MOUSE DRAWER SYSTEM (MDS)

EXPERIMENT

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The skeleton prevents the animal from collapsing due to gravity.
Osteoporosis is a systemic skeletal disease resulting from an unbalance of bone remodeling process.

In osteoporosis bone mineral density (BMD) is reduced, bone microarchitecture is deteriorating, and the amount and variety of proteins in bone are altered.

It is prevalent in post-menopause women (up to 3-4% bone loss per year).

Osteoporosis can be associated with particular conditions such as immobilization and spaceflight.
OSTEOPOROSIS & SPACEFLIGHT

Osteoporosis
“the silent disease”

Space environment is considered as an accelerated and reversible model of terrestrial osteoporosis.

An astronaut loses up to 1.5% a month, as much as a post-menopausal woman loses per year.

Cortesy of S. Pugh

Bone Density (g/cm³)

<table>
<thead>
<tr>
<th>Average Astronaut</th>
<th>Average Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>0.8</td>
</tr>
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</table>

Osteoporosis

Mars Mission Duration

31 months
Main goal of the MDS initial proposal

To investigate space related bone alterations at structural, cellular and molecular levels in mice that remained in space for a long period.

Multiple genetic loci influence bone mass. An additional goal was to investigate bone alterations in transgenic mice with an increased bone density, on the assumption that these mice are likely to be better protected from osteoporosis.
MDS is an ASI funded program aimed at the development of a facility to perform scientific experiments with mice on board the International Space Station established in 1998 to support the bone research proposed by the Genoa University.

Nevertheless, MDS represents a facility that can be used by a large number of scientists in different research fields.

MDS experiment nominal duration could be up to 100 days with possible extension up to 180 days.

MDS design takes into account animal welfare guidelines and recommendations used in on-ground laboratories.
Mice for a total mass of 240 grams can be housed. Mice can be housed either individually (maximum 6) or in groups.

The Mice Chamber is divided in 2 habitats configured as follows:

✓ as a unique cage of about 364 x 98 mm floor area
✓ as 2 equal cages each one of about 178 x 98 mm floor area
✓ as 3 equal cages each one of 116 x 98 mm floor area (current configuration)

Each habitat provide mice with basic services:

✓ 3 FEV’s (Food Envelopes) for food delivery
✓ 3 drinking valves for water delivery
✓ 3 cameras for video observation
✓ LED’s for illumination
✓ sensors for air composition control
  (temperature, rH, CO₂ and NH₃)
Food is supplied to each cage independently with 2 food bars integrated within a Food Envelope (FEV). The mass of each pair of food bars is about 90 grams.

Food bars composition can be decided by researchers according to scientific protocol needs. Solid additives can be added.

Once empty, FEV’s can be exchanged with new ones through six doors located in the MDS Front Panel.

Water is provided “ad libitum”
Mice selection for the MDS experiment

Pleiotrophin (Osteoblast stimulating factor-1) stimulates proliferation and differentiation of human osteoprogenitor cells “in vitro”.

Are PTN Tg mice less susceptible to the negative effects of microgravity on bones?

Transgenic mice over-expressing pleiotrophin (PTN) in bone, compared to Wt mice, appear more protected from aging bone loss and female are more protected from ovariectomy induced osteoporosis.
MDS experiment
- Mice for the first time on the International Space Station
- Animals for the first time 91 days in space

After preliminary behavior tests, BDF PTN-transgenic mice developed by Hashimoto-Gotoh (Kyoto, Japan) were backcrossed in C57Bl/J10

6 mice employed for the “flight experiment”

3 transgenic mice

(PTN Tg)

3 wild type mice

(C57BL/J10)

Age of flight mice:
at launch = 2 months; at landing = 5 months
MDS Mission Scenario

- Experiment Pre-Launch preparation at KSC (August 2009)
  - Training session of mice (i.e. use of food and water dispensers, cage volume etc.)
- Pre-Launch operations (August 22-27, 2009)
- Launch and Ascent (STS 128 Discovery; August 28, 2009)
- Transfer from Mid deck to Express Rack (September 1, 2009)
- On Orbit Operations (September - November 2009)
- Transfer from Express Rack to Mid deck (November 24, 2009)
- Return to KSC (STS 129 Atlantis; November 27, 2009)
- Post-Landing Operations
Pre-Launch preparation at KSC

- Training session of mice (use of food and water dispensers and cage volume)
- Measurement of food and water uptake by each animal
- Selection of flight and spare flight animals

Each mice received a “drinking training” before the insertion in the MDS by drinking water from bottles connected to a commercial stem activated valve at the bottle spout.
Launch and Ascent
(STS 128 Discovery; August 28, 2009)
MDS installed in ISS into Express Rack 4 in Japanese module (JEM)
Earth Control and Flight Operations

MDS - User Home Base (UHB)
Univ. Degli Studi di Genova

MDS - Payload Support Center (PSC)
Thales Alenia Milano

Pre-processed TM Video & Voice

TM, TC, Video & Voice

ASI NET

NASA - Marshall Space Flight Center (MSFC)
JSC Houston

NASA Payload Operations Center (POC)
JSC Houston

MDS - User Support Operations Center (USOC)
Telespazio Napoli
Monitoring of Flight Operations

Water consumption Flight Mice

Water consumption (ml)

Tg 1 2 3  Wt 1 2 3

Mouse

Drinking profile mouse 1
Camera observation:
2 hours with cage rotation 3 times a day
(recorded)
Unfortunately during this period 3 mice died due to:

- a spinal cord lesion possibly occurred during the shuttle lift off  \textit{(Wt 3 at day 16)}

- a possible liver pathology  \textit{(Tg 3 at day 24)}

- a failure of the food cassette system  \textit{(Wt 1 at day 44)}
Wild Type 2 mouse after about 3 hours from landing
All flight mice had essentially normal hematological parameters, but a higher erythrocyte concentration with a hematocrit near or above 50%.
Tissue Sharing Program Goals & Objectives:

• The MDS experiment was also expected to contribute data to the current body of research on microgravity effects on the skeletal, cardiovascular, and immune systems, liver and kidney function as well as other physiological systems through a tissue sharing program.

• Every effort has been made to harvest as many different samples and types of tissue from the mice.

• Positive results from this research may advance our understanding of mechanistic changes that occur in various physiological systems after exposure to microgravity and support overall efforts to reduce health risks to crewmembers.
TSP – Tissue Sharing Program

17 groups
6 countries
Tissue Sharing Program investigators and tissues of interest

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Country</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambesi</td>
<td>Italy</td>
<td>Tyroid</td>
</tr>
<tr>
<td>Cancedda</td>
<td>Italy</td>
<td>Serum, Bones, Bone marrow</td>
</tr>
<tr>
<td>Capasso</td>
<td>Italy</td>
<td>Urine, Kidney</td>
</tr>
<tr>
<td>Dinardo</td>
<td>Italy</td>
<td>Heart, Thoracic diafragm</td>
</tr>
<tr>
<td>Misericocchi</td>
<td>Italy</td>
<td>Lung, Trachea</td>
</tr>
<tr>
<td>Pippia</td>
<td>Italy</td>
<td>Intestine, Stomach</td>
</tr>
<tr>
<td>Rizzo</td>
<td>Italy</td>
<td>Serum, Red blood cells</td>
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<tr>
<td>Santucci</td>
<td>Italy</td>
<td>Tongue, Brain, Surreno glands, Hind paw</td>
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<tr>
<td>Schiaffino / Conte Camerino</td>
<td>Italy</td>
<td>Tongue, Limb muscles</td>
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<tr>
<td>Strollo / Masini</td>
<td>Italy</td>
<td>Pituitary gland, Pancreas, Kidney, Liver, Testis, Stomach, Forepaw</td>
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<tr>
<td>Bateman</td>
<td>U.S.A.</td>
<td>Bones</td>
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<tr>
<td>Blottner</td>
<td>Germany</td>
<td>Tongue, Limb muscles</td>
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<tr>
<td>Boyle / Shin-ici</td>
<td>U.S.A.</td>
<td>Skull</td>
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<tr>
<td>Green Johnson</td>
<td>Canada</td>
<td>Lymph nodes, Colon</td>
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<tr>
<td>Nusgens</td>
<td>Belgium</td>
<td>Skin</td>
</tr>
<tr>
<td>Ohira</td>
<td>Japan</td>
<td>Brain, Testis, Spinal chord, Limb muscles</td>
</tr>
<tr>
<td>Yufang</td>
<td>U.S.A.</td>
<td>Tymus, Spleen</td>
</tr>
</tbody>
</table>
A 1 g ground replica of the flight experiment (“ground control”) was performed at the University of Genova from November 2009 to the second week of February 2010.

As additional control tissue samples were collected also from mice maintained in standard vivarium IVC cages (“vivarium control”).
ANALYSIS PERFORMED ON BONE SAMPLES

1) Computed microtomography (μCT) analysis on weight-bearing bones
   - Femur
   - Lumbar spine (7th vertebra)

2) Computed microtomography (μCT) analysis on non weight-bearing bones
   - Calvaria

3) Histology
   - Femur

4) Molecular analysis of bone formation and resorption markers
   - Alkaline phosphatase (ALP)
   - Collagen type I (Coll I)
   - Osteocalcin (OC)
   - Rank ligand (RankL)
   - Tartrate-resistant acid phosphatase (TRAP)
   - Cathepsin K (CTK)
   - Osteoprotegerin (OPG)
Main findings

- For both Tg and Wt strains, a decrease of the trabecular number as well as an increase of the mean trabecular separation were observed after flight, whereas trabecular thickness did not show any significant change.
- Non weight-bearing bones were not affected.
- Microgravity-induced bone loss was due to both an increased bone resorption by osteoclasts and a decreased bone deposition by osteoblasts.
- The PTN-Tg mice exposed to normal gravity presented a poorer trabecular organization than Wt mice, but the expression of the PTN transgene during the flight resulted in some protection against the negative effect of microgravity.
- Apparently, the PTN transgene protection was the result of a higher osteoblast activity in the flight mice.
THE MICE DRAWER SYSTEM EXPERIMENT

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ALL THE TISSUE SHARING TEAM!!