of microtubules, stress fibers, intermediate filaments, and focal adhesions.
- Transcription is altered as nucleus shape is changed.
- Fibrillogenesis (a tension-dependent process) limited; ECM not properly synthesized and lost its mechanical properties appearing softer for mesenchymal stem cells, reinforcing adipogenesis

Guignandon et al., *Faseb* 2014
Louis et al., *BioMed Res. Int.* 2015

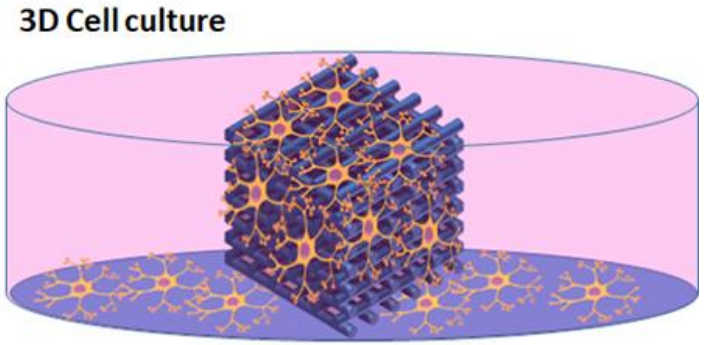
In vitro bone



The models were aimed at better understanding bone loss at the cellular level to better achieve bone generation.

- develop 3D multicell models mimicking bone marrow environment
- provide a complete model of bone remodeling in a very well controlled environment

… a challenge!



In vitro bone

Needs – supporting technology

3D macroporous substrates
HA/βTCP/PLA composition and geometry
Mechanical properties

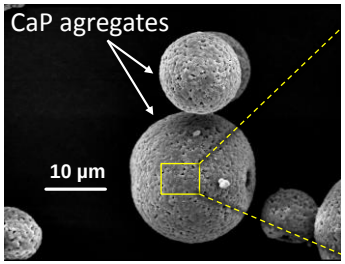
Culture chambers
Fitting with fragile scaffolds,
allowing perfusion and micromechanical stimuli

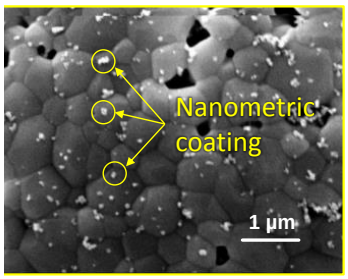
Culturing conditions
Culture medium, gaz exchange, closed systems

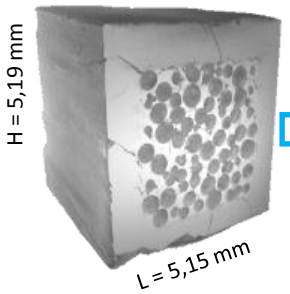
CO_3^{2-}, PO_4^{3-}, SiO_4^{4-}

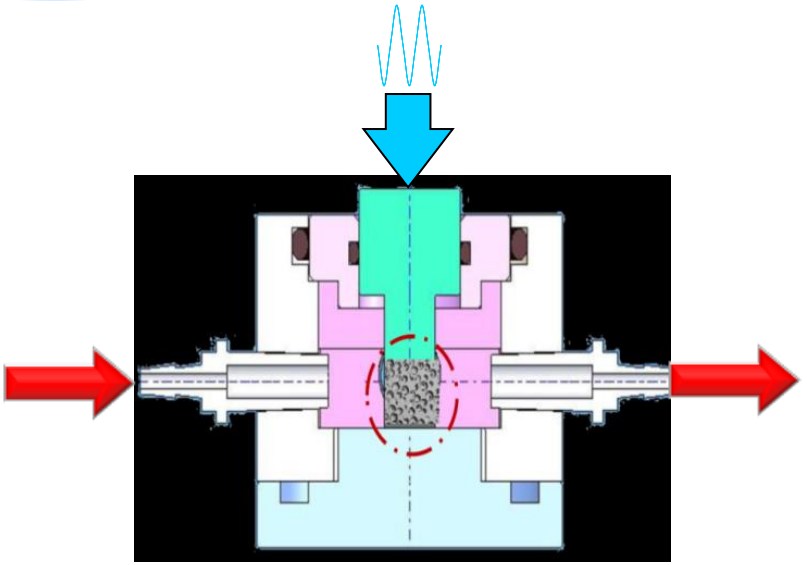
Ca_{10} $(XO_4)_6$ $(Y)_2$

OH^-, CO_3^{2-}





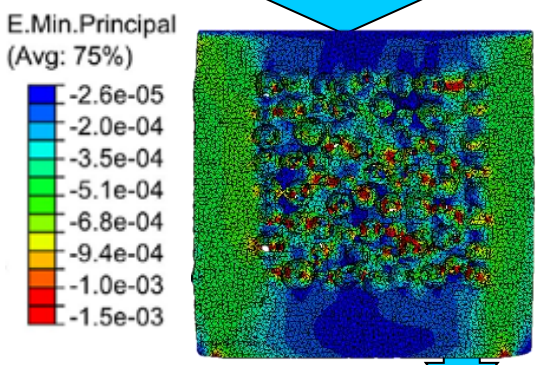


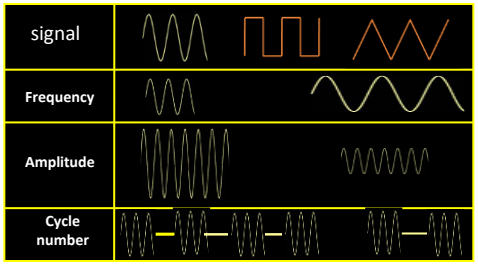


Bouet G et al., *Eur Cell Mater*. 201

In vitro bone

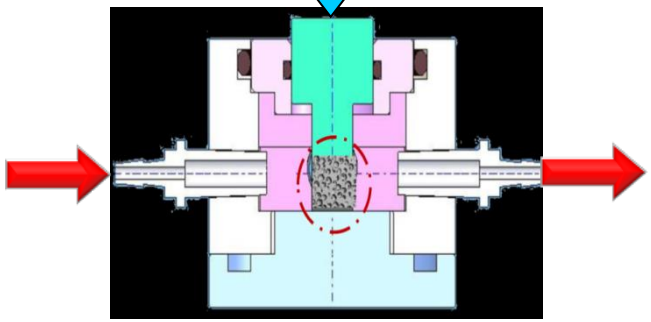
20 N

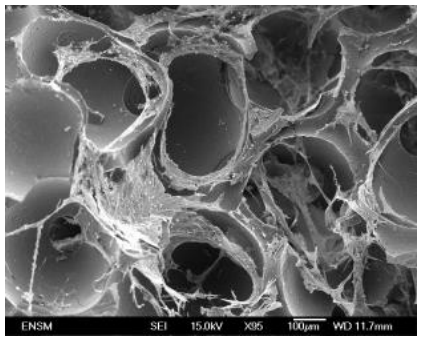


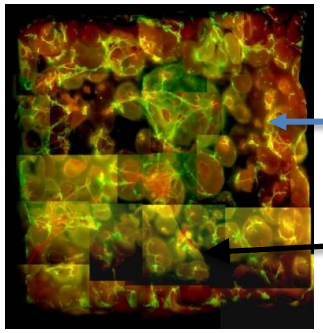


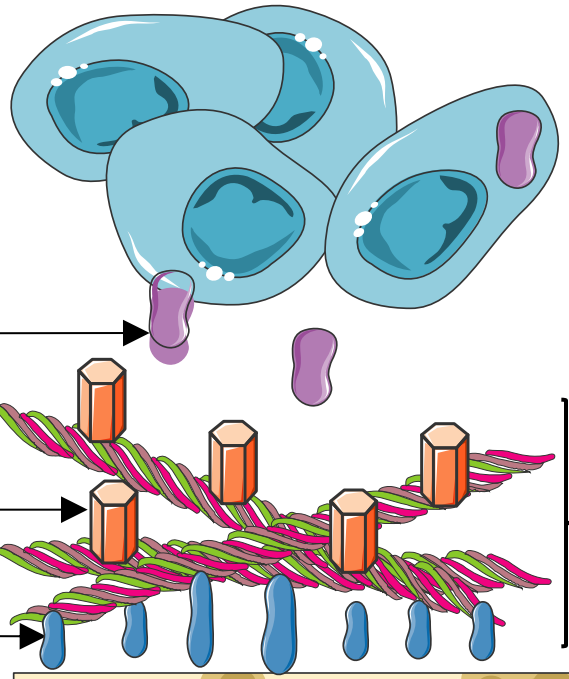
Cell culture conditions

- Co-culture Osteoblasts-cytes/clasts/endothelial cells
- Hormones/growth factors/drugs
- Micromechanical stimuli perfusion









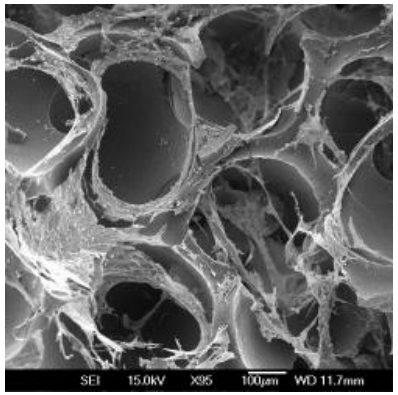
Bouet G et al., *Eur Cell Mater*. 2015

In vitro bone

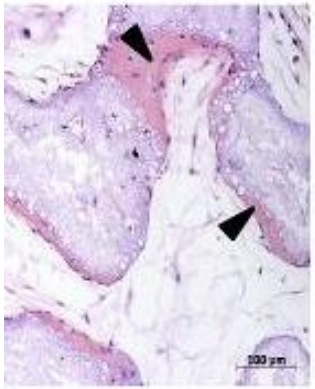
Characterization

Cell – Cell interactions Cell – tissue – scaffold interactions	Chemical analyses of harvested Medium	µCT, holotomography OCT imaging

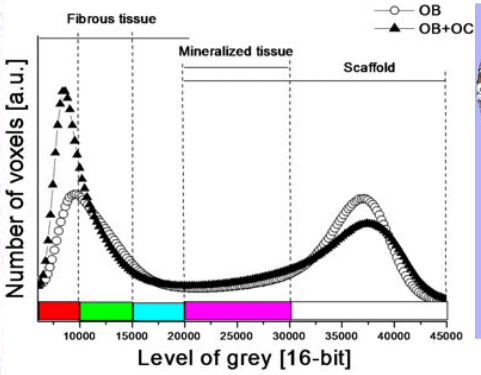
Phase CT, ESRF Grenoble , Fr

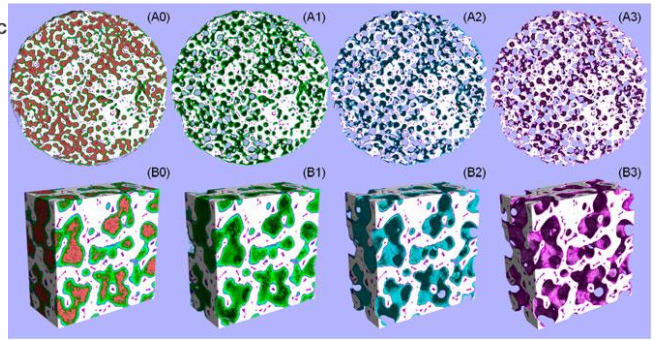


Bouet et al., Eur Cell Mater. 2015

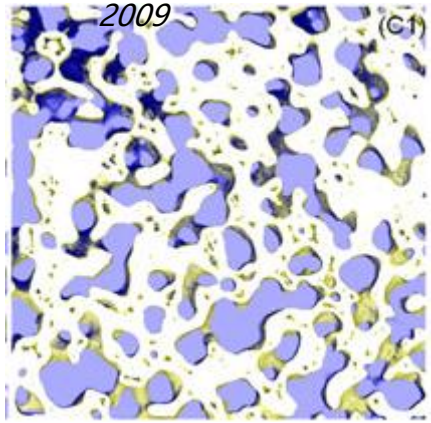


Tortelli et al., Tissue Eng, Part A, 2009





Ruggiu et al., J Tissue Eng Regen Med. 2014

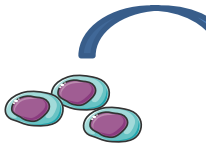


3D display of a scaffold before seeding with OB and OC and subsequent in vitro culture.
(yellow=volumes with decreased density;
blue=scaffold volumes representing matrix deposition)

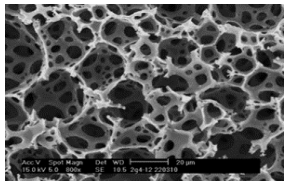
Ruggiu et al., J Tissue Eng Regen M

Invitrobone III – Adiposteo, mission spatiale eOSTEO

- Cellules amplifiées sur supports durs qui les reprogramment (perte de stemness).
- Comparaison entre cellules naïves et programmées => déterminer si les conditions de µpesanteur **restaurent la stemness et/ou limitent la sénescence** (régulation épigénétique, gènes de mécano-transduction et de sénescence).

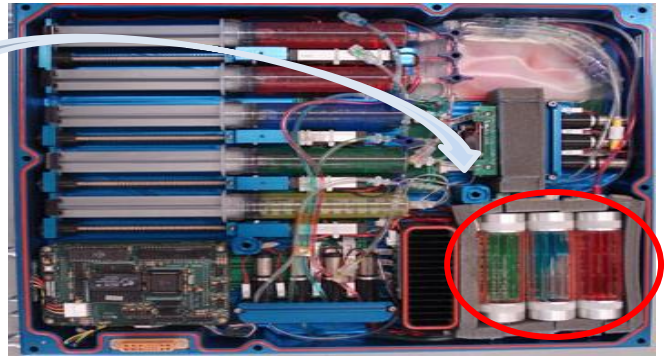


cellules souches squelettiques humaines, différents passages.



Hepes, Glucose

Integration des bioréacteurs **eOSTEO dans le tray (T° fixe et changements de milieu).**

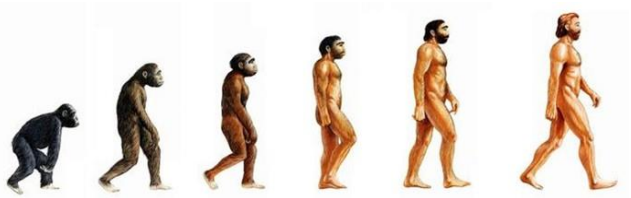


SPACEX *mars 2018.*

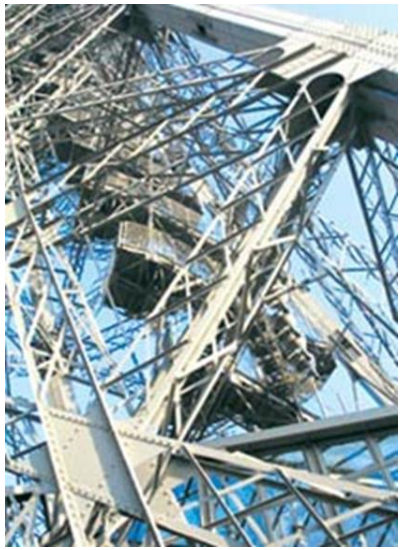
CALM TECHNOLOGIES INC

A continuum from organs to cells

Structural hierarchies composed of a tense network of muscles, bones, extracellular matrices, cells, and cytoskeletal filaments are influenced and shaped by constant 1-G on Earth. Changing the G level and direction is thus a unique challenge spanning several size scales from organ to organelle and whose mechanisms of adaptation to altered gravity have begun to be studied.



From *Homo erectus* toward *Homo ingravitus*?



Tour Eiffel

Many Thanks to:

The crewmembers for their time, effort, and dedication to the success of these studies

From Apollo 8 dec 1968

Devices for perfused 3D cultures with load generation

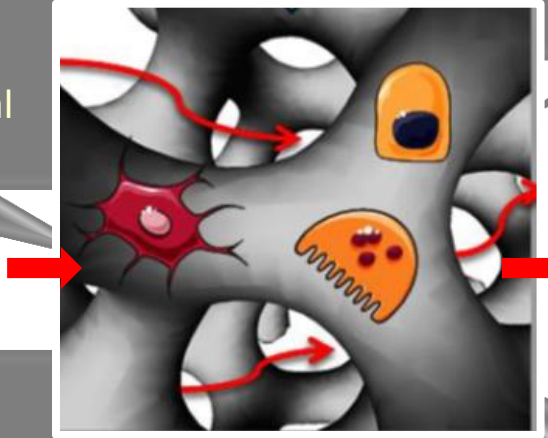
Scaffold

- Controlled macro- and micro-porous architecture
- Optimized mass transport and mechanical properties
- Fitted in culture chamber
- Accurately characterized (experimental and computational methods)

Cells

- Cell types (cell line, primary cells)
- Suitable medium
- homogeneous cell seeding

loading



flow

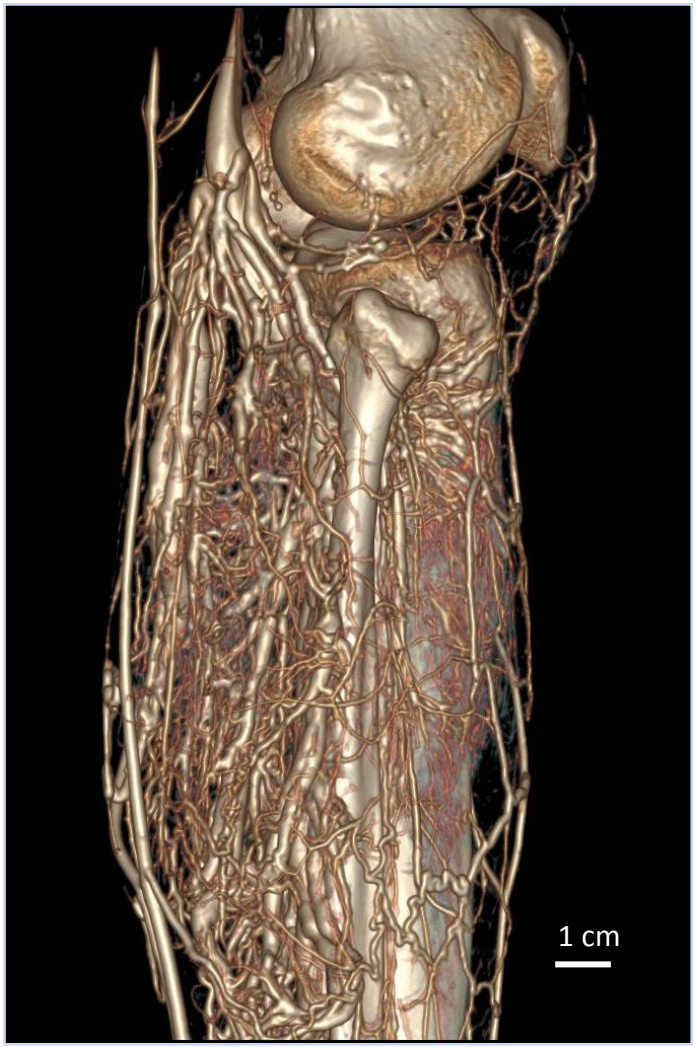
Bioreactor

- Perfused
- Mechanical stimulation via loading and/or shear stress
- Specific design for on-line monitoring
- Low cost production
- Easy handling (limit contamination)
- Sterilizable

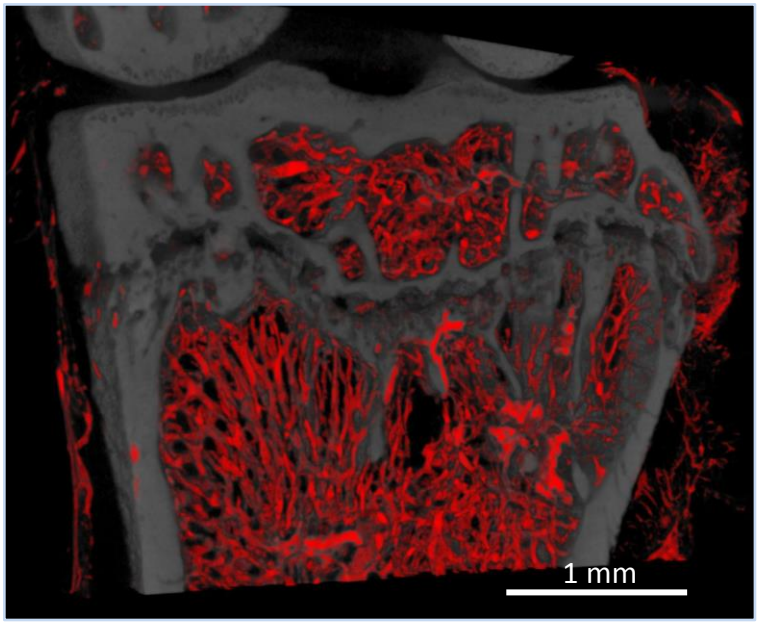
In situ characterization

- Culture medium analyses : cell metabolism, pH, gaz exchanges
- Micro-CT for mineralized scaffolds
- 3D Imaging (holotomography, optical coherence tomography, confocal microscope)

The transition to microgravity eliminates the hydrostatic gradients in the vascular system

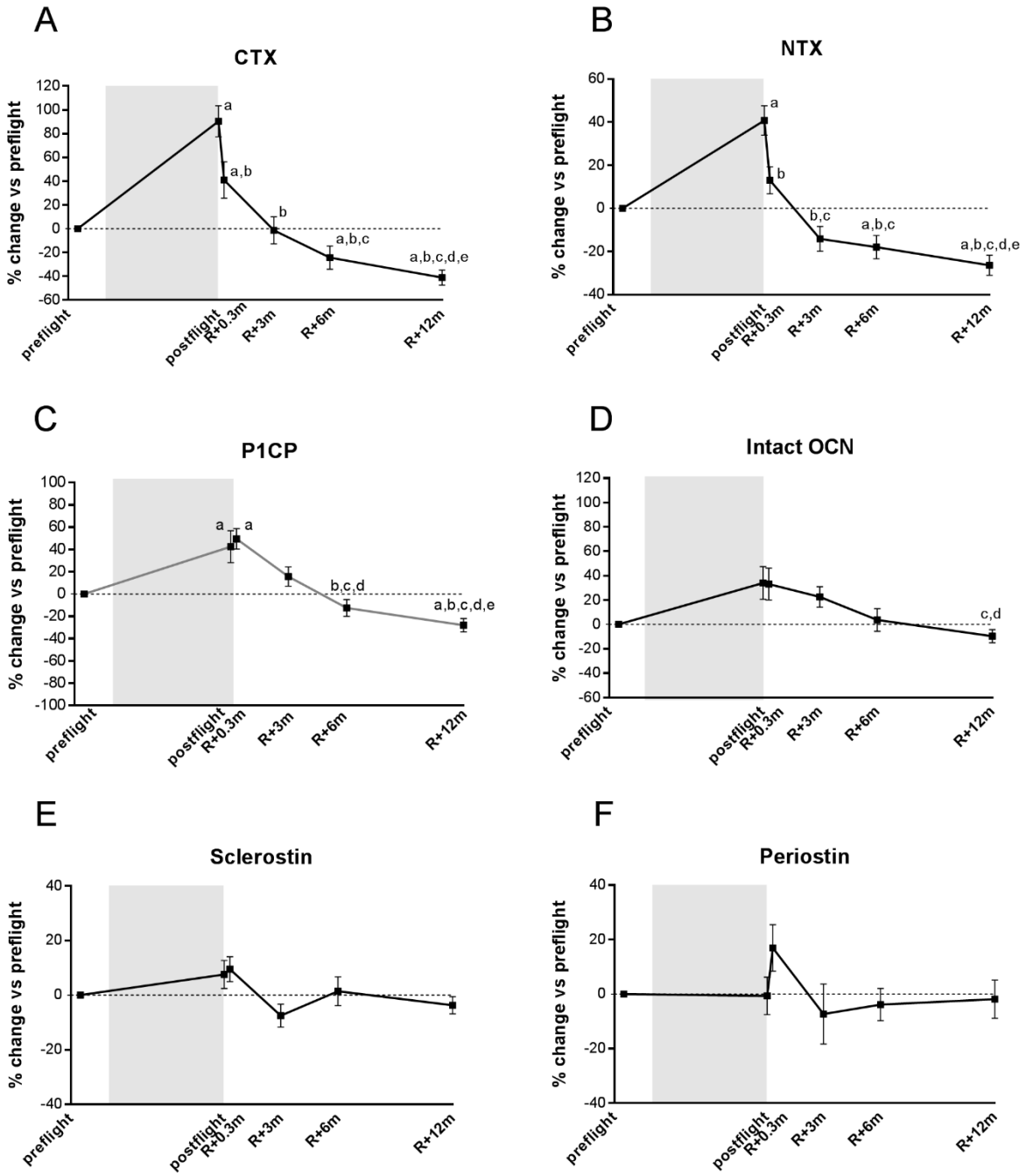


Human leg



Mouse proximal tibia

BONE MARKERS

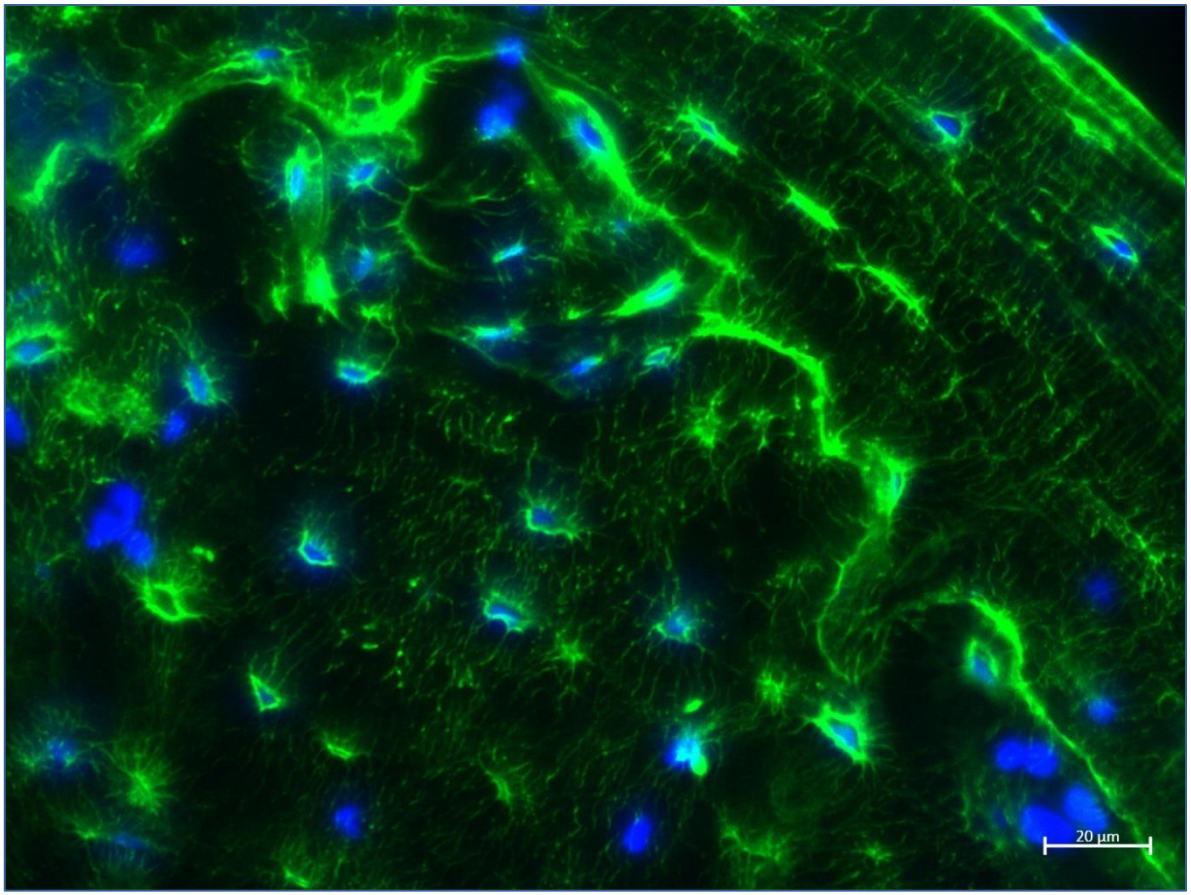


Differences in % vs preflight (median±quartile), $p \leq 0.05$

a vs. preflight,
b vs. postflight,
c vs. 0.5-mo,
d vs. 3-mo,
e vs 6-mo,

Vico et al., *JBMR*, 2017

Osteocyte Network



- Blaber et al., *PlosOne*, 2013
- Berg-Johansen et al., *J Orthop Res,* 2016
- Gerbaix et al., *Sci Rep*, 2017

- 14-d STS-131 mission: ↗osteocyte lacunae size, ↗MMP10 → **osteocytic osteolysis**
- 30-day Bion M1 mission: ↘lacunar volume, lacunae more spherical, ↗empty lacunae
- 91-day ISS mission, osteocyte lacunae acquired a rounder shape