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Effects of spaceflight on the murine mandible: Possible factors mediating skeletal changes in non-weight bearing bones of the head

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ABSTRACT

Spaceflight-induced remodeling of the skull is characterized by greater bone volume, mineral density, and mineral content. To further investigate the effects of spaceflight on other non-weight bearing bones of the head, as well as to gain insight into potential factors mediating the remodeling of the skull, the purpose of the present study was to determine the effects of spaceflight on mandibular bone properties. Female C57BL/6 mice were flown 15 d on the STS-131 Space Shuttle mission ($n = 8$) and 13 d on the STS-135 mission ($n = 5$) or remained as ground controls (GC). Upon landing, mandibles were collected and analyzed via micro-computed tomography for tissue mineralization, bone volume (BV/TV), and distance from the cemento–enamel junction to the alveolar crest (CEJ–AC). Mandibular mineralization was not different between spaceflight (SF) and GC mice for either the STS-131 or STS-135 missions. Mandibular BV/TV (combined cortical and trabecular bone) was lower in mandibles from SF mice on the STS-131 mission (80.7 ± 0.8%) relative to that of GC ($n = 8$) animals (84.2 ± 1.2%), whereas BV/TV from STS-135 mice was not different from GC animals ($n = 7$). The CEJ–AC distance was shorter in mandibles from STS-131 mice (0.217 ± 0.004 mm) compared to GC animals (0.283 ± 0.009 mm), indicating an anabolic (or anti-catabolic) effect of spaceflight, while CEJ–AC distance was similar between STS-135 and GC mice. These findings demonstrate that mandibular bones undergo skeletal changes during spaceflight and are susceptible to the effects of weightlessness. However, adaptation of the mandible to spaceflight is dissimilar to that of the cranium, at least in terms of changes in BV/TV.

1. Introduction

Bone loss is considered to be one of the most serious and potentially intractable biomedical consequences of human spaceflight. However, microgravity-induced changes in bone are not uniform along the skeletal axis. For instance, while bone mineral density (BMD) decreases in the lower extremities [1,2], it increases in the skull [3,4]. Similar patterns of region-specific changes in bone properties have been reported to occur with head-down bedrest [5,6], a ground-based model used to simulate a weightless environment in humans. These regional changes in bone properties have been proposed to be associated with the cephalic fluid shifts that occur in a microgravity environment [7–9].

Flight studies with rats have likewise shown regional changes in bone properties, including bone loss in the long bones of the hindlimbs and increases in BMD and Ca^{2+} and P content in the skull [10,11]. Rats subjected to hindlimb unloading (HU), a ground-based animal model which produces skeletal unloading and a headward fluid shift [12–14], have similarly demonstrated a loss of bone mineral content and density in hindlimb bones [15–17] and increases in bone mass, BMD, and Ca^{2+} content in both the skull and mandible [12,18–21]. These studies all support the notion that bone properties change in both actual and simulated microgravity and are associated with changes in tissue fluid pressure redistribution.

In HU rats, shifts in arterial blood pressure also occur throughout the body correspond to distinct patterns of arterial remodeling. Specifically, with HU, elevations in arterial pressure in the head result in increases in cerebral artery wall thickness [22,23], whereas reductions in arterial pressure in the hindlimbs result in a thinning of the vessel wall in bone and skeletal muscle resistance arteries [24,25]. Similar patterns of arterial remodeling do not occur in mice with either spaceflight [26,27] or hindlimb unloading [27], suggesting that the small body size of the mouse does not evoke significant arterial blood pressure shifts and corresponding tissue fluid pressure alterations during spaceflight or with a change in body orientation. Thus, it was surprising when mice flown on the STS-131 Space Shuttle mission were reported to experience increases in skull bone volume [28]. With a presumed absence of significant arterial pressure shifts in mice, these changes in the properties of non-weight bearing bones in the head suggest an influence from other factors, such as increases in bone blood flow or intracranial

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pressure, both of which could modulate the increase in calvaria bone volume reported to occur in these animals.

To further investigate the effects of spaceflight on the properties of non-weight bearing bones of the head, as well as to gain insight into potential factors mediating the skeletal changes of the mouse skull in a weightless environment, the purpose of the present study was to determine the effects of spaceflight on the bone properties of the mouse mandible. Mandibles were collected from two groups of mice flown on the STS-131 and STS-135 Space Shuttle missions, the former group being the same animals demonstrating an increase in calvaria bone volume [28]. Given the lack of evidence for a significant fluid pressure shift in mice with spaceflight [26,27], we hypothesized that murine mandibular bone volume would be unchanged with spaceflight.

2. Methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the National Aeronautics and Space Administration (NASA) and conformed to the U.S. National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* (8th ed., 2011).

2.1. Animals

Experiments were performed on animals from two separate NASA space shuttle missions. Female C57BL/6 mice (23 wk. old; $n = 8$; Charles River, Raleigh, NC, USA) were flown for 15 days on the STS-131 mission, while female C57BL/6 mice (9 wk. old; $n = 5$) were flown for 13 days on the STS-135 mission. Spaceflown (SF) mice on both missions were housed in NASA's animal enclosure modules (AEMs) located on the orbiter's middeck, maintained on a 12:12-h light–dark cycle, and provided food and water ad libitum as previously described [26,27,29]. SF mice were weighed, anesthetized with isoflurane, euthanized via exsanguination at the NASA Kennedy Space Center (KSC), and bone and soleus muscle samples were collected within 3–4 h of landing. Age-matched ground-based control (GC) mice were housed in identical AEMs within an orbital environment simulator at KSC to mimic the temperature, humidity, and CO_2 levels of the space shuttle middeck. GC mice during the STS-131 ($n = 8$) and STS-135 ($n = 7$) missions were housed for the duration of each mission beginning 48 h after launch and ending 48 h after landing. All animals were part of NASA's Biospecimen Sharing Program. Mandibles were snap frozen and stored at −80 °C until analyzed and soleus muscle samples were weighed.

2.2. Microcomputed tomography assessment of mandibular bones

Mandible properties were assessed using high-resolution micro-CT (Skyscan 1172). Bones were wrapped in parafilm and scanned at 60 kV with a 6-μm isotropic pixel size. Images were reconstructed and analyzed using standard Skyscan software (NRecon and CTAn, respectively). A single slice from the central region of the first mandible molar was analyzed for three parameters: total bone volume (both cortical and trabecular bone, excluding the molar and incisor) and lingual cementum–enamel to alveolar bone crest distance (CEJ–AC), and tissue mineralization (gray scale value on a 0–255 scale) (Fig. 1) as previously described [30,31]. In this context, the tissue mineralization is the average gray scale value of tissue classified as bone and represents an index of the level of tissue mineralization. CEJ–AC, as its name indicates, is the measured distance from the cementum edge on lingual tooth surface to the alveolar bone apex.

2.3. Statistical analysis

Unpaired t-tests were used to compare body and tissue masses and microCT parameters between SF and GC mice. Due to the age differences of the mice studied from the STS-131 (23 wks) and STS-135 (9 wks) missions, bone parameters from the two flights were analyzed separately. All values are presented as means ± SE. Significance was set at $P < 0.05$.

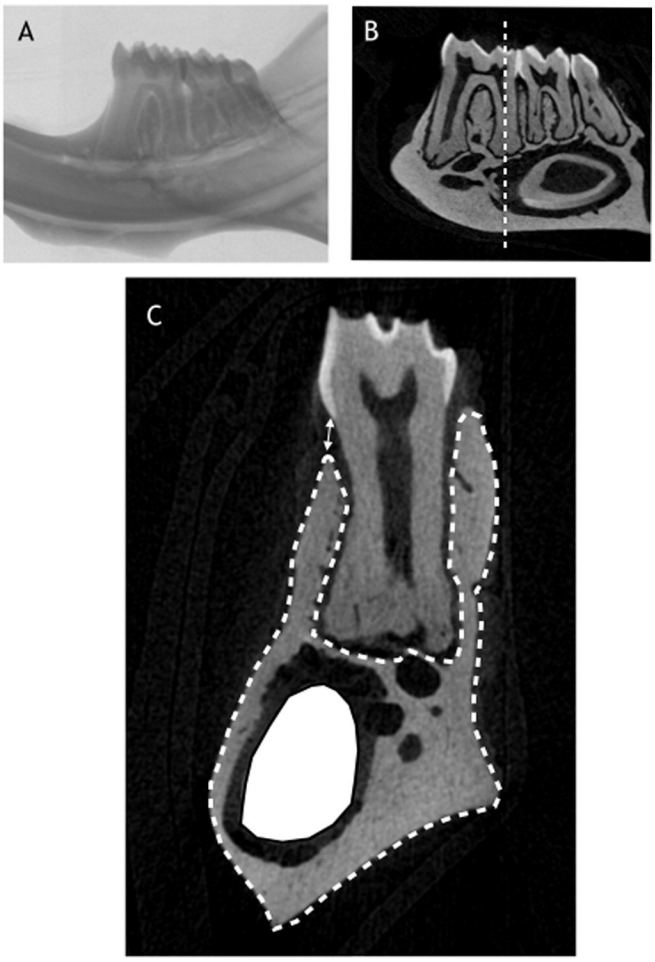

Fig. 1. Mandible CT workflow. Raw images were scanned to encompass the entire molar region (A). Reconstructed images were oriented and a section through the second root of the first molar was saved for analysis (B). The body of the mandible was isolated by tracing the periosteal surface and excluding the incisor, all bone within the region of interest (depicted by the white lines) was assessed for bone volume/total volume (C). The distance from the cementum–enamel junction to the alveolar crest (CEJ–AC) was measured (arrow).

3. Results

3.1. Body and tissue mass

Total body mass did not differ between GC and SF mice from both the STS-131 (GC: 21.2 ± 0.3 g; SF: 20.0 ± 0.6 g) and STS-135 (GC: 19.5 ± 0.5 g; SF: 18.3 ± 0.7 g) missions [29]. Soleus muscle mass was lower in SF mice relative to GC animals for both the STS-131 (GC: 14.3 ± 0.8 mg; SF: 10.9 ± 0.7 mg) and STS-135 (GC: 6.1 ± 0.4 mg; SF: 4.7 ± 0.2 mg) missions [29], indicating that there was a physiologically significant unloading of hindlimb muscles and bones with spaceflight.

3.2. Mandible characteristics

There were no differences in tissue mineralization with spaceflight in either mission (Fig. 2). However, mandible bone volume (BV/TV, %) was lower in SF mice from STS-131 (Fig. 3A). Mandibles from SF mice from the STS-135 mission had no difference in BV/TV (Fig. 3B). The