Craniofacial bones and teeth in spacefarers: systematic review and meta-analysis

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1 Abstract

1.1 Abstract (English)

Estimating the risk of dental problems in long duration space missions to Moon and Mars is critical for avoiding dental emergencies in an environment that does not support proper treatment. Previous risk estimates were constructed based on the experience in short duration space mission and isolated environments on Earth. However, previous estimates did not account for potential changes in dental structures due to space travel, even though bone loss is a known problem for long duration spaceflights. The objective of this study was to systemically analyze the changes in hard tissues of the craniofacial complex during spaceflights. Comprehensive search of Medline, Embase, Scopus, the NASA Technical Report Server and other sources identified 1585 potentially relevant studies. After screening, 30 articles that presented quantitative data for skull in humans (6/30), and for calvaria, mandible and lower incisors in rats (18/30) and mice (6/30) were selected. Skull bone mineral density showed a significant increase in spacefaring humans. In spacefaring rodents, calvaria bone volume to tissue volume (BV/TV) demonstrated a trend towards increasing that did not reach statistical significance, while in mandibles there was a significant decrease in BV/TV. Dentin thickness, and incisor volume of rodent incisors were not significantly different between spaceflight and ground controls. Our study demonstrates significant knowledge gaps regarding many structures of the craniofacial complex such as the maxilla, molar and canine teeth, as well as small sample sizes for the studies of mandible and incisors. Understanding the effects of microgravity on craniofacial structures is important for estimating risks during long duration spaceflight and for formulating proper protocols to prevent dental emergencies.

1.2 Résumé (French)

L'estimation du risque de problèmes dentaires dans les missions spatiales de longue durée vers la Lune et Mars est essentielle pour éviter les urgences dentaires dans un environnement qui ne prend pas en charge un traitement approprié. Les précédentes estimations des risques ont été construites sur la base de l'expérience de missions spatiales de courte durée et d'environnements isolés sur Terre. Cependant, les estimations précédentes ne tenaient pas compte des changements potentiels dans les structures dentaires dus aux voyages dans l'espace, même si la perte osseuse est un problème connu pour les vols spatiaux de longue durée. L'objectif de cette étude était d'analyser de manière systémique les modifications des tissus durs du complexe craniofacial lors des vols spatiaux. Une recherche complète dans Medline, Embase, Scopus, le serveur de rapports techniques de la NASA et d'autres sources a identifié 1585 études potentiellement pertinentes. Après sélection, 30 articles présentant des données quantitatives pour le crâne chez l'homme (6/30) et pour les calvaires, la mandibule et les incisives inférieures chez le rat (18/30) et la souris (6/30) ont été sélectionnés. La densité minérale osseuse du crâne a montré une augmentation significative chez les humains spatiaux. Chez les rongeurs spatiaux, le volume osseux/volume tissulaire de la calvaire (BV/TV) a montré une tendance à l'augmentation qui n'a pas atteint la signification statistique, tandis que dans les mandibules, il y avait une diminution significative de BV/TV. L'épaisseur de la dentine et le volume des incisives des rongeurs n'étaient pas significativement différents entre les vols spatiaux et les contrôles au sol. Notre étude démontre des lacunes importantes dans les connaissances concernant de nombreuses structures du complexe craniofacial telles que les dents maxillaire, molaires et canines, ainsi que de petites tailles d'échantillons pour les études de la mandibule et des incisives. Comprendre les effets de la microgravité sur les

structures cranio-faciales est important pour estimer les risques pendant les vols spatiaux de longue durée et pour formuler des protocoles appropriés pour prévenir les urgences dentaires.

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3 Contribution of Authors

Mahmoud S. Moussa, Master of Science Candidate: Contributed to study idea and design, performed screening, study characterization, data-extraction, critical appraisal and synthesis, and preparation of the final figures and manuscript.

Matthew Goldsmith: Contributed to study idea and design, performed screening, data extraction and meta-analysis calculations.

Svetlana V. Komarova: supervisor, contributed to study idea and design, critical appraisal and synthesis and was involved in manuscript preparation and editing.

4 Abbreviations

4.1 Missions

BION/Cosmos: Russian Biocosmos Flights

CRS: Commercial Resupply Services

STS: Space Transportation System

SL: Sky Lab

ISS: International Space Station

NASA: National Aeronautics and Space Administration

4.2 Hard Tissue Parameters

BV/TV: Bone Volume/Tissue Volume

TMD: Tissue Mineral Density

CEJ AC: Cemento-Enamel Junction to Apical Crest

Oc.S/B.S: Osteoclast Surface to Bone Surface

Oc.N: Osteoclast Number

Cs.Th: Calvarium Thickness

5 Introduction and Objective

5.1 Introduction

Microgravity induced bone loss is a well-known and still unmitigated effect of long-term spaceflight on the human and rodent skeleton (Fu et al. 2021; Stavnichuk et al. 2020), however the effect of space travel on the oral cavity and jaw bones is far less studied. Hard tissues of the oral cavity are constantly used for vital tasks such as mastication, speech, respiration and deglutition. Understanding the effect of spaceflight on the overall health of the oral cavity and its supporting structure is critical for avoiding dental emergencies in an environment that does not support proper treatment. This becomes especially important when planning for long duration mission to the Moon and Mars.

Dental issues reported in space travel include dental caries, crown displacement, and lost fillings (Menon 2012). However, when preflight and postflight dental events in astronaut corps were taken into account, several cases of pulpitis and dental abscesses were recorded, which may result in severe health consequences when untreated (Menon 2012). Documented dental emergencies accounted for 1% of all medical events aboard the Mir space station in a 3-year period (Gontcharov et al. 2005), as well as a case of a cosmonaut who spent 2 weeks of the 96 days in space in incapacitating pain (Ball et al. 2001). Based on the estimates developed for isolated Earth-based environments such as expeditions (Brown et al. 1977; Kupper et al. 2014), the frequency of potential dental emergencies will increase to substantial levels in longer term spaceflights. Taking into account the potential length of the space mission to Mars of 9-12 months, and a team of the 10 space travellers, these risk estimates translate into at least 1 certain event resulting in significant discomfort to crew members. However, none of the previous estimates accounted for potential changes in dental structures due to space travel that may affect the risk estimates.

5.2 Rationale and Objectives

Understanding how microgravity influences craniofacial hard structures is essential for estimating risks during long duration spaceflight and for formulating proper protocols to prevent dental emergencies. The objective of this study is to systemically review the literature for changes in hard tissues of the craniofacial complex during spaceflights and use meta-analytic approaches to quantify these changes.

6 Review of Literature

6.1 History of dentistry in space programs

The US Apollo missions were the first to take humans to the moon. It was the third US space program following Mercury and Gemini, running from 1968 to 1972. A year following the end of the Apollo program, the first US space station Skylab launched into orbit. Until 1979, Skylab hosted 3 missions with a total crew of 9 astronauts. After Skylab came the shuttle program that operated for 30 years. During that time, the soviet union launched their own space station program with Salyut in 1971, which ran for 15 years. The US and Russia then collaborated in 1993 to send 11 missions to the Mir space station. Nowadays the whole international community collaborates aboard the international space station (ISS). The Apollo program did not present any dental events that impacted the functionality of crew members. During the preflight period 5 of 33 astronauts required dental intervention. One of these crew members suffered from pulpitis case that occurred prior to and following their mission (Johnston et al. 1975). In the Skylab series there was an introduction of an inflight medical support system that included an onboard physician or scientist pilot. For the first time a dental kit was made available onboard along with a catalog complete with intraoral radiographs of all crew members. Aboard the Salyut and Mir missions, reported lost fillings due to take off vibrations and a case of caries that was treated in space using temporary fillings (Menon). A Russian cosmonaut aboard Salyut-6 spent 2 weeks of his 96-day flight in debilitating extreme pain, which he kept secret from ground control until his fellow astronauts disclosed the information. At the time there was no protocol for dental emergencies in space and evacuation was not a possibility (Seedhouse 2011). This kind of event is a clear indicator that dental emergencies may happen at any time even during spaceflight and even to an individual who underwent extensive medical examination. Nowadays, aboard the ISS there is a crew medical officer (CMO) who trains in different medical disciplines (Häuplik-Meusburger et al. 2016). They conduct regular medical examinations including radiation monitoring and physical tests. The ISS medical equipment and instruments are available to be used for multiple procedures such as crown replacement, avulsion, exposed pulp, injection technique, temporary filling, extraction and toothache. Preforming dental procedures is a very technical task and becomes even more challenging in an isolated environment that lacks gravity. Space missions will increase in duration in the future and it might become more difficult to manage dental emergencies. Especially when these long missions will have less access to mission control for assistance and no option of evacuation.

6.2 Risk Estimates

6.2.1 Risk of dental emergencies in space

Most recently, integrated medical models have been used to predict the occurrence of dental events during space missions. The actual reported incidence of dental events indicates very low risks (Menon). However, it was suggested that this may be due to severe under reporting of dental events before and during spaceflight. Based on the dental observations in the entire space corps, it was predicted that risk of dental caries in flight is 0.39 events per person year and 0.02 events per person year for dental abscess or exposed pulps (Menon). For the mission to Mars of 10 crewmembers for 2 years, this risk translates into 8 new instances of caries and 0.4 events of pulpitis. While the risk of pulpitis does not reach one, it is important to note that this condition is extremely painful and, if left untreated, may lead to a true medical emergency (Hodapp 2008).

Thus, it is important to understand the factor that may affect this risk estimate. Astronauts undergo extensive medical examinations prior to flight including dental examination. Though these examinations can significantly reduce the occurrence of some dental events they cannot preclude the occurrence of others such as fractures due to trauma or changes and decay in dental materials. Taking into account the duration of mission is also important, as dental prophylaxis prior to flight is unlikely to prevent dental events from occurring in long duration missions such as flights to Mars.

6.2.2 Risk of dental emergencies in isolated environments

Although, the risks of dental emergencies in space are not well investigated due to poor documenting, we can look at similar isolation conditions here on earth to evaluate risk of dental events. In a published review by Lloro et al (Lloro et al. 2019) dental incidences are investigated in isolated conditions, including Antarctic and submarine missions. This systematic review pooled together data from 7 submarine missions and 3 Antarctic deployment to Australian bases. This review identified 70 dental events in Antarctica and 813 in submarines. They found that the risk of dental event is 0.467 per person year in Antarctic missions, while significantly lower in submarine missions at 0.00239 events per person year (Lloro et al.). This significantly lower rate in submarine missions may be explained by an under-reporting of smaller-scale events by military personnel. In Antarctic conditions acute events such as fracture of teeth presented 51.4% of all events, while caries surprisingly accounted for only 10% of all dental events in Antarctica. While in submarines, caries presented 40% of all dental events (Lloro et al.). This difference may be explained by several factors, such as dental education and access to care for the personnel in Antarctic missions, who all were scientific personnel and older in age compared to the submarine crew. The cold weather in Antarctica may be another factor with adverse effects on dental materials leading to their clinical failure or fracture (Fletcher 1983). Importantly, none of these missions lasted for more than 3 months in isolated conditions, thus it is difficult to establish risk estimates for long duration isolation. Trekkers in Nepal arriving from different countries, predominantly Europe, are a population that has recently been studied for the risk of dental events during their trekking expeditions. Similar to spaceflight, expeditions of this nature are physically and psychologically demanding on its participants. The drastic changes in environment can lead to changes in hygiene routines and introduce additional risks. It was calculated that the risk of any dental problem occurring is 15 events per person year (Kupper et al. 2014). Trekkers that visited the dentist 6 months or less prior to their trip experienced significantly fewer dental problems. Interestingly, oral hygiene measures decreased while trekking in comparison to participants home routines (Kupper et al. 2014). The decrease in oral hygiene may be due to lack of usual facilities, fatigue or stress from busy nature of the trip, which can also be said for astronauts who have hectic schedules in space. Oral hygiene is of extreme importance to reduce the risk of dental events. Thus far, there has not been a study comparing dental hygiene in astronauts in orbit and on Earth. Thus, the risk of dental events is determined by the health status of travelers before spaceflight, as well as by the changes that occur during the actual flight. In addition to changes in hygiene, changes in dental tissues, similar to bone loss reported for space travellers (Stavnichuk et al.) may negatively affect dental health in space. Although dental tissues do not express the same remodeling capacities as bone, and are not generally actively changed through cellular activities, changes in mineral content in dental tissues are possible. In addition, lack of remodeling suggests that the negative effects of spaceflight specifically on tooth structure may not be recovered in a similar fashion to bone. Currently, risk of dental event in spaceflight estimated based only on prior events will underestimate the actual risk significantly if changes during spaceflight are not taken into consideration.

6.3 Bone Health in Spaceflight

6.3.1 Overview of spacefarers

For the past 60 years humanity has been exploring the vast cosmos. During this time there has been 565 spacefaring humans as of 2020 (Corlett et al. 2020). During the past 2 decades there has been increases in the time spent in spaceflight and conducting other activities such as space walks (Corlett et al. 2020). While the population of human space travelers is considerably large, the limitations of analyzing this population arise from the small sample size per mission and deficiencies in reporting (Corlett et al. 2020). With regards to animals, there have been plenty of spacefaring species including rodents, primates, fish, birds and dogs. Over 90% of the literature on the changes in bone in space animals was focused on rodents such as rats and mice (Fu et al. 2021). Therefore, humans and rodents are two abundant populations of spacefarers that can be examined for the changes in the craniofacial complex in space.

6.3.2 Spaceflight induced changes in bone health.

Bone loss is a known and still unmitigated complication of space travel. In humans, previous meta-analysis of spacefaring population found that bone loss is more pronounced in the lower skeleton and less so in the upper skeleton. The rate of bone loss in the lower limbs was calculated at approximately 0.8% loss per month, intriguingly an increase was observed in the bone density of the skull region. While resorption markers in astronauts increased significantly during the first month of spaceflight and then plateaued, formation markers kept increasing gradually throughout the whole flight (Stavnichuk et al. 2020). In rodents, it is suggested that spaceflight induced bone loss is occurring more in trabecular bone and less in cortical bone. The sub-group analysis of spacefaring rodents found that the distal skeleton such as the tibia and femur exhibited more bone loss than the axial skeleton (Fu et al. 2021). Therefore, it is evident that spaceflight-induced bone loss in humans and rodents is region dependent. Different bones (tibia vs. ribs) as well as different regions (trabecular vs. cortical) within them respond differently to spaceflight. Thus, it is important to understand how craniofacial hard tissues react under microgravity.

6.4 Dental health indicators in spacefarers

6.4.1 Differences in dentitions between humans and rodents

Teeth are formed of a crown composed of enamel covering dentin that surrounds dental pulp and below the crown lies a root composed of cementum covering dentin. Periodontal ligaments are responsible for anchoring teeth in the jaw bone. Enamel and dentin are deposited by ameloblast and odontoblasts respectively. In humans, ameloblasts disappear at tooth eruption but in rodents remain active following eruption and are responsible for the continuous eruption of incisor teeth. Therefore, enamel is continuously deposited in rodent incisors and they are characterized by being continuously erupting. In contrast, molar teeth in rodents are of limited growth and may present a closer model to changes occurring in humans. While humans have two sets of dentitions throughout their lifespan, rodents only have a single set of teeth.

6.4.2 Indicators of potential dental problems.

The three most common dental issues occurring in isolated environments include caries, periodontal problems, and fractures. Spaceflight associated changes in bone microarchitecture (Fu et al. 2021; Stavnichuk et al. 2020) are known to predispose for bone fractures (Mikolajewicz et al. 2020). Caries is a progressive disease that can have dire consequences if left untreated. It is one of the most prevalent chronic diseases in the world (Benjamin 2010). Bacteria release acids that demineralize tooth structure and help penetrate further into the tooth (Abou Neel et al. 2016). It is important to therefore understand how spaceflight might affect the mineralization of different tooth structures. It is known that a calcium alterations exists in spacefarers (Wronski and Morey 1983). These alterations can pose challenges in newly formed dentin in space potentially becoming more susceptible to bacterial infection. Periodontal problems can be presented in the form of bleeding gums known as gingivitis or a more severe form known as periodontitis that is associated with irreversible damage to the periodontal apparatus. Good oral hygiene measures help slow down the progression of periodontitis (Watt and Petersen 2012). The resultant damage from periodontitis is loss of attachment as well as bone in the apical crest region. It is therefore important to investigate the changes in apical crest given the known influence of spaceflight on bone regions and the periodontal region is no exception since it has a very high rate of turnover and is susceptible to local and systemic changes (Jonasson et al. 2018).

7 Methods

This study was performed in compliance with the Preferred Reporting Items for Systematic Reviews and Meta- analysis (PRISMA) statement (PRISMA Checklist is provided in **Appendix note 1**).

7.1 Search strategy, inclusion and exclusion

A systematic search strategy (**Appendix note 2**) containing relevant terms for spaceflight, space missions, teeth and all cranial bones was constructed and reviewed by a medical librarian. The **search was performed in Medline, Embase and Scopus on June 9th, 2021, and was complemented** by a manual search of the NASA technical report server. Title and abstract screening were carried out by two independent reviewers (MG and MSM). Articles in any language were included for full text screening if they alluded to any craniofacial structure in any species that has experienced spaceflight. Articles describing simulated microgravity were excluded. Included papers were scored for reporting quality (**Appendix note 3**).

7.2 Data Extraction

From studies with quantitative parameters for craniofacial structures, we recorded authors, year of publication, mission, duration of spaceflight, species, craniofacial structures analyzed, type of quantitative measurement, and control groups involved in study. For studies included in the meta-analysis we also extracted sample sizes for spaceflight and comparison group(s), craniofacial structure and its region being assessed, and for each parameter we extracted mean spaceflight group (SF) values and the mean comparison control (CC) group values (pre-flight measurements for humans, or ground control (GC) and vivarium control (VC) for animals), along with corresponding measure of variance (standard errors, standard deviations or interquartile ranges). When articles presented similar data for two identical populations we selected the one with higher quality score.

7.3 Measurement level outcomes

Two types of control groups (CC) were used: ground control (GC), where some aspects of spaceflight environment excluding microgravity were mimicked; and vivarium control (VC), where animals lived in standard laboratory environment. When possible, GC was used as comparison to SF animals, with additional analysis conducted when VC data were presented. Data were processed as previously described (Fu et al. 2021; Mikolajewicz and Komarova 2019). Briefly, percentage difference between SF and CC for an individual measurement θ_j was calculated from the mean SF values, μ_{SF_j} and the mean CC value μ_{CC_j} using **equation (1)**.

$$\theta_j = \frac{\mu_{SF_j} - \mu_{CC_j}}{\mu_{CC_j}} \times 100\% \quad \textbf{(1)}$$

Standard errors se_j for each measurement in SF or CC group were extracted or calculated based on the provided data (Mikolajewicz and Komarova 2019), and then normalized as $SE_j = se_j/\mu_{CC_j}$. To calculate the standard deviation for percentage difference of a measurement σ_j, SF and CC groups were assumed to be independent, allowing to use the **equation (2).**

$$\sigma_j = \sqrt{{SE_{SF_j}}^2 + {SE_{CC_j}}^2} \times 100\% \quad \textbf{(2)}$$

For BMD measurements, different bone regions in the same group of animals were combined as unweighted averages.

7.4 Meta-analytic model, global outcome and heterogeneity

We used the random effects model to calculate global effect size $\hat{\theta}$ and the corresponding $SE(\hat{\theta})$ with DerSimonian and Laird τ^2 estimator. METALAB, a freely available custom software for MatLab developed by N Mikolajewicz (Mikolajewicz and Komarova 2019) was used to run the meta-analytic model. The 95% confidence intervals (CI) was determined as 95% CI= $\hat{\theta} \pm z_{(1-\alpha/2)} \times SE(\hat{\theta}) = \hat{\theta} \pm 1.96 \times SE(\hat{\theta})$.

7.5 Heterogeneity and bias

To quantify heterogeneity, we calculated H^2 and I^2 as described previously (Mikolajewicz and Komarova 2019). Potential bias was assessed using the largest bone volume/tissue volume (BV/TV) dataset, in which we preformed single dataset exclusion analysis and funnel plot for publication bias (**Appendix Figure 1**).

7.6 Additional analysis

Subgroup analysis was performed for species (humans, rats, mice) and bone type (calvaria, mandible) by combining mission-level outcomes and standard error within each category.

8 Results

8.1 Overview of relevant literature

Systematic search conducted in Medline, Embase and Scopus retrieved 1577 candidate articles. Eight additional sources were from the NASA Technical Report Server. Following the screening, 30 articles that discuss any hard structure of the skull that has experienced spaceflight were included in the systematic review (**Fig. 1A**). Included articles described studies in rats (18/30)(Davis et al. 1998; Hatton et al. 2002; Keune et al. 2015; Kleber et al. 1989; Prokhonchukov et al. 1978a; Prokhonchukov et al. 1977; Prokhonchukov et al. 1978b; Roberts et al. 1987; Roberts and Mozsary 1981; Roberts et al. 1981; Rosenberg et al. 1984; Savostin-Asling 1978; Simmons et al. 1990a; Simmons et al. 1990b; Simmons et al. 1981a; Simmons et al. 1980; Simmons et al. 1981b; Simmons et al. 1983; Tran Van et al. 1981; Volozhin et al. 1989), mice (6/30) (Dadwal et al. 2019; Dagdeviren et al. 2018; Ghosh et al. 2016; Macaulay et al. 2017; Maupin et al. 2019; Zhang et al. 2013), and humans (6/30) (Grigoriev et al. 1998; Miyamoto et al. 1998; Oganov 2003; Oganov et al. 2005; Oganov et al. 1992; Shigematsu et al. 1997). The most studied hard structures within the skull were the mandible, calvaria and the lower incisors (**Fig. 1B**), which were first studied in Cosmos missions in the 1970's, and continued with space transportation system (STS), Russian Biocosmos (BION) and commercial resupply services (CRS) flights (**Fig. 1C**).

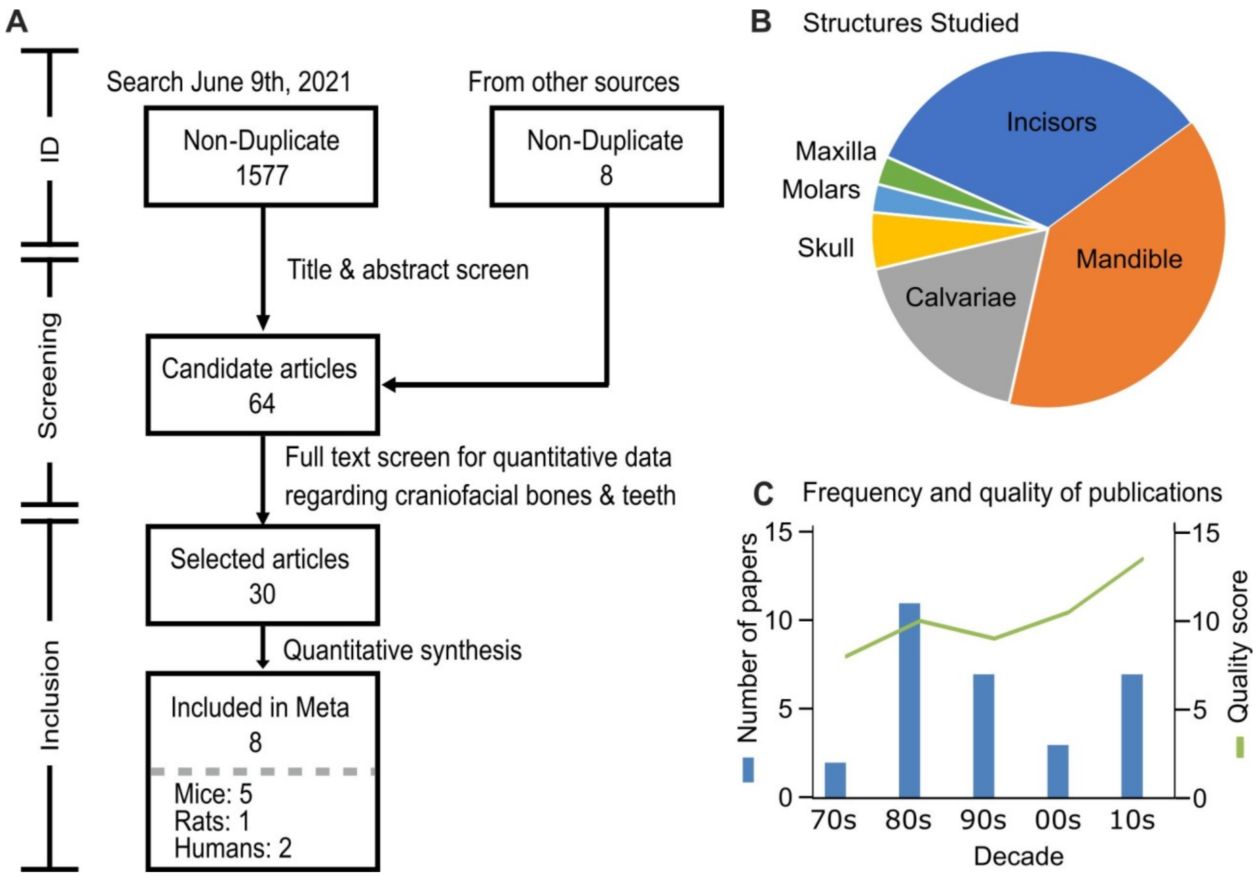

Figure 1. Systematic review information flow and PRISMA diagram. (A) PRISMA diagram. **(B)** Craniofacial hard tissue structures studied in spacefaring vertebrates in selected articles. **(C)** Number of selected papers per decade.

Thirty-two papers reported the changes in craniofacial structures from 15 missions that took to space rats (9 missions), mice (4 missions), and humans (multiple Mir and Space Shuttle missions) (**Table 1**). The earliest Cosmos missions 605, and 782 studied incisors and mandible in rats. Unfortunately, the reporting of these mission was not sufficient to extract quantitative data, with some articles not presenting the measure of variance and others not distinguishing between craniofacial structures and other bones. Later Cosmos missions 1129, 1667, and 1887, provided clearer reporting on calvaria, mandible, incisors, and, for the first time, molars in spacefaring rats. Our search retrieved multiple articles concerning Cosmos 1129, however there was a large overlap in presented data. We included two articles (Rosenberg et al. 1984; Simmons et al. 1983) that contained most amount of data and scored the highest in quality. Spacelab-3 (SL-3) was the only mission to investigate the maxilla, however reporting was limited to fibroblast like cells in the periodontal ligaments. Although in missions STS 62, 70, and 80 craniofacial structures in rats were assessed, these missions included ovariectomized, pregnant or hypertensive rats without the corresponding untreated controls, therefore these missions were excluded from subsequent analysis. Since 2010, calvaria, mandible and incisors were investigated in mice on 4 missions, STS 131, 135, Bion-M1, and CRS-10. Reporting for these missions was consistent and qualified all articles except for one for further analysis (Dagdeviren et al. 2018; Ghosh et al. 2016; Macaulay et al. 2017; Maupin et al. 2019; Zhang et al. 2013). The remaining paper presented the overlapping data (Dadwal et al. 2019). Four papers described changes in skull in astronauts visiting the Mir space station in the 90's. Since these papers provided updated information for the same population of space travelers, only the last, most complete study (Oganov et al. 2005) was used in the analysis. Two additional papers provided overlapping data for changes in skull in two astronauts that flew aboard Space Shuttle missions; the higher quality study (Miyamoto et al. 1998) was include in the analysis. Human studies generally reported on the skull with respect to the rest of the skeleton and did not provide specifics about individual bones within the skull.

Mission & Year	**Author & Year of Publication**	**Species**	**Days**	**Structure**	**Groups**
Cosmos-605 (1973)	Prokhonchukov 1977	Rat	21.5	Incisors	SF, GC, VC
Cosmos-782 (1975)	Savostin-Asling 1978; Prokhonchukov 1978	Rat	19.5	Incisors, Mandible	SF, GC, VC

Cosmos-1129 (1979)	Simmons 1980, 1981, Van 1981, Roberts 1981; **Simmons 1983**; Rosenberg 1984	Rat	18.5	Incisors Mandible	SF, GC, VC
Cosmos-1667 (1985)	Kleber 1989; Volozhin 1989	Rat	7	Incisor, Mandible Molar	SF, GC, VC
SL-3 (1985)	Roberts 1987	Rat	7	Maxilla	SF, GC
Cosmos-1887 (1987)	Simmons 1990; Kleber 1989	Rat	13	Calvaria, Incisor Mandible, Molar	SF, GC, VC
MIR EO-6-24 (1990-1997)	Oganov 1992; Grigoriev 1998; Oganov 2003; **Oganov 2005**	Human	180[1]	Skull	SF, PF
STS-62 (1994)	Keune 2015	Rat	14	Calvaria	SF, GC
STS-70 (1995)	Davis 1998	Rat	9	Calvaria	SF, GC, VC
STS-80 (1996)	Hatton 2002	Rat	18	Skull	SF, GC, VC
SpaceShuttle (1997)	Shigematsu 1997; **Miyamoto 1998**	Human	9 -15[2]	Skull	SF, PF
STS-131(2010)	**Zhang 2013**; **Ghosh 2016**	Mouse	15	Calvaria, Incisors Mandible	SF, GC
STS-135(2011)	**Ghosh 2016**; **Dagdeviren 2018**	Mouse	13	Mandible, Incisors	SF, GC, VC
Bion-M1(2013)	**Macaulay 2017**; **Dagdeviren 2018**	Mouse	30	Calvaria, Incisors Mandible	SF, GC, VC
SpaceX/CRS-10 (2017)	Dadwal 2019; **Maupin 2019**	Mouse	26[3]	Calvaria, Incisors Mandible	SF, GC

Table 1. Overview of studies that reported spaceflight induced changes in craniofacial bones. SF: Spaceflight; GC: Ground Control; VC: Vivarium Control; PF: Pre-flight; **Bold Text:** Articles with parameters that can be combined in meta-analysis.

We further assessed the potential for meta-analysis of different parameters for the three most studied craniofacial hard tissues in spaceflight. In mandibles (**Appendix Table 1**), spaceflight-induced changes in mineral content, tissue-level properties and microarchitecture were analyzed.

[1] Average duration spent in space across all astronauts in the study.
[2] Duration for both astronauts involved in the study.
[3] Animals were euthanized between day 24-28 of spaceflight aboard the ISS. Astronauts were limited to the number of animals they can euthanize per day.

From these parameters, only bone volume/tissue volume (BV/TV), tissue mineral density (TMD) and osteoclast surface/bone surface were studied in at least 3 independent missions and were included in meta-analysis. In calvaria (**Appendix Table 2**), mineral content was assessed only in Cosmos-1887 mission, calvaria thickness and BV/TV were reported in three mouse missions and TMD in two missions. Human studies reported on the skull using the DEXA whole body imaging. While this region includes both calvaria and mandible, the contribution from calvaria is larger, therefore we combined these data with calvaria measurements in rodents. Incisors were the only reasonably studied teeth in spaceflight individuals (**Appendix Table 3**), with studies reporting mineral content, dentin and enamel properties, of which we were able to find sufficient datasets for analysis of incisor volume, dentin thickness and cemento-enamel junction to apical crest distance (CEJ-AC). Thus, although data scarcity was very noticeable, after the analysis of quality and overlap in presented data we identify several parameters for which data reported by 8 papers for 7 mission (Dagdeviren et al. 2018; Ghosh et al. 2016; Macaulay et al. 2017; Maupin et al. 2019; Miyamoto et al. 1998; Oganov et al. 2005; Simmons et al. 1983; Zhang et al. 2013) were included in meta-analysis.

8.2 Spaceflight-induced changes in calvaria and mandible

Previously, it was shown that humans experiencing spaceflight exhibit an increase in bone mineral density in the skull region (Oganov et al. 2005; Stavnichuk et al. 2020). We meta-analyzed available data for spaceflight-induced changes in BV/TV in calvaria and mandible of rodents (**Fig. 2A**). While the calvaria demonstrated a trend towards increasing BV/TV, in mandibles there was a significant decrease in BV/TV in SF animals compared to GC. Combining TMD data for any space-traveling vertebrates, demonstrated no significant effect of spaceflight (**Fig. 2B**). However, separating the bones for mouse studies demonstrated a significant increase in calvaria TMD, while mandible TMD was not affected in SF animals compared to GC (**Fig. 2B**). Calvarium thickness was not significantly affected by spaceflight (**Fig. 2C**). Heterogeneity was low among BV/TV and TMD and moderate in Cs.Th. Other parameters measured only once or twice and not included in the meta-analysis were calcium, phosphorus and magnesium content which were not significantly different between SF and CC rats (Simmons et al. 1990a).Taken together, these data suggest that spaceflight affects the bones of the skull differently, resulting in bone gain in calvaria and bone loss in mandible.

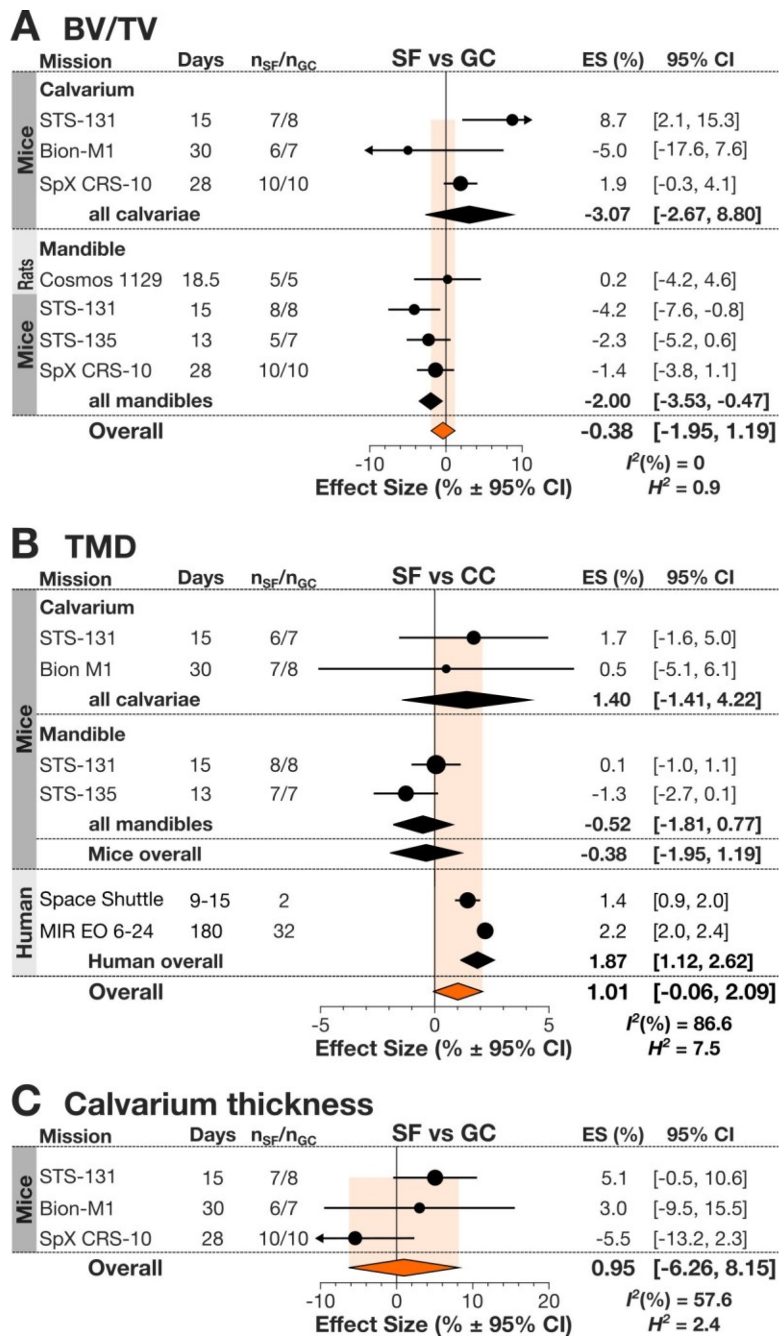

Figure 2. Spaceflight related changes in bones of the skull. (A) Forest plot of changes in calvaria and mandible BV/TV in rodents (GC). (**B**) Changes in skull TMD in humans, and mice. **(C)** The subgroup analysis for TMD in mouse calvarium and mandible. **(D)** Changes in calvarium thickness in mice. Changes were calculated as the percentage difference between spaceflight (SF) and comparison control animals (CC), which was ground control for rodents and pre-flight values for humans. Indicated are the species of spacefarers, missions, days spent in space, number of spaceflight to ground control animals (n_{SF}/n_{GC}) or number of astronauts. Circles and lines represent mission's effect size (%) and 95% confidence interval (CI), the size of the circle is dependent on

n_{sf}. Overall effect sizes and CI presented by diamonds. I^2 and H^2 were calculated for each parameter analyzed.

8.3 Spaceflight-induced changes in incisors of spacefaring rodents

Incisor volume, dentin thickness and cemento-enamel junction to apical bone crest distance (CEJ-AC) were not significantly different between SF and GC rodents (**Fig. 3**). Heterogeneity was low for dental thickness, moderate for incisor volume data, and high for the CEJ-AC datasets. Other parameters not included in the meta-analysis assessed incisor mineral content, length and pulpal space. Incisor calcium and phosphorus content was examined in two studies: while it was found to be generally unchanged in SF animals on Cosmos-1887 mission (Simmons et al. 1990a), the Cosmos-1129 study demonstrated a more complex relationship (Rosenberg et al. 1984). The later study demonstrated that while overall Ca and P content in the entire dentin was greater in SF compared to GC, Ca in the inner half of dentin (formed during flight) was relatively deficient (Rosenberg et al. 1984). This is consistent with the observation that in SF mice on CRS-10 the pulpal space area was significantly increased, also potentially suggesting deficient dentin formation in SF animals (Maupin et al. 2019). Incisor length was increased in SF animals in STS-131 mission but not in Bion-M1. Thus, there are indications that SF induces specific changes in teeth, however more studies are needed to understand spaceflight-induced changes in and around dentition.

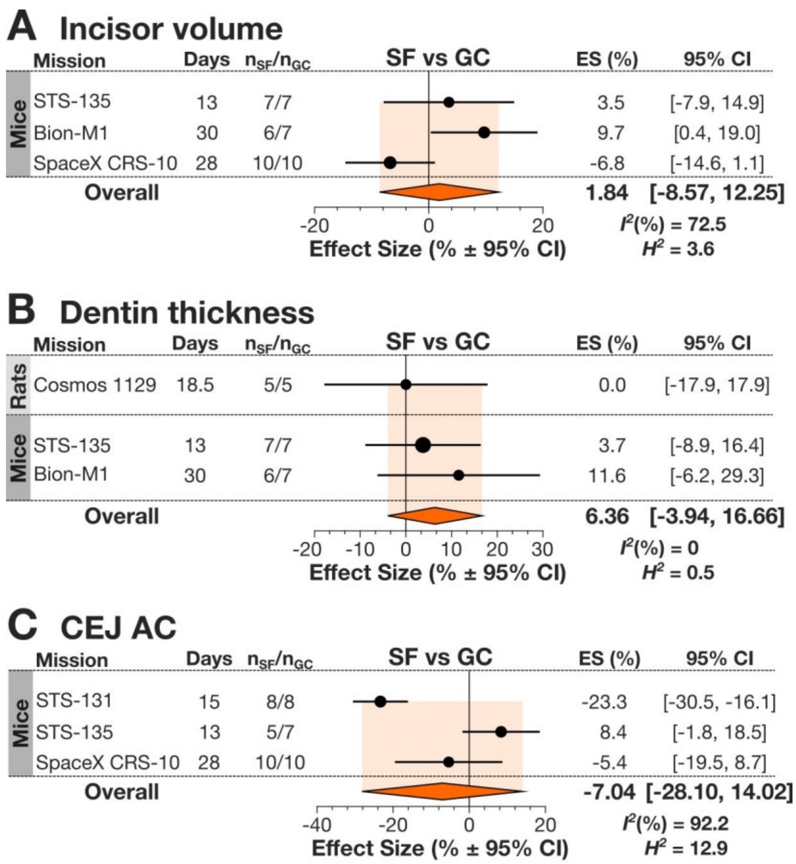

Figure 3. Spaceflight induced changes to mandibular incisors in spacefaring rodents. (A) Forest plots for changes in incisor volume, **(B)** dentin thickness and **(C)** cemento-enamel junction to apical crest distance (CEJ-AC). Indicated are the species of spacefarers, missions, days spent in space, number of spaceflight to ground control animals (n_{SF}/n_{GC}). Circles and lines represent mission's effect size (%) and 95% confidence interval (CI), the size of the circle is dependent on n_{sf}. Overall effect sizes and CI presented by diamonds. I^2 and H^2 were calculated for each parameter analyzed.

8.4 Spaceflight-induced changes in craniofacial bone turnover

The only bone turnover parameter suitable for the meta-analysis was the osteoclast surface per mandibular bone surface (Oc.S/B.S) reported on the septal bone of the first and second molar as well as the inter-radicular surface between the roots of the second molar. Overall Oc.S/B.S was not significantly different between SF and GC animals (**Fig. 4**). For this dataset we also analyzed the difference between ground control and vivarium control animals, which helps to account for factors other than microgravity that are associated with spaceflight. While overall GC and VC were not significantly different, at least in one mission (Bion-M1) Oc.S/B.S was significantly higher in GC animals compared to VC. Heterogeneity was low when comparing SF vs GC but moderate when comparing GC vs VC. Slight and non-significant decrease in other resorption parameters such as osteoclast number (Oc.N) and increase in osteoid thickness on formation side of tooth sockets were noted in flight group aboard Cosmos-1129, potentially suggesting decreased posterior drift of teeth (Simmons et al. 1983). Bone formation parameters were measured in rats in Cosmos-1129 mission(Simmons et al. 1983), where mean calcification rates were decreased in the areas without muscle attachment. Thus, there are changes in mandibular bone remodeling in spaceflight, however more studies are needed to fully characterize them.

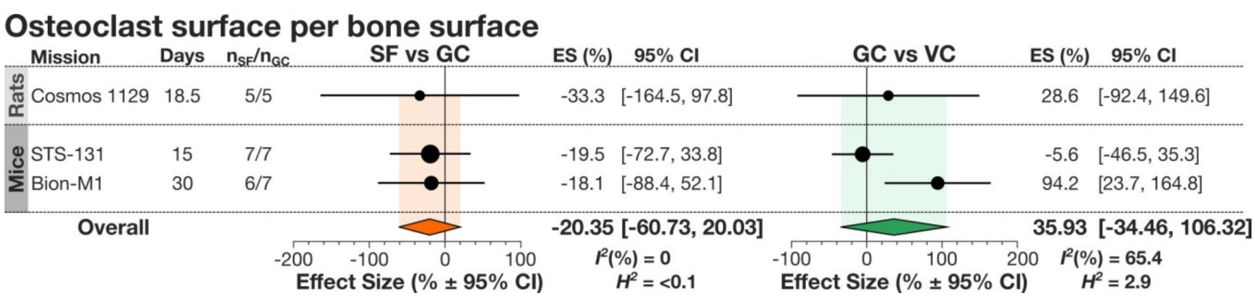

Figure 4. Changes in alveolar bone resorption in spacefaring rodents. Forest plot for changes in osteoclast surface per bone surface between spaceflight (SF) and ground control (GC) (left) and GC and vivarium control (VC) (right). Indicated are the species of spacefarers, missions, days spent in space, number of spaceflight to ground control animals (n_{SF}/n_{GC}). Circles and lines represent mission's effect size (%) and 95% confidence interval (CI), the size of the circle is dependent on n_{sf}. Overall effect sizes and CI presented by diamonds. I^2 and H^2 were calculated for each parameter analyzed.

9 Discussion

We systematically reviewed and quantitatively synthesized available literature on craniofacial bone and teeth changes in spacefarers. We report that craniofacial bones react differently to spaceflight. We show that in calvaria there is a trend towards increased BV/TV and TMD in rodents and a significant increase in TMD in humans. In contrast, BV/TV significantly decreased compared to ground control in mandibles of spacefaring rodents. Rodent incisors demonstrated no significant differences from ground control in incisor volume or dentin thickness, however they were investigated only in a small number of missions. We identified a significant gap in knowledge regarding changes in mandible, maxilla and dentition in humans, for which only the TMD for the whole skull was reported for a limited number of missions. Similarly, no data were found for changes in rodent maxilla or molars. Thus, we demonstrate that space travel may induce unexpected changes in craniofacial bones, and identify a substantial gap in our knowledge of space-related changes in craniofacial hard tissues.

Previously an association between bone loss and bone position relative to gravitational vector was identified in human space travellers (Oganov et al. 2005; Stavnichuk et al. 2020), where bone loss was most pronounced in lower limbs and bone gain was identified in the upper skeleton. We also demonstrated potential differences between the distal and axial regions in rodents (Fu et al. 2021). However, we now identify that two bones of the axial skeleton, calvaria and mandible, respond differently to spaceflight, with mandible significantly loosing bone in space. The mandible, though non-weight bearing is subject to mastication-induced mechanical loads, which may be negatively affected by spaceflight diet. Spaceflight diets evolved from the paste diets used early in missions to the rice based solid diets, and then to food bars developed by NASA to avoid crumbling while providing the consistency important for rodent teeth maintenance (Sun et al. 2014). It is known that on Earth the use of soft diets for rats results in significant decreases in bone parameters in mandible but not maxilla (Shimizu et al. 2013). A decrease in masticatory force in the mandible has been shown to decrease mandibular growth (Bresin 2001). In our dataset, the mice aboard Bion M-1 were feed a paste diet and those on STS-131 and STS-135 were fed the NASA food bar (Dagdeviren et al. 2018). If it is the softer food that results in lower mandibular mechanical loading leading to the mandibular bone loss, then we should observe higher bone deficits in Bion M1 mission compared to STS-135 mission. However, examination of data suggests that similar mandibular bone deficits were observed in Bion M1 and STS-135 missions, suggesting that diet may not be the primary contributor to the mandibular bone loss. Another difference between the the alveolar bone of the mandible and maxilla and other regions in craniofacial skeleton is in the remodeling rates. Rodent molars exhibit a natural physiological distal drift (Mednieks and Hand 2019), supported by high alveolar bone turnover rate (Vignery and Baron 1980), which potentially can make it more susceptible to environmental changes. Our analysis of bone turnover data indicates potential trends to decreased resorption and formation. This suggests a potential scenario where bone remodeling is suppressed in both mandible and calvaria in spaceflight, however in mandible higher initial bone turnover rates result in its increased susceptibility to bone loss, while calvaria is either unaffected or exhibits anabolic response in space. More data directly comparing responses to spaceflight of different bones and bone regions in the craniofacial skeleton, while taking into account diet, age and sex of the animals, are needed to answer this question.

Although we did not identify significant differences in incisor parameters in SF rodents, these data cannot be considered conclusive, since multiple confounding factors could not be taken into account with the available data. First, the diet was significantly different between the missions, as previously discussed. Second, age and sex of animals for which incisor parameters were reported were very different. If we consider the changes separately, we can see that incisor volume in SF group was increase in older males (Bion M-1), tended to decrease in younger males (CRS-10), and was unchanged in younger females (STS-135). To further complicate the interpretation, only the older male group was on the paste diet during the flight. Similarly, although no significant difference was found in the CEJ-AC is indicative periodontal health, in one mission, which also was the only mission with older females (STS-131), CEJ-AC was significantly decreased (Ghosh et al. 2016). Thus, it is premature to conclude that teeth are not affected by the spaceflight and more studies are needed to take into account all the potential confounding factors. In fact there are several findings from individual studies that suggest a potential detrimental effect of SF on teeth. In Cosmos-1129 mission, calcium content in dentin formed in space was demonstrated to be lower than in dentin formed on Earth (Rosenberg et al. 1984). In CRS-10 mission, incisors of SF animals demonstrated an increase in pulp area with a corresponding decrease in hard tissue area in the cross-sectional samples, which maybe suggestive of altered morphology (Maupin et al. 2019). Thus, more data are needed to fully understand the effect of spaceflight on teeth structure. It is also important to note that incisors in rodents are exceptionally different from humans, they are continuously erupting and tooth enamel and dentin are constantly deposited by ameloblasts and odontoblasts respectively (Goldberg et al. 2014). Unlike incisors, molar teeth in rodents demonstrate limited eruption and do not renew their dental tissues, and thus are more similar to human teeth in their response to environmental changes (Goldberg et al. 2014). Thus, molars may present a more stable model for studying the effects of spaceflight on dental tissues.

Several molecular players have been suggested to play a role in mediating spaceflight induced bone loss. One of the recent studies investigated for the first time the role of sclerostin in bone loss in spacefaring mouse . Sclerostin is encoded by Sost gene and is known to inhibit bone formation by supressing Wnt signaling (Robling et al. 2006). In spacefaring mice Sost mRNA expression was increased 16-fold in comparison to control Earth-based groups (Macaulay et al. 2017). This finding is contrary to the presumed hypothesis that decreased Sost levels should correspond to increase bone formation. It is noteworthy that Sost mRNA levels in these animals did not correspond to local hard tissue changes, but may instead reflect total bone changes in whole skeleton as sclerostin circulates in blood (Modder et al. 2011). Thus, more studies are required to understand the roles of different pathways implicated in bone mechanoadaptation.

The main limitation of this study was the lack of sufficient data reported on craniofacial hard structures in space missions for the past 50 years. Furthermore, no reporting was found for many craniofacial hard structures such as the maxilla, base of the skull, molar teeth, canine teeth, and the temporomandibular joint in any spacefarer. This scarcity in reporting prevented us from preforming analysis on many parameters measured in one or two missions. For animal studies, very few confounding factors could be investigated, since even for the very important ones, such as spaceflight duration, animal sex and age, the data lacked sufficient spread. For example, no data were available for long duration (>30days) missions with rodents; the oldest animals in the dataset were 23-week-old at the time of launch, which is barely reaching skeletal maturity, and no data for female rats was available. Thus, animal studies aiming to comprehensively assess the effects of spaceflight on the craniofacial complex are needed. For humans, even less data was available, with no information for specific craniofacial regions, or for any teeth.

Our study suggests that to properly estimate the risk of dental event in space, in addition to mission duration and the number of travelers (Menon 2012), it may be important to account for spaceflight-induced changes in craniofacial structures. In similarly isolated Earth-based conditions, such as Antarctic and submarine missions, estimated dental incidence was reported to be the highest in Antarctic missions, compared to submarines and non-isolation conditions (military deployment and maneuvers) (Lloro et al. 2019). In other physically demanding conditions such as trekkers in Nepal, it was calculated that a dental event can occur once every 23.7 trekking days; suggesting that a decrease in oral hygiene among travelers on trip may contribute to the development of dental problems (Kupper et al. 2014). The most common dental issues were caries, tooth fractures and periodontal health problems (Kupper et al. 2014; Lloro et al. 2019). Our data suggest that spaceflight-induced changes, including significant bone deficits in mandible and change in teeth morphology and composition observed in some studies, may act to increase the risks of fractures and caries progression.

10 Conclusion

We investigated the available literature on the effect of spaceflight on craniofacial skeleton. Our study demonstrates that this effect is complex, and the underlying mechanisms remain obscure. Based on the previous studies that implicated fluid shifts as a strong contributor to bone loss in spaceflight (Colleran et al. 2000; Oganov et al. 2005; Stavnichuk et al. 2020), we had expected to see similar changes in all bones in the skull. However, the mandible showed a tendency for bone loss, while calvaria demonstrated bone gain, suggesting an interplay between different driving forces. Importantly, our study demonstrates significant knowledge gaps regarding many structures of the craniofacial complex such as the maxilla, molar and canine teeth, as well as small sample sizes for the studies of mandible and incisors. Concrete understanding on the effects of microgravity on craniofacial structure is important for understanding the risks of space travel for oral health, and for developing the strategies to mitigate these risks as humanity continues to explore the cosmos.

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12 Appendix

Appendix Note 1. PRISMA Checklist:

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	4,5
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	10
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	10
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	The study was not registered
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	17,18
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	17,18
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Refer to Appendix
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	18,Figure 1, Appendix note 3
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	18
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Appendix tables 1-3

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	20, Appendix figure 1
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	19
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	18-19
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Appendix Note 3
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	20
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	21,22,Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	21,22, Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Figure 5
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figures 2-4
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	24-29, Figures 2-4
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Appendix Figure 1
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression see Item 16.	Figure 2
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	30-33
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	33

Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	34
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	7

Appendix Note 2. Search Strategy

Searched in MEDLINE (June 9th 2021)

1. exp Spaceflight/
2. exp Weightlessness/
3. exp Extraterrestrial Environment/
4. ((soyuz* or apollo* or gemini or "international space station" or saluyt or skylab or shenzhou or voskhod or euroMir or NASA or voskhod or tiangong or Mir or mercury or shuttle or ISS or ESA or CNSA or NASDA) and (space* or orbit* or station* or mission*)).ti,ab,kf.
5. (space adj5 (flight* or travel* or explor* or outer)).ti,ab,kf.
6. or/1-5
7. exp Skull/
8. (mandible or jaw or maxilla or maxillofacial or tooth or teeth or edentulous or alveolar or ridge or tempromandibular or TMJ or TMD or joint or palat* or glenoid or diaphyses or epiphyses or hyoid or cranium or cranial or occipital or basilar or foramen or basicranium or sphenoid or mastoid or petrous or odontoid or parietal or fossa or skull or sphenoid or vomer or zygoma).ti,ab,kf.
9. or/7-8
10. 6 and 9

These searches were then transcribed for two other databases, Embase and Scopus.

Appendix Note 3. Quality checklist

Quality score checklist for included articles:

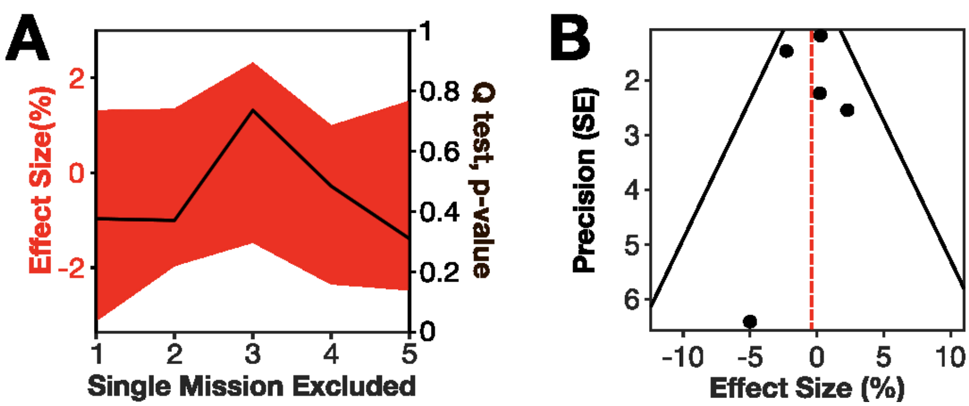
(Total of 18)

1. Mission title & flight duration are clearly stated (1)
2. Clear indication of: (maximum of 4)
   sex (1), age (1), weight postflight (1), and sample size: n_{SF} (1) of spaceflight groups.
3. Study contains the following control groups: (maximum 2 points)
   For animal studies: ground control group (1), vivarium control group (1)
   For human studies: pre-flight values (2) change from pre-flight (1)
4. Specify housing conditions for animal studies of: (maximum 2 points, human studies automatically receive 2 points)

spaceflight group: group vs single housing (0.5) and specific habitat (0.5);
ground control group: specific conditions in reference to spaceflight group (1)

5. Diet of space travelers and controls is clearly stated (1)
6. All data was presented in a table (1) or in a graph (0) form
7. Clearly indicate all measurement units (including type of data spread) correctly (1)
8. Specific bone region from which measurements are taken is defined (1) and measurement
   techniques used are indicated (1)
9. Data regarding the following bone parameters shown: (maximum 3 points)
   Bone turnover parameters (1), density parameters (1), mineral composition parameters
(1)

Appendix Figure 1. Heterogeneity and sensitivity analysis for BV/TV dataset



Appendix Figure 1. Heterogeneity and sensitivity analysis for the BV/TV dataset. (A) Single mission exclusion analysis. Red area: 95% CI for global effect size (left axis); P-value (right axis). **(B)** Funnel plot.

Mission	Days	$N_{SF}/N_{GC}/N_{VC}$	Meta-analysed measure	Other measures
Cosmos-782	22	6/6/6	-	Coefficient of Mineralization
Cosmos-1129	18.5	5/5	Oc.S/BS (%) Bone Density (%)	Ca, P, Hyp content (%) Osteoid Th Osteoclast Number & Nuclei MAR (um/day) Mineral content by Density Gradient
Cosmos-1667	7	10/5	-	Microhardness (kgf/mm^2) CO_3/PO_4 ratio Carbon apatite content
Cosmos-1887	13	10/5/5	-	Ca, P, Mg content (%) Ca/P (molar ratio) Ca/Mg (weight ratio) Ash Weight (%) X-ray diffraction of bone ($b_{1/2}$, D-002, D-130) Carbon apatite content
STS-131	15	8/8	BV/TV (%)	Tissue Mineralization (pixel intensity)
STS-135	13	7/7/7	BV/TV (%) Oc.S/B.S. (%) Tissue Density (mgHA/cm^3)	BV (mm^3) Tissue Mineralization (pixel intensity)
Bion-M1	30	6/7/7	Oc.S/B.s (%)	BV (mm^3)
CRS-10	26	10/10	B.Ar/T.Ar (%)	T.Ar (mm^2) B.Ar (mm^2) M.Ar (mm^2)

Appendix Table 1. Quantifiable parameters measured in space flown mandibles.

Mission	Days	$N_{SF}/N_{GC}/N_{VC}$	Meta-analysed measure	Other measures
Cosmos-1887	13	10/10	-	Ca, P, Mg content (%) Ca/P (molar ratio) Ca/mg (weight ratio) Ash Weight (%) X-ray diffraction of bone ($b_{1/2}$, D-002, D-130)
STS-62	14	12/12	-	Parietal Thickness
STS-70	9	10/10/10	-	Matrix Thickness
STS-131	15	7/8	BV/TV (%) Cs.Th (mm) TMD (g/cm^3)	-
Bion-M1	30	6/7	BV/TV (%) Cs.Th (mm) TMD (g/cm^3)	-
CRS-10	26	10/10	BV/TV (%) Cs.Th (mm)	TV (mm^3) BV (mm^3) Marrow Volume (mm^3)

Appendix Table 2. Quantifiable parameters measured in space flown calvaria.

Mission	Days	$N_{SF}/N_{GC}/N_{VC}$	Meta-analysed measure	Other measures
Cosmos-605	21.5	5-7	-	Ca content P content Ash content
Cosmos-782	19.5	6/6	-	Coefficient of mineralization Optical density of enamel and dentin
Cosmos-1129	18.5	5/5	Dentin Thickness (mm)	Mineral apposition rate (um/day) Ca, P, Hyp content (%) Ca/P ratio Periodontal ligament length Nuclear volume of fibroblastic cells in PDL
Cosmos-1667	7	10/5	-	Carbon apatite content
Cosmos-1887	13	10/5/5	-	Ca/P ratio Ca/Mg ratio Ca/Zn ratio Carbon apatite content
STS-131	15	8/8	CEJ-AC (mm)	-
STS-135	13	7/7	Incisor E+D Volume (mm^3) Dentin Thickness (mm) CEJ-AC (mm)	Incisor Length (mm) Enamel Thickness (mm)
Bion-M1	30	6/7/7	Incisor E+D Volume (mm^3) Dentin Thickness (mm)	Dentin Tissue Density ($mgHA/cm^3$) Enamel Thickness (mm)
CRS-10	26	10/10	CEJ-AC (mm) E+D.Ar (mm^2)	Pulp space area (mm^2) T.Ar (mm^2) E+D.Ar/T.Ar (%)

Appendix Table 3. Quantifiable parameters measured in space flown incisors: