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Responses to spaceflight of mouse mandibular bone and teeth

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ABSTRACT

Objective: To determine if spaceflight and microgravity affect non-weight bearing bones and development and mineralization of teeth, reasoning that combining an organ and a cellular level approach can lead to greater insights about these effects.

Design: Mandibles and incisors of mice flown on the US STS-135 space shuttle mission and the Russian Bion-M1 satellite were studied using micro-computed tomography and immunohistochemistry. Ground controls were mice housed in standard vivarium cages and flight habitats.

Results: Incisor length was greater in the 13-day STS-135 flight mice than in either control group. Initial incisor mineralization occurred more posteriorly, and incisor, enamel and dentin volumes and enamel and dentin thicknesses were greater in the 30-day Bion-M1 flight and habitat control mice than in vivarium control mice. Mandibular bone volume (BV) was increased in STS-135 flight and habitat groups and decreased in Bion-M1 flight and habitat groups compared to vivarium controls. No significant histological alterations occurred, but changes were seen in the bone and tooth proteins dentin sialoprotein, amelogenin and the type II regulatory subunit of protein kinase A. The percentage of sclerostin positive osteocytes was greatest in flight mice, and greater in STS-135 flight and habitat control mice than in the corresponding Bion-M1 groups. TRAP staining, representing osteoclastic bone remodeling, differed between the two flights and corresponded with changes in BV.

Interpretation of the findings was limited by a small number of flight mice, different sex and ages of the mice in the two missions, and different habitats and diets.

Conclusions: Microgravity has measurable effects on mandibular bone physiology and incisor development and mineralization. The results also showed that the habitat had an effect either in flight or ground control samples, as demonstrated by the changes in BV and apparent slowing of incisor eruption. Therefore, developing appropriate habitats is critical for future spaceflight missions.

1. Introduction

As the Earth's resources diminish, space exploration and colonization are increasingly important considerations. Spaceflight has a variety of effects on human physiology including bone and muscle loss, cardiovascular deconditioning, increased risk of renal stone formation, altered immune function, vision changes and disruption of taste (Demontis et al., 2017; Novoselova et al., 2015; Smith et al., 2015; Stein, 2013). Bone loss is one of the most well-known effects of prolonged spaceflight (Bikle, Sakata, & Halloran, 2003; Cavanagh, Licata, & Rice, 2005; Demontis et al., 2017; Nagaraja & Risin, 2013; Orwoll et al., 2013; Sibonga, 2013; Smith et al., 2015; Stein, 2013). Skeletal effects on weight-bearing bones such as the lower limbs and spine are well established; namely, imbalance in bone formation and resorption resulting in bone loss in astronauts (Sibonga, 2013; Spector, Smith, & Sibonga, 2009). These changes can be a restrictive factor for sending humans to space because of increased risk of fractures after return to Earth. Studies on the effects of spaceflight on human health

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are of great importance in order to develop effective countermeasure to prevent bone loss and other detrimental changes induced by microgravity or the spaceflight environment.

Data from animal experiments have shown that rodents also undergo microgravity-related bone loss (Bikle et al., 2003; Vajda, Wronski, Halloran, Bachus, & Miller, 2001; Vico, Lafage-Proust, & Alexandre, 1998). Depending on the mechanical load imposed on the skeleton, bone mass changes can vary greatly from weight bearing bone to non-weight bearing bone. Although previous studies have shown that weight bearing bones were more affected than non-weight bearing bones (Simmons et al., 1983), recent studies of the calvaria and mandible revealed that non-weight bearing bones also are affected by spaceflight (Ghosh, Stabley, Behnke, Allen, & Delp, 2016; Zhang, Cory, Bhattacharya, Sah, & Hargens, 2013).

Few studies have examined the effects of spaceflight on tooth development and mineralization, and the findings have been inconsistent. Some reports indicated increased calcium and phosphorus content of incisor dentin (Rosenberg, Campbell, & Simmons, 1984), while others reported unchanged values (Prokhonchukov, Tigranian, Kolesnik, Novikov, & Timofeeva, 1977; Prokhonchukov, Komissarova, Zhizhina, & Volozhin, 1980; Simmons et al., 1983; Simmons, Grynpas, & Rosenberg, 1990). Rodent incisor teeth are useful models for studying the effects of microgravity on tooth development and mineralization. The continuous growth of rodent incisor teeth allows observation of the successive stages of odontogenesis along the entire length of the tooth.

Understanding the underlying cellular and biochemical mechanisms for microgravity-induced/spaceflight associated changes is necessary in order to devise effective countermeasures, especially for long-term (e.g., interplanetary) flights. The working hypothesis in this study is that spaceflight has measurable effects on non-weight bearing bones and the development and mineralization of teeth, and that the extent of the changes are correlated with the length of the flight. To test this hypothesis, the effects of a 13-day flight on the US space shuttle Atlantis and a 30-day flight on the Russian Bion-M1 biosatellite were investigated using the mandible and incisor teeth of mice at the organ, tissue and cellular levels of organization employing radiographic and immunohistochemical approaches.

2. Material and methods

2.1. Animals and experimental conditions

The use of mice in these investigations was approved by the Institutional Animal Care and Use Committee at the National Aeronautics and Space Administration (NASA), the Institutional Animal Care and Use Committee of Moscow State University Institute of Mitoengineering (Protocol No–35, 1 November, 2012), and the Biomedical Ethics Commission of the Institute of Biomedical Problems (Moscow) (protocol No–319, 4 April, 2013). The specimens were made available through the NASA Biospecimen Sharing Program.

Mandibles containing molar and incisor teeth were collected from 7 adult female C57Bl/6 J mice housed in Animal Enclosure Modules (AEMs) (Morey-Holton, Halloran, Garetto, & Doty, 2000) on the 13-day STS-135 mission. Mandibles were obtained from 6 adult male C57Bl/6 N mice housed in cylindrical, acrylic “BOS” flight habitats (Andreev-Andrievskiy et al., 2014) on the 30-day Russian Bion-M1 mission. The AEMs and the BOS flight habitats are enclosed rodent habitats, of two different designs, that provide ventilation, lighting, waste collection, food and water for the rodents. Mandibles also were collected from 7 age and gender-matched ground control mice housed in the AEMs and 7 age and gender-matched ground control mice housed in the BOS flight habitats for the same length of time as each flight. The AEM-housed flight and ground control mice were fed (ad libitum) NASA rodent food bars (Sun, Tou, Yu, Girten, & Cohen, 2014), mounted on the sides of the AEM. Mice housed in the BOS habitats were fed a paste diet (Andreev-Andrievskiy et al., 2014; Novoselova et al., 2015), provided at 4-hour intervals. The mice were adapted to the paste diet during the 2-week period prior to launch. Mandibles were collected for each flight from 7 additional age and gender-matched ground control mice maintained in plastic cages under standard vivarium conditions and fed standard rodent chow. A summary of the animal data and experimental parameters is provided in Table 1.

Table 1
Animal Data.

Parameter	STS-135	Bion-M1
Flight Length	13 days	30 days
Strain	C57Bl/6 J	C57Bl/6 N
Sex	Female	Male
Flight Mice		
Number	7	6
Age / Weight at Launch	9 weeks / 20.74 g	15-16 weeks / 26.78 g
Age / Weight at Landing	11 weeks / 18.43 g	19-20 weeks / 29.37 g
Diet / Moisture Content	NASA Rodent Food Bars / 28%	Paste / 74.6%
Food / Water Consumption	4.1 g / 2.73 ml[a]	1.27 g/4.25 g[b]
Habitat Control Mice		
Number	7	7
Beginning Weight	20.61 g	27.29 g
Ending Weight	19.56 g	29.20 g
Diet / Moisture Content	NASA Rodent Food Bars / 28%	Paste / 74.6%
Food / Water Consumption	4.1 g / 3.38 ml[a]	1.27 g/4.25 g[b]
Vivarium Control Mice		
Number	7	7
Ending Weight	19.76 g	28.89 g
Diet / Moisture Content	Rodent Chow / 10%	Rodent Chow / 10%

[a] Per day, per animal.
[b] Per day, per 10 g body weight (calculated from percentage of water in the paste), determined in preliminary experiments prior to flight (Andreev-Andrievskiy et al., 2014).

2.2. Tissue collection and sample preparation

The flight mice were transported to the laboratory approximately 3 h after landing of the 13-day STS-135 flight (at the Kennedy Space Center in Florida) and 13 h after landing of the 30-day Bion-M1 flight (due to initial field inspections and transport from the landing site in the Orenburg region of Russia to the dissection site in Moscow). The STS-135 mice were euthanized by anesthesia with isoflurane and exsanguination; under the supervision of a NASA veterinarian, the Bion-M1 mice were killed by cervical dislocation followed by decapitation. The mandibles were removed, split into hemimandibles, trimmed of excess soft tissue and fixed in 4% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. After fixation overnight at 4 °C, the samples were transferred to 1% paraformaldehyde and shipped cold to UConn Health.

2.3. Micro-computed tomography (MicroCT)

The hemimandibles were scanned in 70% ethanol at a resolution of 12 μm voxels using a Scanco μCT 40 (Scanco Medical, AG, Bassersdorf, Switzerland) at the UConn Health MicroCT Imaging Core Facility. Serial images were acquired transverse to the longitudinal axis of the hemimandible at 55 kVp (145 μA), employing 1000 conebeam projections per revolution at an integration time of 300 ms within a 12.3 mm field of view. Three-dimensional images were reconstructed using standard convolution back-projection algorithms with Shepp and Logan filtering, and rendered at isometric 12 μm voxels. The morphometric variables analyzed included bone volume (mm^3) and mineral density (mg hydroxyapatite [HA]/cm^3), incisor enamel volume (mm^3) and incisor