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Review article

Cosmic chronometers: Is spaceflight a catalyst for biological ageing?

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ABSTRACT

Astronauts returning from space missions often exhibit health issues mirroring age-related conditions, suggesting spaceflight as a potential driver of biological ageing and age-related diseases. To unravel the underlying mechanisms of these conditions, this comprehensive review explores the impact of the space "exposome" on the twelve hallmarks of ageing. Through a meticulous analysis encompassing both space environments and terrestrial analogs, we aim to decipher how different conditions influence ageing hallmarks. Utilizing PubMed, we identified 189 studies and 60 meet screening criteria. Research on biological ageing in space has focused on genomic instability, chronic inflammation, and deregulated nutrient sensing. Spaceflight consistently induces genomic instability, linked to prolonged exposure to ionizing radiation, triggers pro-inflammatory and immune alterations, resembling conditions in isolated simulations. Nutrient sensing pathways reveal increased systemic insulin-like growth-factor-1. Microbiome studies indicate imbalances favoring opportunistic species during spaceflight. Telomere dynamics present intriguing patterns, with lengthening during missions and rapid shortening upon return. Despite a pro-ageing trend, some protective mechanisms emerge. Countermeasures, encompassing dietary adjustments, prebiotics, postbiotics, symbiotics, tailored exercises, meditation, and anti-inflammatory supplements, exhibit potential. Spaceflight's impact on ageing is intricate, with diverse findings challenging established beliefs. Multidisciplinary studies provide guidance for future research in this field.

1. Introduction

The dawning era of space exploration has brought humanity to the brink of unprecedented frontiers, with ambitious plans to transport humans to the moon and Mars by 2020 and 2030 (NASA Lunar Programs: Improved Mission Guidance Needed as Artemis Complexity Grows | U.S. GAO). These endeavors hold the promise of not only expanding human presence beyond Earth but also fostering new opportunities for living, working, and even tourism in space. However, this celestial voyage carries an inherent peril, as space itself presents a formidable and inhospitable environment now known as the "space exposome". This exposome comprises a compendium of daunting challenges, including microgravity, radiation, harsh workload, circadian disruptions, isolation, and confinement, all of which are classified as "red risks" due to their capacity for inflicting severe impacts on human health (Cucinotta, 2014; Patel et al., 2020).

Upon returning from prolonged space missions, astronauts have been observed to endure a spectrum of health issues strikingly reminiscent of those seen in the elderly (Strollo et al., 2018). The effects extend to various physiological systems, affecting the immune system, bones, muscles, eyes, and cardiovascular balance and coordination (Strollo, 1999; Vernikos and Hosie, 2004; Vernikos and Schneider, 2010). These health challenges not only compromise mission performance but also cast a shadow on the long-term quality of life after the mission (Afshinnekoo et al., 2020; Patel et al., 2020). Thus, a profound association emerges between spaceflight and a process akin to accelerated ageing, rendering space missions a potential risk factor for age-related diseases.

The ageing process is characterized by a gradual decline in physiological integrity, leading to impaired function and heightened vulnerability until death. Pioneering research by López-Otín and colleagues unveiled nine fundamental molecular hallmarks of ageing, collectively contributing to and defining the ageing phenotype (López-Otín et al., 2013). Recently, these hallmarks were further expanded with the addition of three new hallmarks: disabled macroautophagy as primary hallmarks, chronic inflammation, and dysbiosis as integrative hallmarks

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(López-Otín et al., 2023). These hallmarks are classified into three distinct groups: primary, antagonistic, and integrative, with the primary hallmarks causing cellular damage, the antagonistic hallmarks responding to and potentially exacerbating the damage, and the integrative hallmarks governing the ageing-associated functional decline (López-Otín et al., 2023).

This comprehensive review embarks on an exploration of the burgeoning literature, delving into the impact of spaceflight on each of these ageing hallmarks, including the newly identified ones. Our analysis encompasses both the space environment and ground research analogs, designed to uncover the specific risks posed to human health by spaceflight. Notably, we explore isolation and confinement models, encompassing bed rest (BR) and dry immersion, which mimic altered microgravity and isolated, confined, and controlled (ICC) analogs, providing valuable insights into the adaptability of the human body to weightlessness while excluding confounding radiation exposure (Cromwell et al., 2021).

By evaluating the short and long-term exposure to the spatial exposome, we seek to elucidate how exposure duration influences the effects on ageing hallmarks. Our ultimate mission is twofold: to identify the primary ageing pathways influenced by spaceflight and to pinpoint potential targets for mitigating biological ageing, thereby ensuring the safety and well-being of astronauts during and after their missions. As ageing represents the main risk factor for major human diseases, such as cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions (Guo et al., 2022), human spaceflight investigations hold the key to unraveling the mechanisms underlying these diseases.

In a cosmic quest to unveil the enigmatic connection between spaceflight and ageing, our journey through the depths of scientific inquiry serves not only to fortify humanity's pursuit of cosmic exploration but also to illuminate groundbreaking pathways to combat age-related afflictions on our terrestrial abode.

2. Search strategy and selection criteria

2.1. Search strategy

PubMed was used as the search engine where term "spaceflight" (term used to narrow the field of our interest) and the specific terms for each out of the twelve hallmarks of (biological) ageing, including "genomic instability", "telomere length", "epigenetic alterations", "loss of proteostasis", "deregulated nutrient sensing", "mitochondrial dysfunction", "cellular senescence", "stem cell exhaustion" "intercellular communication", "disabled macroautophagy", "chronic inflammation", "dysbiosis", were respectively combined. The search strategy displayed relevant articles published up to January 4, 2024.

2.2. Selection criteria and Screening process

Starting from this point, a work of screening and filtering was carried out and relevant papers were selected considering the following inclusion criteria shaped for this review. Articles were included if they reported human studies and data upon the impact of spaceflight and ground-based studies or analog missions on human hallmarks of biological ageing, with particular attention of telomere length and DNA methylation age as primary biomarkers of biological ageing and biological features of spaceflight. In particular, for ground-based studies or analog missions, only the studies funded by space agencies and relevant institutions in this field, were considered. Further inclusion criteria were applied: original articles (not reviews, editorials, brief communications or conference abstracts), English language, and working on human samples. No restrictions were used for study designs, population characteristics, and the number of included subjects. Furthermore, cited papers in the selected publications and the reference lists of relevant reviews during the screening process were scanned and also considered, even if without relevant results.

3. Results

Fig. 1 shows the search strategy with key terms and the screening process. We found 189 studies related to the key terms used, which were subdivided into the main themes, including each of the twelve hallmarks of ageing, categorised as primary, antagonistic and integrative hallmarks. Finally, after the screening process, also considering the additional articles cited in the selected publications and in the reference lists of relevant reviews, we identified a total of 60 studies that met the inclusion criteria, i.e. investigated the effects of spaceflight on the twelve selected hallmarks of human biological ageing. Our results include 30 articles on space missions and 30 articles on space analogs or ground-based studies. These articles have been grouped into 12 pathways, as seen in Fig. 1 and in the following paragraphs, with reference to the different hallmarks of ageing. All the studies included were carried out between 1976 and 2023 July, funded by space agencies and relevant institutions in this field. Some papers were reported twice or more because they assessed more than one characteristic of ageing.

4. Spaceflight and hallmarks of biological ageing

4.1. Spaceflight and genomic instability

The time-dependent accumulation of genetic damage throughout life (Moskalev et al., 2013) is one of the common traits of ageing and it is considered one of the primary hallmarks (López-Otín et al., 2023). Several exogenous physical, chemical, and biological factors, as well as endogenous factors, including DNA replication errors, spontaneous hydrolytic reactions, and reactive oxygen species (ROS), can affect DNA integrity and stability (Hoeijmakers, 2009). Point mutations, translocations, chromosomal gains and losses and gene disruption induced by virus or transposon integration are all examples of genetic lesions caused by extrinsic or intrinsic damage. To limit these lesions, organisms have developed a complex network of DNA repair systems capable of coping with most of nuclear DNA damage (Hoeijmakers, 2009; Lord and Ashworth, 2012).

Table 1 shows studies (n=14) on the effects of spaceflight on genomic instability. All, excepted one, are longitudinal (before and after) space mission studies conducted in a restricted set of healthy male astronauts (from n=2 to n=38) and healthy age- and sex-matched ground controls, with different duration, ranging between 10 days, in the space shuttle mission (George et al., 2001), and one-year, in the International Space Station (ISS) mission (Garrett-Bakelman et al., 2019; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021). Just one study was a ground-based study that investigated the influence of 60 days BR period in n=8 healthy young European women (Chopard et al., 2009). All studies were performed on DNA from peripheral blood leukocytes (PBL) (Durante et al., 2004; Fedorenko et al., 2001; Feiveson et al., 2021; Garrett-Bakelman et al., 2019; George et al., 2013, 2010, 2005, 2004, 2001; Greco et al., 2003; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021) as the most easy available tissues, excepted the ground-based study that was performed on soleus and vastus lateralis muscles DNA (Chopard et al., 2009). All PBL and muscles samples were collected always before and after (Chopard et al., 2009; Durante et al., 2004; Fedorenko et al., 2001; Feiveson et al., 2021; Garrett-Bakelman et al., 2019; George et al., 2013, 2010, 2005, 2004, 2001; Greco et al., 2003; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021) and sometimes at different time points spanning the missions and ground-based simulations (Garrett-Bakelman et al., 2019; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021). The cytogenetic analysis of DNA damage was mainly performed by using fluorescence in situ hybridization (FISH) with chromosome painting probes (Durante et al., 2004; Feiveson et al., 2021; George et al., 2013, 2010, 2005, 2004, 2001; Greco et al., 2003), but also strand-specific directional genomic hybridization (dGH) (Garrett-Bakelman et al., 2019; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021) and Giemsa (Durante et al.,

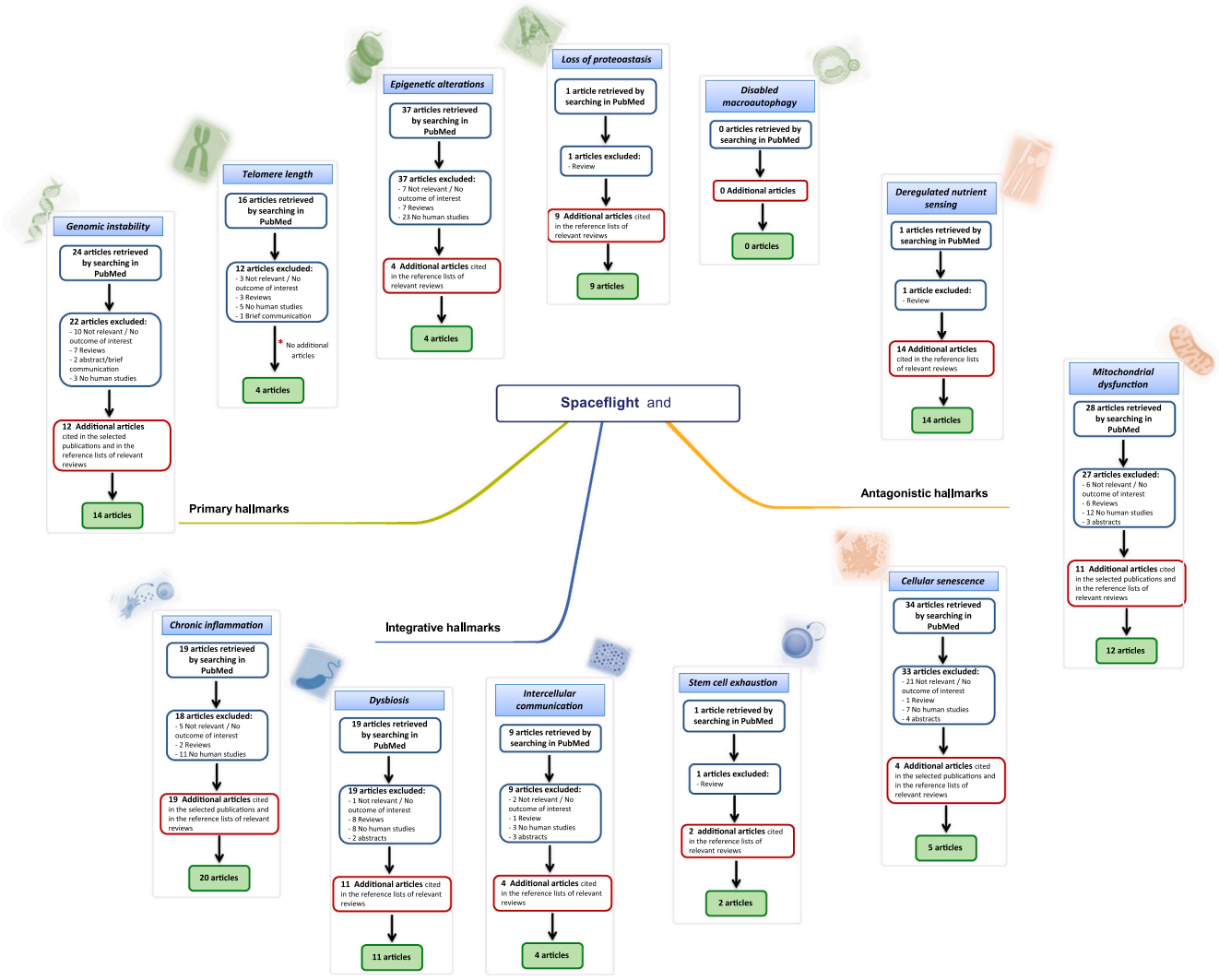

Fig. 1. Flow diagrams of search strategy and study selection. Flow diagrams of the search strategy and study selection displayed for each of the twelve hallmarks of ageing categorized by different colors into three different branches: primary (green), antagonistic (orange) and integrative (blue) hallmarks. The middle box shows the specific term used to narrow the field, which was added to each of the twelve different hallmarks of ageing shown in the blue boxes at the apex of the flowcharts. The green squares show the articles found for each of our searches.

2004; Fedorenko et al., 2001). While, altered transcript levels involved in RNA damage and repair were analyzed with Microarrays and then verified by RT-PCR assay (Chopard et al., 2009).

Six out of 14 studies, comparing data from pre- and post-flight samples, coherently reported an increase in chromosomal aberration during and after ≥10 days of space missions (Durante et al., 2004; Fedorenko et al., 2001; George et al., 2013, 2010, 2001; Greco et al., 2003). There was however no pre- and post-flight difference in chromosome aberration in two studies conducted on 12 and 6 astronauts with different mission duration (George et al., 2005, 2004) and, even considering chromosome aberration rate (CAR) shortly after mission and at > 6 months post-mission (Feiveson et al., 2021). The authors argue that it is the result of the damage caused in bone marrow cells (Feiveson et al., 2021), since chromosome aberrations of clonal origin have been reported in some post-flight blood samples from astronauts (George et al., 2004). Comparing pre-flight with inflight and post-flight, increased frequencies of inversion and translocation were found (Garrett-Bakelman et al., 2019; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021), consistent with inflight ionizing radiation (IR) exposure, the high linear energy transfer (LET) space radiation monitored by physical dosimeter. The dose 76.18 milligrays and effective dose 146.34 millisieverts were recorded by NASA (Garrett-Bakelman et al., 2019). In particular, inversions (intrachromosomal rearrangements) occurred at much higher frequencies than translocations (interchromosomal rearrangements) and persisted even more after spaceflight (Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021).

Interesting, an upregulation of muscle samples (soleus and vastus lateralis) transcripts levels involved in the network of RNA damage and repair was found in the only ground-based study after 60 days of BR period simulating the effect of microgravity (Chopard et al., 2009), suggesting an activation of DNA repair pathways even in the absence of IR.

In summary, these studies reveal that genomic instability, as the early determinant step in ageing mechanism, occurs in short- and long-term space missions and is related to inflight IR exposure monitored during spaceflights. DNA lesions can be caused by external UV/IR radiation, and the ROS formed by the deposition of the energy of IR (Auger electrons) in intracellular water, and the consequent replication mistakes, and spontaneous reactions (Hoeijmakers, 2009). This suggests that protecting astronauts from radiation injury remains one of the most important steps in order to preserve DNA stability. On the other hand, reducing ROS levels and implementing the antioxidants mechanisms with meals rich in bioactive compounds (polyphenols, vitamins and mineral salts) and nutraceutical supplements with high antioxidant

Table 1
Spaceflight and genomic instability.

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Chopard et al., 2009	SS	N=8 healthy young European women	60 days of BR period	Multiple time points: 2 days before reambulation and on day 59 of the BR period	Soleus and vastus lateralis muscle	Microarray RT-PCR	mRNAs involved in RNA repair transcripts upregulated after BR period.
Durante et al., 2004	SM	N=33 cosmonauts involved in long-term space missions on Mir or ISS, and on short-term taxi flights	Different duration mission (>3 and <3months) over 11 years	Multiple time points: 2–4 months before mission, and day after landing	PBMCs	Giemsa staining or FISH painting.	↑ Chromosomal aberrations following long-term (>3 months) space missions at first flight. No significant changes in aberration frequencies for short-term (<3 months) taxi flights. Frequency of chromosomal aberrations is lower than expected for cosmonauts involved in multiple space missions (up to five). ↑ Radioresistance after multiple space flights observed by changes in the immune system in microgravity and/or adaptive response to space radiation.
Fedorenko et al., 2001	SM	N=22 cosmonauts	4–6 months	Before and a day after mission.	PBMCs	Giemsa staining	↑ Dicentrics and centric rings scored after long-term space flights vs prior to the flights.
Feiveson et al., 2021	SM	N=38 astronauts Five astronauts participated in two space missions, increasing the number of crew-missions from 38 to a total of 43	ISS missions between 2 and 7 months	Multiple time points: before, after 2 weeks and 6–12 months mission	PBMCs	FISH with chromosome painting probes	Post-flight observations of CAR above the pre-flight dose response line are reflecting a RBE of about 3.1 compared to the ex vivo dose response to gamma irradiation. Post-flight observations of CAR can be predicted by the radiosensitivity determined pre-flight, in combination with the dose received during the mission and the background CAR. ↔ CAR observed shortly after mission and at > 6 months post-mission.
Garrett-Bakelman et al., 2019	SM	NASA twin astronauts: (male monozygotic), one twin in space (TW) and one twin on Earth (HR)	A 340-day mission	Multiple time points: before (pre-flight), during (inflight), and after flight (post-flight), for a total of 25 months	PBMCs	Strand-specific dGH paints for chromosomes 1, 2, and 3	***Pre-flight:*** Frequencies of structural variants were similar for the two subjects, with inversions more frequent than translocations. ***Inflight:*** ↑ TW's inversion frequencies at a greater rate than translocations, consistent with inflight IR exposure, particularly to LET space radiation. ↑ Translocations compared with preflight. ***Post-flight:*** ↑ TW's inversion frequencies. ↑ Translocations compared with pre-flight. No statistically significant results in interchromosomal reciprocal translocation and intrachromosomal inversion frequencies for HR and TW inflight and post-flight.
George et al., 2001	SM	N=8 astronauts	A 10-day shuttle mission	Multiple time points: before and after 10 days mission	PBMCs	FISH with chromosome painting probes	↑ Aberrations after mission. The ratio of aberrations identified as complex was slightly higher after flight.
George et al., 2004	SM	N=12 astronauts	3-month mission	Multiple time points: before and a day after mission	PBMCs	FISH with chromosome painting probes for chromosomes 1, 2, 4 and 5	Cells with clonal aberrations were identified in three of the twelve individuals both before and after space flight. ↑ Aberrations than previously reported for healthy individuals

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Table 1 (*continued*)

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
							in the age range (40–52 years of age).
George et al., 2005	SM	N=6 astronauts	NA	Multiple time points: before and after (from 5 months to more than 5 years)	PBMCs	FISH with chromosome painting probes	Temporal declines for five astronauts in yields of chromosome damage with individual half-lives ranging from 10 to 58 months. No data concerning chromosome aberration in post flight in respect pre-flight.
George et al., 2010	SM	N=16 astronauts	Long-duration of 3 months or more (from 95 days to 215 days)	Multiple time points: before and after mission	PBMCs	FISH with chromosome painting probes for chromosomes 1, 2, 4 and 5	↑ Frequency of chromosome exchanges within a month of return from space compared with preflight yield. ↓ Trend in total chromosome exchanges after flight (not seen for all astronauts), in particular for data collected more than L+220, although with inter-individual differences. Large inter-individual differences were in the temporal response of chromosome aberration yields after spaceflight.
George et al., 2013	SM	N=5 astronauts	Two flights of a few months or more	Multiple time points: before and after first and second mission	PBMCs	FISH with chromosome painting probes for chromosomes 1, 2 and 3	↑ Chromosome aberration in all individuals after both first and second flights.
Greco et al., 2003	SM	N=9 cosmonauts	Different duration on ISS and on the Mir: from 9 to 312 days	Multiple time points: pre-flight L- 341, post-flight within 19 days from landing	Blood sample	FISH with chromosome painting probes for chromosomes 1 and 2	↑ Chromosome damage after flight. No correlation between chromosome damage and flight history, in terms of number of flights at the time of sampling, duration in space and extra-vehicular activity.
Luxton et al., 2020a	SM	N=3 NASA astronauts (aged 35–55 year) N=11 healthy age- and sex-matched ground control subjects (NASA volunteers)	One-year mission (n=1) and six-month mission (n=2)	Multiple time points: before (L-270, L-180, L-60), during (Flight Day ~45, 90 and 140 or 260), after (L+1–7, L+60, L+180, L+270) spaceflight	PBMCs	Strand-specific dGH paints for chromosomes 1, 2, and 3, together with chromosome-specific subtelomere probes	↑ Inversions during spaceflight for all three astronauts. Increased frequencies of inversions persisted after spaceflight for all three crewmembers.
Luxton et al., 2020b	SM	N=11 NASA astronauts (males and females aged 35–55 year) N=11 healthy age- and sex-matched ground control subjects (NASA volunteers)	One-year mission (n=1) and six-month mission (n=10)	Multiple time points: before (L-270, L-180, L-60), during (Flight Day ~45, 90 and 140 or 260), after (L+1–7, L+60, L+180, L+270) spaceflight	PBMCs	Strand-specific dGH	***Inflight:*** SCE along the length of chromosomes or into subtelomeric regions were not significantly increased during spaceflight. ↑ Frequencies of terminal SCE during spaceflight, specifically occurring at the subtelomeric heterochromatin junction of chromosome 2p. ↑ Frequencies of chromosomal inversions. ***Postflight:*** Frequencies of terminal SCE remained elevated. Frequencies of inversions approximately ↑ double between pre- and post-spaceflight measurement, ↓ compared to inflight and converged to relatively similar and elevated levels post-flight.
Luxton and Bailey, 2021	SM	The NASA twin astronauts: (male monozygotic), one	A 340-day mission	Multiple time points: before (preflight), during (inflight), and	PBMCs	Strand-specific dGH paints for chromosomes 1, 2, and 3	↑ Inversion and translocation frequencies during spaceflight. Inversions (intrachromosomal

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Table 1 (*continued*)

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
		twin in space (TW) and one twin on Earth (HR)		after spaceflight (post-flight)			rearrangements) occurred at much higher frequencies than translocations (interchromosomal rearrangements) for both twins. TW's inversion frequencies remained elevated post-flight. Translocation frequencies were more variable than inversions but ↓ post-flight compared with inflight.

Abbreviations:
BR= Bed rest.
CAR= Chromosome aberration rate.
dGH= Directional genomic hybridization.
FISH= Fluorescence in situ hybridization.
IR= Infrared radiation.
ISS= International Space Station.
L− = days before launch.
L+ = days after launch.
LET= Linear energy transfer.
NA= Not available.
NASA= National Aeronautics and Space Administration.
PBMCs= Peripheral blood mononuclear cells.
RBE= Relative biological effectiveness.
RT-PCR= Real time polymerase chain reaction.
SCE= Sister chromatid exchange.
SM= Space Mission.
SS= Space Simulation.

power, may be an excellent countermeasure for this and other sings of biological ageing. Notably, the only BR study simulating microgravity demonstrates an activation of DNA repair pathways even in the absence of radiation exposure (Chopard et al., 2009). BR studies are also needed to disentangle the contribution and the mechanism of microgravity on genomic instability.

4.2. Spaceflight and telomere length

Accumulating evidence has revealed that telomere length (TL) attrition can be regarded as the early pillar of biological ageing and the origin of cellular dysfunction, inducing senescence and/or apoptosis (López-Otín et al., 2013). Telomeres are short DNA repeats (TTAGGG) that join several proteins in a complex that is crucial for maintaining the stability of cells' genome (Blackburn et al., 2015). Telomeric repeats in normal somatic cells are reduced by 30–200 bp after each mitotic division. However, radiation and genotoxic substances, and the consequent oxidative stress, and inflammation damage telomeres, which, being triple G-containing sequences, are a sensitive target for these factors that directly accelerate telomere shortening. TL in leukocytes shortens with age (Müezzinler et al., 2013), and the telomere shortening rate constitutes a robust biomarker of ageing and disease (Pavanello et al., 2021).

Table 2 shows studies (n=4) on TL measure in spaceflight. All are longitudinal (before and after) studies conducted in a restricted set of male astronauts, and healthy age- and sex-matched ground controls, over different space mission duration, from 6 months to one year (Garrett-Bakelman et al., 2019; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021). All studies, excepted one on urine samples (Luxton et al., 2020a), were performed on DNA from PBL (Garrett-Bakelman et al., 2019; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021) as the most easily available tissue. PBL and urine samples were collected at different time points spanning the mission: before (pre-fligth), during (inflight), and after (post-fligth) spaceflight (Garrett-Bakelman et al., 2019; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021). Mean TL was measured by the multiplexed quantitative polymerase chain reaction (qPCR) method developed by Cawthon (Cawthon, 2009, 2002). The telomere fluorescence *in situ* hybridization (telo-FISH) was also performed on metaphase chromosomes to further investigate the TL distributions and to monitor shifts in populations of short and long telomeres over time for each astronaut (Garrett-Bakelman et al., 2019; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021). In addition, nanopore sequencing was used to confirm the TL throughout spaceflight (Luxton et al., 2020a, 2020b).

All studies presented in Table 2 report a spaceflight-specific TL elongation irrespective of mission duration, samples (including urine (Luxton et al., 2020a)), and mission duration compared to both pre-flight and post-flight measures (Garrett-Bakelman et al., 2019; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021). However, after return to Earth, a rapid TL shortening has been observed if compared to pre-flight. TL in the healthy age- and sex-matched ground controls remained instead stable over the course of the study (Garrett-Bakelman et al., 2019; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021). This telomeres' dynamics, which lengthen inflight and rapidly shorten post-flight, was also confirmed by telo-FISH, showing a temporal shift toward an increased number of longer telomeres inflight, while after returning to Earth, the distribution shifted back toward increased amounts of shorter telomeres (Garrett-Bakelman et al., 2019; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021).

All these data coherently reveal that the spaceflight is characterized by specific telomere dynamics with TL elongation occurs during spaceflight regardless of mission duration, whereas TL shorten happens following return to Earth. These data should also be confirmed by model studies, such as those of BR, in order to check the role of microgravity (immobility) as determinant of TL shortening and distinguish it from other spaceflight-specific stressors such as radiation exposure. Our previous study (Pavanello et al., 2019) and others (Conklin et al., 2018; Thimmapuram et al., 2017), shown a beneficial effect of meditation practices on TL in healthy subjects, by evoking the relaxation response

Table 2
Spaceflight and Telomere length.

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Garrett-Bakelman et al., 2019	SM	NASA twin astronauts (male monozygotic): one twin in space (TW) and one twin on Earth (HR)	A 340-day mission	Multiple time points: before (preflight), during (inflight), and after flight (postflight), for a total of 25 months	PBMCs	qRT-PCR Telo-FISH	***Pre-flight:*** HR and TW had similar TLs at baseline. ***Inflight:*** ↑ TW's TL vs TW's preflight and postflight TLs and with HR's TL. Shift in TW's inflight TL distribution compared to his pre-flight (L−162) distribution. ***Post-flight:*** ↓ TW's TL (R+48 h) and stabilized to near preflight averages within months. Shift in TW's TL distribution (R+190) with ↑ numbers of shorter telomeres vs pre-flight. ***Ground control:*** ↔ HR's TL over the duration of the study.
Luxton et al. 2020a	SM	N=3 NASA astronauts (aged 35–55 year) N=11 healthy age- and sex-matched ground control subjects (NASA volunteers).	One-year mission (n=1) and six-month mission (n=2).	Multiple time points: before (L-270, L-180, L-60), during (Flight Day ~45 or 90, 140 or 260), after (L+1–7, L+60, L+180, L+270) spaceflight.	PBMCs	qRT-PCR Telo-FISH Nanopore sequencing	***Pre-flight:*** TLs baseline. ***Inflight:*** ↑ TL and ↑ numbers of longer telomeres during spaceflight vs pre-flight and post-flight. Inter-individual differences in the magnitude of telomere elongation. ***Post-flight:*** ↓ TL (days) vs TL inflight and pre-flight. Shift in TL distribution with ↑ numbers of shorter telomeres vs pre-flight. Inter-individual differences in TL distributions. ***Grround control group:*** ↔ TL in the ground control cohort over the course of the study.
Luxton et al. 2020a	SM	NASA twin astronauts (male monozygotic): one twin in space (TW) and one twin on Earth (HR).	A 340-day mission.	Multiple time points: before (L-270, L-180, L-60),during (Flight Day ~45, 90 and 140 or 260), after (L+1–7, L+60, L+180, L+270) spaceflight.	Urine samples	qRT-PCR	***Pre-flight:*** TLs value at baseline. ***Inflight:*** ↑ TW's TL vs TL pre-flight. ***Post-flight:*** ↓ TW's TL vs TL inflight and pre-flight. ***Ground control:*** ↔ HR's TL over the duration of the study.
Luxton et al. 2020b	SM	N=11 NASA astronauts (males and females aged 35–55 year). N=11 healthy age- and sex-matched ground control subjects (NASA volunteers).	One-year mission (n=1) Six-month mission (n=10).	Multiple time points: before (L-270, L-180, L-60), during (Flight Day ~45, 90 and 140 or 260), after (L+1–7, L+60, L+180, L+270) spaceflight.	PBMCs	qRT-PCR Telo-FISH Nanopore sequencing	***Pre-flight:*** ↓ TL at baseline for the astronauts vs TL for the ground control cohort. ***Inflight:*** ↑ TL for whom inflight samples were available (n=3). ***Post-flight:*** ↓ TL (R+1→R+7) vs TL inflight and pre-flight. Inter-individual differences in TL distributions: ↑ TL for group 1 (n=3), ↓ TL for group 2 (n=5), and ↔ TL distributions for group 3 (n=3) at R+270. ***Ground control group:*** ↔ TL in the ground control cohort over the course of the study.

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Table 2 (*continued*)

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Luxton and Bailey, 2021	SM	NASA twin astronauts (male monozygotic): one twin in space (TW) and one twin on Earth (HR)	A 340-day mission	Multiple time points: before (preflight), during (inflight), and after spaceflight (postflight)	PBMCs	qRT-PCR Telo-FISH	***Pre-flight:*** HR and TW had similar TLs at baseline. ***Inflight:*** ↑ TW's TL vs TW's preflight and post-flight TLs and with HR's TL. Shift in TW's TL distribution (R+190) with ↑ numbers of longer telomeres vs TL pre-flight distribution. ***Post-flight:*** ↓ TW's TL (R+48 h) and stabilized to near pre-flight averages within months. Shift in TW's TL distribution (R+190) with ↑ numbers of shorter telomeres vs pre-flight. ***Ground control:*** ↔ HR's TL over the duration of the study.

Abbreviations:
L− = Days before launch.
L+ = Days after launch.
NASA= National Aeronautics and Space Administration.
PBMCs = Peripheral blood mononuclear cells.
qRT-PCR = Quantitative real-time polymerase chain reaction.
SM= Space Mission.
Telo-FISH = Telomere fluorescence in situ hybridization.
TL= Telomere length.

(RR) (Lazar et al., 2000) and reducing levels of stress hormones, inflammation and oxidative stress (Black and Slavich, 2016; Kaliman et al., 2014; Paul-Labrador et al., 2006), that are molecular pathways involved in cellular ageing processes (Epel and Lithgow, 2014). These non-pharmacological practises would be employed during flight and after landing to mitigate TL shortening.

4.3. Spaceflight and epigenetic alterations

Epigenetics is an inherited and reversible mechanism that influences gene expression without altering DNA sequences (Zhang et al., 2020). During the life, all cells and tissues are affected by a series of epigenetic changes that consist in: alterations of DNA methylation patterns, post-translational histone modifications, chromatin remodelling and transcriptional changes (López-Otín et al., 2013). DNA methylation, namely the addition of methyl groups to cytosine residues throughout which the DNA expression may be altered (Moore et al., 2013), is considered one of the primary biological hallmarks of ageing (López-Otín et al., 2023) and the most promising molecular marker for monitoring biological ageing and predicting life expectancy (Bell et al., 2019). DNA methylation levels at specific sites correlated with chronological age have been used to create an "epigenetic clock" able to evaluate the epigenetic age, also defined DNA methylation-based age (DNAmAge), with the aim to build a predictive model of age evaluation (Hannum et al., 2013; Horvath, 2013; Horvath and Raj, 2018; Zbieć-Piekarska et al., 2015), morbidity and mortality (Fransquet et al., 2019; Perna et al., 2016). In humans, DNA methylation changes begin early in life, as demonstrated by longitudinal studies of infants' blood (Herbstman et al., 2013; Martino et al., 2011). Notably, these early epigenetic profiles continue to accumulate changes with age, even more so in twins who do not share the same habits and/or environments (Fraga et al., 2005; Tan et al., 2016), indicating that ageing-associated DNA methylation changes are caused by environmental factors too.

Table 3 summarize studies (n=4) concerning the effects of spaceflight on epigenetic alterations. All are longitudinal studies (before and after) conducted in a restricted set of male astronauts (from n=2 to n=11) and healthy age- and sex-matched ground controls. Two studies regard space missions with different duration, from six months (Capri et al., 2019) to one-year of ISS mission (Garrett-Bakelman et al., 2019). Two are instead ground simulation experiment (Ade and Bemben, 2019; Nwanaji-Enwerem et al., 2020). All studies were performed on PBL samples (Ade and Bemben, 2019; Capri et al., 2019; Garrett-Bakelman et al., 2019; Nwanaji-Enwerem et al., 2020) as the most easily available tissues, although one associated soleus muscles tissue (Capri et al., 2019) to PBL samples. All samples were collected at multiple time points, before and after the mission (Ade and Bemben, 2019; Capri et al., 2019; Garrett-Bakelman et al., 2019; Nwanaji-Enwerem et al., 2020). Different analyses were used to measure several outcomes. Five diverse blood DNA-methylation-based metrics were applied by Nwanaji-Enwerem et al. (Nwanaji-Enwerem et al., 2020) to computed epigenetic age during 520-day ground simulation experiment. Principal component analysis (PCA) and Jensen-Shannon Distance method (JSD) were used to analyse the distances derived from average CPG methylation levels (Garrett-Bakelman et al., 2019). RT-PCR was used for circulating miRNA (Ade and Bemben, 2019; Capri et al., 2019).

The mission duration of 520-day of simulated interplanetary mission was associated with significant decreases in epigenetic ageing biomarkers, including DNAmPhenoAge, a morbidity biomarker (Levine et al., 2018) that remained significant reduced even in post-mission (day 527) (Nwanaji-Enwerem et al., 2020). Garrett-Bakelman et al. (Garrett-Bakelman et al., 2019) reported no significant changes during one-year ISS mission in the astronaut twin's global DNA methylation compared with the ground twin.

The large and growing universe of non-coding RNAs, including microRNAs (miRNAs) play an important role in gene expression regulation and influence biological ageing too (López-Otín et al., 2023). We found two studies on transcriptional alterations modulated by miRNAs that reports increased circulating levels of miRNAs related with skeletal

Table 3
Spaceflight and epigenetic alterations.

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Ade and Bemben, 2019	SS	N=11 participants	30-days head-down tilt BR	Multiple time points: 3 days prior to BR and immediately following BR	Blood	RT-PCR	Multiple cardiovascular-related c-miRNA significantly upregulated following BR.
Capri et al., 2019	SM	N= 2 crewmembers	6-months ISS mission	Multiple time points: preflight between L-76 and L-79 and postflight R+1 and R+15	Blood/ plasma Soleus muscle tissue samples	RT-PCR	↑ Myo-miR-206 in crewmember A at both R+1 and R+15, ↑ only at final recovery time in crewmember B. No significant changes of Myo-miR-133a-3p and miR-363–3p in both crewmembers. ↑ Inflamma-miR-21–5p and miR-122–5p in crewmember A at R+15, no significant changes in crewmember B. ↑ Inflamma-miR-126–3p and inflamma-miR-146a-5p in crewmember A at R+1, the ↑ in crewmember B at R+1 was completely recovered at R+15. ↑ MiR-145–5p at landing time in both crewmembers.
Garrett-Bakelman et al., 2019	SM	NASA twin astronauts (male monozygotic): one twin in space (TW) and one twin on Earth (HR)	A 340-day mission	Multiple time points: before (preflight), during (inflight), and after flight (postflight), for a total of 25 months	PBMCs	PCA of distances derived from average CpG methylation levels in 1-kb intervals along the chromosomes. JSD method	Global DNA methylation changes in TW were within the range of variation seen in HR throughout the study.
Nwanaji-Enwerem et al., 2020	SS	N=6 Mars-500 mission crewmembers	520-day ground simulation experiment of interplanetary mission from Earth to Mars	Six time points: L-7, on days 60, 168, 300, and 512 of the mission; and R+7 (day 527)	Whole blood	Blood DNA-methylation-based metrics: DNAmGrimAge, DNAmPhenoAge, DNAmTL, Mitotic clock epiTOC2, PoA	***Inflight e post-flight:*** Compared with baseline: - mission day 168 was associated with a 5.90-year ↓ in DNAmPhenoAge . This difference remained significant post-mission (day 527). - mission day 168 was associated with a 4.50-year ↓ in DNAmGrimAge . This difference was no significant post-mission (day 527). - mission day 168 was associated with ~481 fewer divisions per stem cell per year (epiTOC2). This difference was not significant post-mission (day 527). - DNAmTL and PoA were not significant.

Abbreviations:
BR= Bed rest.
DNAmTL= DNA-methylation-based estimator of telomere length.
ISS= International Space Station.
L− = Days before launch.
JSD= Jensen-Shannon distance.
NASA= National Aeronautics and Space Administration.
PBMCs= Peripheral blood mononuclear cells.
PCA= Principal components analysis.
PoA= Pace of Ageing.
R+ = Days after return.
RT-PCR= Real time polymerase chain reaction.
SM= Space Mission.
SS= Space Simulation.

muscle (c-miRNA-206), inflammation (c-miRNA-126–3p,146a-5p), and cell proliferation (c-miRNA-145–5p) during and after six months of ISS mission (Capri et al., 2019), and multiple cardiovascular-related c-miRNA upregulated after 30-days Head-down tilt BR (Ade and Bemben, 2019). The impacts of all the aforementioned epigenetic variables converge on gene expression level modification, leading to transcriptional noise and aberrant products and maturation of numerous mRNAs, which are a typical expression of ageing (Bhadra et al., 2020; Hernando-Herraez et al., 2019).

Literature is still scanty on DNA methylation and non-codind RNAs epigenetic changes, and data on histone modification and chromatin remodeling is lacking. Additional research is therefore required to thoroughly study these specific features and to introduce possible countermeasures.

4.4. Spaceflight and loss of proteostasis

Dysfunction in protein homeostasis (proteostasis) leads to the accumulation of protein aggregates. Proteostasis involves processes for the stabilization of appropriately folded proteins, most notably the heat-shock protein family (HSPs), as well as systems for protein degradation via the proteasome or the lysosome (Hartl et al., 2011; Koga et al., 2011; Mizushima et al., 2008). Ageing is linked to a decrease in the cell's ability to maintain correct protein homeostasis with the accumulation of misfolded proteins in tissues over time (Hipp et al., 2019), a harmfully cause of a progressive cell functional loss during ageing. Table 4 shows studies (n=9) examining the effects of spaceflight on loss of proteostasis. All are longitudinal (before and after), one is conducted in a restricted set (n=2) of astronauts over a six months mission in ISS (Capri et al., 2019), the others are ground-based study on BR conducted in healthy volunteers (from n=5 to n=12) (Brocca et al., 2012; Brooks et al., 2010; Chopard et al., 2009; Fernandez-Gonzalo et al., 2020; Hafen et al., 2019; Ogawa et al., 2006; Reich et al., 2010). Samples collected in BR studies were from vastus lateralis muscle biopsy (Brocca et al., 2012; Brooks et al., 2010; Chopard et al., 2009; Fernandez-Gonzalo et al., 2020; Hafen et al., 2019; Ogawa et al., 2006; Reich et al., 2010). In the study conducted on astronauts, blood and soleus muscle samples were both collected (Capri et al., 2019). Loss of proteostasis assessed by accumulation of misfolded proteins was determined by RT-PCR for gene expression (Brocca et al., 2012; Brooks et al., 2010; Chopard et al., 2009; Ogawa et al., 2006; Reich et al., 2010), microarrays (Brooks et al., 2010; Chopard et al., 2009; Fernandez-Gonzalo et al., 2020; Ogawa et al., 2006; Reich et al., 2010), MALDI ToF/ToF Mass Spectrometer for protein presence (Capri et al., 2019), and Western blot (Brocca et al., 2012; Brooks et al., 2010; Hafen et al., 2019; Ogawa et al., 2006; Reich et al., 2010) and enzyme-linked immunosorbent assay (ELISA) to measure protein levels (Capri et al., 2019).

Heat shock protein family B (HSPB1) increased in 2 astronauts after a 6-months ISS mission, as well as C-proteasome, while individual changes in HSPA2 and HSPA5 were observed (Capri et al., 2019). In six astronauts HSP27 and HSP90 genes were upregulated already after short-term shuttle missions (Barrila et al., 2016). In contrast, HSP70 and HSP90 levels remained instead unchanged after 10 days of immobilization in 11 healthy volunteers (n=5 women and n=6 men) (Hafen et al., 2019), while other HSPs, including chaperonins, start to be downregulated in 5 healthy volunteers after 20 days of BR in the vastus lateralis (Ogawa et al., 2006). HSP27 and HSP70 decrease after 35 and 24 days of BR in two groups of 9 young men (Brocca et al., 2012). In addition, BR studies demonstrated an increase in indicators of muscle proteolysis detected by a high number of polyubiquitinated proteins (Brocca et al., 2012; Ogawa et al., 2006), and an upregulation of genes encoding for protein ubiquitination such as tripartite motif-containing protein 32 (TRIM32) in middle-aged men (Fernandez-Gonzalo et al., 2020) and cbl proto-oncogene b (cbl-b) in young men (Ogawa et al., 2006). Furthermore, an increase in the muscle mRNA levels of atrogin-1 (also known as muscle atrophy F-box – MAFbx), an important E3 ubiquitin ligase involved in the pathways controlling muscular atrophy, was also found after BR (Chopard et al., 2009; Ogawa et al., 2006; Reich et al., 2010), but not in the study conducted by Brocca et al. (Brocca et al., 2012). Muscle RING finger-1 (MuRF1), another central ubiquitin ligase implicated in muscle atrophy, appeared to be less affected by BR showing no significant changes (Brocca et al., 2012; Brooks et al., 2010; Ogawa et al., 2006; Reich et al., 2010). Finally, although Brocca et al. (Brocca et al., 2012) reported an increased upregulation of skeletal muscle relevant indicator of autophagy such as beclin-1, BR stimulates the activation of the ubiquitin-proteasome system rather than the autophagy-lysosomal system.

These studies coherently show dysfunction in protein homeostasis (i.e. proteostasis) during spaceflight, both in short-term Shuttle missions and long-term missions, and in BR studies as early as 20 days of immobilisation. In particular, HSPs increase after spaceflight in response to loss of proteostasis, whereas ground-based BR studies, which are useful for testing microgravity effects, show unchanged or decreased levels of HSPs, including chaperonins. BR studies on the other hand, show an increase in indicators of muscle proteolysis and increased expression of genes related to protein ubiquitination, which is associated with muscle atrophy. Further studies with other protein aggregates are needed to understand which is the most sensitive and appropriate indicator, but also to better understand the mechanisms underlying proteostasis dysregulation and identify potential countermeasures to mitigate its effects.

4.5. Spaceflight and deregulated nutrient sensing

Deregulated nutrient sensing is one of the antagonistic hallmarks of ageing (López-Otín et al., 2023). Nutrient-sensing pathways have been widely studied in both humans and model species, demonstrating the significant impact of trophic and bioenergetic pathways on longevity (Barzilai et al., 2012). In mammals, the somatotrophic axis consists of growth hormone (GH), that is released by the anterior pituitary, and insulin-like growth factor 1 (IGF-1) which is in turn synthetized in response to GH by several cell types and activates the same intracellular signaling route of the insulin. The "insulin and IGF-1 signaling" (IIS) pathway is the most conserved ageing-controlling pathway in evolution, with multiple targets including the forkhead box O (FoxO) family of transcription factors and the mTOR complexes, both of which are implicated in ageing process (Barzilai et al., 2012; Fontana et al., 2010; Kenyon, 2010).

Table 5 shows studies (n=14) investigating the impact of spaceflight on nutrient sensing pathway. All are longitudinal studies performed on a restricted set of participants (from n=4 to n=26). Three studies concerned astronauts during space shuttle mission, ranging between 9 days (McCall et al., 1999; Stein et al., 1999) and six months of ISS mission (Hughson et al., 2016). Eleven studies were ground-based, which include nine studies on BR (Biolo et al., 2008; Boesen et al., 2014; Brocca et al., 2012; Brooks et al., 2010; Downs et al., 2020; Fernandez-Gonzalo et al., 2020; Heer et al., 2014; Hughson et al., 2016; Yang et al., 2014), one study on dry immersion (Linossier et al., 2017) and other one on ICC analog (Strollo et al., 2018). Eleven studies analyzed blood samples (Boesen et al., 2014; Brooks et al., 2010; Linossier et al., 2017; McCall et al., 1999; Yang et al., 2014), one of these also included breath samples (Downs et al., 2020), while the other three studies were performed on (vastus lateralis) muscle tissue samples (Brocca et al., 2012; Brooks et al., 2010; Fernandez-Gonzalo et al., 2020), and other one analyzed urine samples (Stein et al., 1999). All samples were gathered at various time points, before and after (Biolo et al., 2008; Boesen et al., 2014; Brocca et al., 2012; Brooks et al., 2010, 2014; Downs et al., 2020; Fernandez-Gonzalo et al., 2020; Heer et al., 2014; Hughson et al., 2016; Linossier et al., 2017; McCall et al., 1999; Stein et al., 1999; Strollo et al., 2018; Yang et al., 2014). Deregulated nutrient sensing, evaluated by the circulating IGF-1 levels and insulin concentration was detected by different methods, including chemiluminescence immunoassay (Brooks

Table 4
Spaceflight and loss of proteostasis.

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Brocca et al., 2012	SS	N= 9 subjects (group A) N= 9 subjects (group B)	Two BR campaigns: one lasting 35 days (group A) and one 24 days (group B)	Group A: pre-BR, post-8d BR and post-35d BR. Group B: pre-BR, post-8d BR and post-24d BR	Vastus lateralis muscle	Proteome analysis in 2-DE. Immunoblot analysis. RT-PCR	***Subject group A:*** HSP27 and HSP70 downregulated post-8d BR and post-35d BR compared to pre-BR. ***Subject group B:*** ↑ ubiquitin conjugates post-24d BR compared to pre-BR. mRNAs levels of MuRF-1 and atrogin-1 were not significantly higher post-24d BR compared to pre-BR, even if there was a trend towards ↑. ↑ mRNAs and protein content for Beclin1 post-24d BR compared to pre-BR. The increase in the ratio LC3II/LC3 I and in p62 and protein level did not reach statistical significance, as well as the Cathepsin-L mRNA levels and bnip3 protein content. ↓ HSP70 post-24d BR compared with pre-BR.
Brooks et al., 2010	SS	N= 7 healthy males	49 days: 7 days of baseline, 28 days of BR, 14 days of active recovery	Multiple time points: baseline prior to randomization, post-28d BR, and after 14 days of active recovery	Vastus lateralis muscle	RT-PCR Western blot	↔ MuRF1 transcript levels over the course of the study. MAFbx transcript levels were not affected by BR.
Capri et al., 2019	SM	N= 2 crewmembers	6-months ISS mission	Multiple time points: between L-76 and L-79, R+1 and R+15	Blood/plasma Soleus muscle tissue samples	ELISA PMF utilizing MALDI ToF/ToF MS	↑ C-proteasome in both crewmembers at R+1, but subject A had not recovered at R+15 and c-proteasome ↑, whereas subject B did recover. ↑ HSPB1 in both crewmembers at R+1 and recovery. ↑ HSPA2 and GSTM2, ↓ HSPD1 in crewmember A at recovery time. ↑ PARK7 and HSPA5 in crewmember B at R+1 and recovery, respectively.
Chopard et al., 2009	SS	N= 8 healthy young women	60 days of BR	Multiple time points: 2 days before reambulation and on day 59 of the BR period	Soleus and vastus lateralis muscle	Microarrays RT-PCR	↑ MAFbx/atrogin-1 mRNA.
Fernandez-Gonzalo et al., 2020	SS	N= 12 healthy males	84 days of BR	Multiple time points: before and post-84d BR	Vastus lateralis muscle	Microarray	Transcription of TRIM32 (E3 ubiquitin-protein system) was upregulated following BR.
Hafen et al., 2019	SS	N= 11 healthy volunteers	5 days of recovery and 10 consecutive days of immobilization	Multiple time points: on days 1 and 17	Left vastus lateralis muscle	Protein immunoblotting	↔ HSP70 and HSP90 following the 10-day immobilization period, with a trend toward ↓ HSP90 protein expression following immobilization.
Ogawa, et al., 2006	SS	N=5 healthy volunteers	20-day BR	Multiple time points: before and at the end of BR	Vastus lateralis muscle	RT-PCR Western blot Microarray	↑ Ubiquitinated proteins after BR compared with before BR. ↑ Expression of Cbl-b and atrogin-1 transcripts by BR, expression of MuRF-1 and Siah-1A ↔ after BR compared with before BR. ↓ Expression of HSP and chaperonin after BR.
Reich, et al., 2010	SS	N= 7 sedentary men	48 h UL via unilateral lower limb suspension and 24 h RL	Multiple time points: before UL, 24 h RL and 48 h UL	Left vastus lateralis muscle	Microarray RT-PCR Western blot	↑ mRNA for ubiquitin proteasome pathway-related E3 ligase Atrogin1 (but not accompanying increases in protein products). No significant difference in MuRF1 mRNA levels via RT-PCR following 48 h UL or 24 h RL. HSPB8 upregulated under both the UL and RL conditions.

Abbreviations:
2-DE= Two-dimensional gel electrophoresis.
bnip3= Bcl-2 nineteen-kilodalton interacting protein 3.
BR= Bed rest.
Cbl-b= Casitas B-lineage lymphoma proto-oncogene-b.
ELISA= Enzyme-linked immunosorbent assay.
GSTM2= Glutathione S-transferase Mu 2.
HSP= Heat shock protein.
HSPA2= Heat shock-related 70 kDa protein 2.
HSPA5= Heat Shock Protein Family A (Hsp70) Member 5.
HSPB1= Heat shock protein family B member 1.
HSPB8= Heat shock protein family B member 8.
HSPD1= Mitochondrial 60 kDa heat shock protein.
ISS= International Space Station.
L− = Days before launch.
LC3= Microtubule-associated protein 1 A/1B-light chain 3.
MAFbx= Muscle atrophy F-box.
MALDI= Matrix-assisted laser desorption/ionization.
MuRF-1= Muscle RING-finger protein-1.
PARK7= Protein/nucleic acid deglycase DJ-1.
PMF= Peptide mass fingerprinting.
post-d BR= Following days of bed rest.
pre-BR= Before bed rest.
R+ = Days after return.
RL= Reloading.
RT-PCR= Real time polymerase chain reaction.
Siah-1A= Seven in absentia homolog 1 A.
SM= Space Mission.
SS= Space Simulation.
ToF MS= Time of fligt Mass Spectrometer.
TRIM32= Tripartite Motif Containing 32.
UL= Unloading.

et al., 2014; Heer et al., 2014; Linossier et al., 2017), ELISA (Boesen et al., 2014; Hughson et al., 2016; Strollo et al., 2018) and radioimmunoassay (Biolo et al., 2008; McCall et al., 1999; Stein et al., 1999; Yang et al., 2014). Plasma glucose concentration was evaluated through commercially enzymatic technique (Biolo et al., 2008; Strollo et al., 2018) and the oral glucose tolerance test (Downs et al., 2020; Heer et al., 2014). The major kinases of the IGF-1/Akt/mTOR signaling pathway were assessed by western blot analysis, and transcript levels of IGF-1 and FOXO3 were determined by RT-PCR (Brooks et al., 2010) and microarray analysis (Fernandez-Gonzalo et al., 2020).

Systemic IGF-1 increased after short-term space shuttle missions in male astronauts (McCall et al., 1999; Stein et al., 1999), as well as after six-months ISS mission, with a greater increase in male than in female astronauts (Hughson et al., 2016). Dry-immersion study reported a progressively increase in IGF-1 already after 3 days (Linossier et al., 2017). Similarly, IGF-1 plasma levels increased approximately fivefold after two weeks of BR immobilization (Boesen et al., 2014), as well as after 28 days of unilateral limb suspension in middle-aged men receiving essential amino acid (EAA) supplementation (Brooks et al., 2014). However, no changes after 60 days BR was reported by Yang et al. (Yang et al., 2014). Regarding the muscle tissue, the levels of IGF-1 transcripts were not affected after 28 days of BR (Brooks et al., 2010). However, Fernandez-Gonzalo et al. (Fernandez-Gonzalo et al., 2020) showed that gene expression of FoxO3 in vastus lateralis muscle samples was upregulated following 84 days of BR. The IGF-1/Akt/mTOR signalling pathway was not activated after 24 days of BR as detected by phosphorylated levels of p70S6K and the upstream regulator Akt, either the ratio between the active (phosphorylated) and total form of AMP-activated protein kinase (AMPK), the key sensor and regulator of the cell's energy balance (Brocca et al., 2012).

Increased levels of blood insulin were observed already after 3 days of dry-immersion (Linossier et al., 2017), while no changes were detected up to the end of ICC analog (Strollo et al., 2018). BR studies present different results, showing an increase in blood insulin levels after 35 and 70 days of BR (Biolo et al., 2008; Downs et al., 2020), no changes after 154 days of BR (Downs et al., 2020), and an oscillation with an initial increase after 4 days and a subsequent reduction after 30 days of BR, returning to normal levels at later timepoints during and after BR (Yang et al., 2014). Furthermore, increased levels of blood glucose were observed already after 3 days of dry-immersion (Linossier et al., 2017) and between 249 and 417 days of ICC analog (Strollo et al., 2018). Divergent results were observed after BR: one study described an increase of glucose concentration after 70 days of BR in both blood and breath (Downs et al., 2020), while other two studies did not report any alterations (Biolo et al., 2008; Heer et al., 2014).

In summary, the systemic IGF-1 levels in nutrient signaling pathway seem to be increased in both space mission and ground-based studies. Contrasting results emerge instead from the assessment of circulating insulin levels in controlled ICC analog and BR studies. Both these extracellular ligands, insulin and IGF, are the apex of the nutrient signaling pathway, which is followed by intracellular signaling cascades involving the PI3K-AKT pathway, as well as transcription factors, including FOXO. However, no changes were described in muscles for the IGF-1/Akt/mTOR signaling pathway, although one BR study reported that FoxO3, a downstream intracellular effector of the IIS pathway that plays a major role in slowing ageing, was upregulated following BR. This network is a central regulator of cellular activity, including glucose which levels are altered in ground-based studies probably due to the prolonged chronic stress. It was in fact determined in the ICC analogs rather than in BR studies. Further studies are necessary to investigate all players in the nutrient sensing pathways and possible countermeasures, which could include an energy-balanced diet and chronic stress management practices that induce RR.

4.6. *Spaceflight and mitochondrial dysfunction*

Ageing process of cells and organisms is characterized by a reduction in the efficacy of the respiratory chain, resulting in increased electron leakage and decreased ATP synthesis (López-Otín et al., 2013), leading to mitochondrial dysfunction. Mitochondrial dysfunction is considered

Table 5
Spaceflight and deregulated nutrient sensing.

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Biolo et al., 2008	SS	N=19 healthy male volunteers (Study A N=10; Study B N=9)	5 weeks of BR	Pre and post BR periods	Blood samples (plasma)	Plasma insulin concentration: radioimmunoassay. Plasma glucose concentration: commercially available kits and autoanalyzer	↑ Insulin concentration after BR, while plasma glucose did not change (↔) after BR.
Boesen et al., 2014	SS	N=6 healthy elderly men	14 days with one leg immobilized followed by 6 weeks of retraining	Multiple time points: baseline, after 14 days of immobilization, after 2- and 6-weeks during rehabilitation	Blood samples	ELISA	↔ Systemic IGF-1 between baseline, immobilization, and retraining.
Brocca et al., 2012	SS	N=9 subjects (group A) N=9 subjects (group B)	Two BR campaigns: one lasting 35 days (group A) and one 24 days (group B)	Group A: pre-BR, post-8d BR and post-35d BR. Group B: pre-BR, post-8d BR and post-24d BR	Vastus lateralis muscle	Western blot analysis	The ratios between the phosphorylated and total Akt and p70S6K, two major kinases of the IGF-1/Akt/mTOR signalling pathway, were not significantly higher post-24d BR compared to pre-BR, even if there was a trend towards ↑. ↔ Ratio of phosphorylated and total form of AMPK post-24d BR.
Brooks et al., 2010	SS	N=7 healthy males	49 days: 7 days of baseline measurements, 28 days of BR, 14 days of active recovery	Multiple time points: baseline prior to randomization, post-28d BR, and after 14 days of active recovery	Vastus lateralis muscle	RT-PCR	↑ IGF-1 levels by end of recovery when compared with baseline.
Brooks et al., 2014	SS	N=7 healthy males	49 days: 7 days of baseline measurements, 28 days of strict BR in the supine position, 14 days of monitored re-ambulation and recovery	Multiple time points: baseline prior to randomization, post-28d BR, and after 14 days of active recovery	Blood sample	Chemiluminescent immunometric assay	↑ Plasma IGF-1 post-28d BR. Values returned to baseline after 14 days of recovery.
Downs et al., 2020	SS	N= 26 healthy volunteers	70-day of HD BR	Multiple time point: 1 day pre-BR, during BR (days 38 and 66), post-12d BR	Blood and breath samples	Oral glucose tolerance test and Breath CO2 Protocols	↑ Glucose levels after 70 days of BR, assessed by 2 hours of Oral glucose tolerance test. ↑ glucose derived CO_2 in breath during HD BR (day 66).
Fernandez-Gonzalo et al., 2020	SS	N=12 healthy males	84 days of BR	Multiple time points: pre-BR and post-84d BR	Vastus lateralis muscle	Microarray	FOXO3 upregulated post-84d BR.
Heer et al., 2014	SS	N=7 healthy males	2 campaigns of BR separated by 154 days. Each campaign: 7 days of pre-BR, 21 days of HDT BR, 6 days of recovery	Multiple time points: 4 days pre-BR, day 21 of HDT BR, day 6 of recovery, after 15 days of HDT BR	Blood samples	Serum glucose: automated analyzer. Serum insulin: ECLIA	↔ Fasting serum glucose and insulin concentrations during BR.
Hughson et al., 2016	SM	N=9 astronauts (4 female)	Six-month spaceflight mission	Multiple time points: pre-fight (24–105 days), inflight (99 and 159 days)	Blood samples	ELISA	↑ IGF-1 inflight with a greater increase in men than women.
Linossier et al., 2017	SS	N=12 healthy male volunteers	7 days: 3 days of baseline measurements, 3 days of DI, 1 day for recovery after the immersion period	Multiple time points: pre-immersion baseline data collection (72–24–0 h), daily during DI (24–48–72 h), after recovery (R+3 h and R+24 h)	Blood sample	Chemiluminescence immunoassay	↑ Blood glucose concentration after 24 h of DI, then it normalized to baseline level after 48 h of DI. ↑ Fasting insulin concentrations during all the DI phases. ↑ Levels of IGF-1 during DI.
McCall et al., 1999	SM	N=4 male astronauts	17-day mission on STS-78 of NASA LMS	Multiple time points: L-30 and L-12, on flight days 2 or 3 and 13 or 14, and after 2, 4, 8, and 15 days of recovery	Blood samples	Radioimmunoassay	↑ Plasma IGF-I levels on flight days after 13- or 14-days exposure to microgravity compared with all other test days.

(*continued on next page*)

Table 5 (*continued*)

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Stein et al., 1999	SM	N= 9 astronauts (3 for SLS; 6 for SLS2)	2 shuttle missions: SLS1 of 9.5 days; SLS2 of 15 days.	Multiple time points: 10 days pre-flight, inflight period, 7 days post-flight	Urine samples	Radioimmunoassay	↔ IGF-1 throughout spaceflight.
Strollo et al., 2018	SS	N=6 male subjects	520-day ground-based space simulation "Mars-500 project"	Multiple time points: 60, 120, 168, 249, 300, 360, 418, 510 and 7 days after the confinement	Blood samples	Standard enzymatic techniques	↑ Fasting plasma glucose between 249 and 417 days, and in the last part of the confinement period. ↔ Plasma insulin levels up to the end of confinement.
Yang et al., 2014	SS	N=7 healthy adult male volunteers	90 days: 15 days of basic data collection prior to BR, 60 days of HDT BR, 15 days of recovery	Multiple time points: day 11 pre-BR, day 4, 14, 30, 60 during BR, post-4d and post-10d BR.	Blood samples	Radioimmunoassay	↑ Serum insulin at day 4 during BR. ↓ Serum insulin at day 30 during BR. It recovered to normal level at the subsequent time points. ↔ IGF-I during the whole BR period.

Abbreviations:
Akt= Protein kinase B.
AMPK= Adenosine monophosphate-activated protein kinase.
BR= Bed rest.
DI= Dry immersion.
ECLIA= Electrochemiluminescence Immunoassay.
ELISA= Enzyme-linked immunosorbent assay.
FOXO3= Forkhead box O3.
HD BR= Head-down bed rest.
HDT BR=head-down tilt bed rest.
IGF-1= Circulating insulin-like growth factor 1.
L− = days before launch.
mTOR= Mechanistic target of rapamycin.
p70S6K= Ribosomal protein S6 kinase beta-1.
post-d BR= following days of bed rest.
pre-BR= Before bed rest.
RT-PCR= Real time polymerase chain reaction.
SLS= Shuttle life sciences mission.
SLS2= Shuttle life sciences mission 2.
SM= Space Mission.
SS= Space Simulation.
STS-78= Shuttle Transport System.

an antagonistic (in responses to the damage) hallmark of ageing (López-Otín et al., 2023), however their connection remains, to date, one of the major scientific concerns in ageing research. There are several indicators of age-related mitochondrial dysfunction that include elevated levels of ROS with decreased activity of antioxidant enzymes, impaired respiratory performance and destabilization of the macromolecular organization of respiratory chain (super)complexes, as well as reduced biogenesis, defective mitophagy, altered mitochondrial dynamics (imbalance of fission and fusion events), increased burden of mutations and deletions in mitochondrial DNA (mtDNA), deterioration of energy metabolism (including the Krebs cycle, beta-oxidation, and ketogenesis), and changes in the lipid composition of mitochondrial membranes (López-Otín et al., 2013; Srivastava, 2017).

Table 6 shows studies (n=12) on spaceflight and mitochondrial dysfunction. They are all longitudinal studies conducted in a restricted set of subjects (from n=2 to n=12). Three studies were conducted on male astronauts involved in ISS space-missions with different duration, i.e. 4, 6 months (Capri et al., 2019; da Silveira et al., 2020) and one-year (da Silveira et al., 2020; Garrett-Bakelman et al., 2019). Nine were ground-based stdies on BR (Brocca et al., 2012; Chopard et al., 2009; Fernandez-Gonzalo et al., 2020; Hafen et al., 2019; Irimia et al., 2017; Ogawa et al., 2006; Reich et al., 2010; Salanova et al., 2015; Salvadego et al., 2018). PBL samples were collected during spaceflight studies as the easiest available tissue (Capri et al., 2019; da Silveira et al., 2020; Garrett-Bakelman et al., 2019), while soleus muscles (Capri et al., 2019; Chopard et al., 2009; Salanova et al., 2015) and vastus lateralis muscles (Brocca et al., 2012; Chopard et al., 2009; Fernandez-Gonzalo et al., 2020; Hafen et al., 2019; Irimia et al., 2017; Ogawa et al., 2006; Reich et al., 2010; Salvadego et al., 2018) were collected in BR studies. All blood and muscles samples were collected before and after, and at different time points spanning the missions and the ground-based studies (Brocca et al., 2012; Capri et al., 2019; Chopard et al., 2009; da Silveira et al., 2020; Fernandez-Gonzalo et al., 2020; Garrett-Bakelman et al., 2019; Hafen et al., 2019; Irimia et al., 2017; Ogawa et al., 2006; Reich et al., 2010; Salanova et al., 2015; Salvadego et al., 2018).

Mitochondrial dysfunction was evaluated by several molecular techniques, including RNAseq (da Silveira et al., 2020; Garrett-Bakelman et al., 2019), qPCR (Brocca et al., 2012; Chopard et al., 2009; da Silveira et al., 2020; Garrett-Bakelman et al., 2019; Irimia et al., 2017; Ogawa et al., 2006) and microarray (Chopard et al., 2009; Fernandez-Gonzalo et al., 2020; Ogawa et al., 2006; Reich et al., 2010; Salanova et al., 2015), and biochemical techniques, such as chemiluminescence (Salvadego et al., 2018), immunoblot or western blot (Brocca et al., 2012; Hafen et al., 2019; Ogawa et al., 2006), ELISA (Capri et al., 2019), Maldi ToF/ToF mass spectrometer (Capri et al., 2019; Salanova et al., 2015), and High resolution respirometry (Salvadego et al., 2018).

Table 6
Spaceflight and mitochondrial dysfunction.

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Brocca et al., 2012	SS	N=9 subjects (group A) N=9 subjects (group B)	Two BR campaigns: one lasting 35 days (group A) and one 24 days (group B).	Group A: pre-BR, post-8d BR and post-35d BR. Group B: pre-BR, post-8d BR and post-24d BR	Vastus lateralis muscle	RT-PCR Immunoblot analysis	***Subject group A*** ↓ MDH post-8d and post-35d BR compared to pre-BR. ↓ SOD1 and PRDX3 post-8d BR and post-35d BR. ***Subject group B*** ↓ PGC-1α mRNA post-24d BR. ↑ SREBP-1 mRNA post-24d BR. ↓ SOD1 and PRDX post-24d BR compared with pre-BR.
Capri et al., 2019	SM	N=2 crewmembers	6-months ISS mission	Multiple time points: between L-76 and L-79, R+1 and R+15	Blood/ plasma Soleus muscle tissue samples	ELISA PMF utilizing MALDI ToF/ToF MS	↓ PRDX6 and SOD2 in crewmember A after landing.
Chopard et al., 2009	SS	N=8 healthy young European women	60 days of BR	Multiple time points: 2 days before reambulation and on day 59 of the BR period	Soleus and vastus lateralis muscle	Microarray RT-PCR	↓ Subclusters of mRNAs that mostly encode proteins involved in oxidative phosphorylation fatty acid metabolism post-60d BR.
da Silveira et al., 2020	SM	Subjects Group A (NASA twin study): n=1 twin in space (TW) n=1 twin on Earth (HR). Subjects Group B	Subjects Group A: a 340-day mission. Subjects Group B: missions of 4–6 months	Subjects Group A: at multiple time points preflight, inflight, and postflight, for a total of 25 months. Subjects Group B: L-45, flight Day 15, 30, 60, 120, and 180, R+1 and R+30	PBMCs	Subjects Group A: RNA-seq qRT-PCR Subjects Group B: NA	**Subjects Group A:** Significant shift in mitochondrial activity from inflight to post-flight compared to pre-flight. ↑ Five mtDNA genes related to OXPHOS in response to spaceflight. ↔ Genes from matched samples for the HR or in GAPDH reference gene. mtDNA gene expression changes returned to baseline levels post-flight within a few weeks. **Subjects Group B:** ↓ Astronauts' antioxidant capacity during spaceflight.
Fernandez-Gonzalo et al., 2020	SS	N=12 healthy males	84 days of BR	Multiple time points: before and post-84d BR	Vastus lateralis muscle	Microarray	↑ Gene expression associated with mitochondrial post-84d BR, in particular genes encoding for mitochondrial electron transport and for TCA cycle.
Garrett-Bakelman et al., 2019	SM	NASA twin astronauts (male monozygotic): one twin in space (TW) and one twin on Earth (HR)	A 340-day mission	Multiple time points: before (preflight), during (inflight), and after flight (postflight), for a total of 25 months	PBMCs	RNA-seq qRT-PCR	↑ mtRNA inflight as compared with pre-flight and post-flight. ↑ mtRNA inflight correlated with time spent on the ISS.
Hafen et al., 2019	SS	N=11 healthy volunteers	5 days of recovery and 10 consecutive days of immobilization	Multiple time points: on days 1 and 17	Left vastus lateralis muscle	Protein immunoblotting	↓ Respiratory capacity after 10 days of immobilization. ↓ Expression among all five of the respiratory protein complexes after immobilization: ↓ CI subunit NDUFB8, CII subunit 30 kDa, CIII subunit Core 2, CIV subunit II, ATP synthase subunit alpha, and in the expression of PGC-1α.
Irimia et al., 2017	SS	N=12 men	84 days head-down tilt BR.	Multiple time points: before BR and at day 84 of BR	Vastus lateralis muscle	RT- PCR	↓ Activity and gene expression of enzymes controlling oxidative metabolism (CS, SDH) after BR. ↓ Gene expression of PGC-1α after BR.

(*continued on next page*)

Table 6 (*continued*)

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Ogawa, et al., 2006	SS	N=5 healthy volunteers	20-day BR	Multiple time points: before and at the end of BR	Vastus lateralis muscle	RT-PCR Western blot Microarray	↓ Expression levels of mitochondrial protein and gene in response to BR.
Reich, et al., 2010	SS	N=7 sedentary men	48 h UL via unilateral lower limb suspension and 24 h RL	Multiple time points: before UL, 24 h RL and 48 h UL	Left vastus lateralis muscle	Microarray	Genes involved in mitochondrial metabolism significantly downregulated at 48 h UL: COQ3, MAOB, HCCS, NDUFS4. Remaining genes involved in mitochondrial metabolism and morphology significantly altered after the subsequent 24 h RL.
Salanova et al., 2015	SS	N=4 healthy males	60 days of BR	Multiple time points: 2 days pre-BR and 2 days before end of BR	Soleus lateralis muscle	Microarray 2D-DIGE protein labelling PMF utilizing MALDI ToF/ToF MS	TCA cycle and oxidative phosphorylation-associated genes significant downregulated. Lipid metabolism and several MRPs altered compared to baseline pre-BR.
Salvadego et al., 2018	SS	N=11 recreationally active men	Three 21-day campaigns: N-BR, H-BR, H-AMB. Interventions separated by a 4-month washout period	Multiple time points: 1 day before and on the last day of each intervention	Vastus lateralis muscle	High-resolution respirometry (O2 consumption) Chemiluminescence method (CS content)	↔ CS expression among the experimental conditions. ↓ Maximal ADP-stimulated mitochondrial respiration and maximal capacity of the electron transport system under all experimental conditions vs baseline data. ↑ Mitochondrial leak respiration in N-BR, ↔ in H-BR or H-AMB. ↓ Degree of coupling of oxidative phosphorylation at a specific substrate supply (glutamate and malate) in N-BR vs baseline data; ↔ between H-BR or H-AMB and baseline data.

Abbreviations:
2D-DIGE= Two-dimensional difference gel electrophoresis.
ADP=Adenosine di-phosphate.
BR= Bed rest.
CI = Mitochondrial Complex I.
CII= Mitochondrial Complex II.
CIII= Mitochondrial Complex III.
CIV= Mitochondrial Complex IV.
COQ3= Coenzyme Q3.
CS= Citrate synthase.
ELISA= Enzyme-linked immunosorbent assay.
GAPDH= Glyceraldehyde-3-Phosphate Dehydrogenase.
H-AMB= Hypoxic ambulatory confinement.
H-BR= Hypoxic horizontal bed rest.
HCCS= Holocytochrome c synthase.
ISS= International Space Station.
L− = Days before launch.
MALDI= Matrix-assisted laser desorption/ionization.
MAOB= Monoamine oxidase B.
MDH= Malate dehydrogenase.
MRPs= Mitochondrial ribosomal protein transcripts.
mtDNA= Mitochondrial DNA.
mtRNA= Mitochondrial RNAs.
NA= Not available.
NASA= National Aeronautics and Space Administration.
N-BR= Normobaric normoxic horizontal bed rest.
NDUFS4= NADH Dehydrogenase Ubiquinone 1 alpha subcomplex 8.
NDUFB8= NADH Ubiquinone Oxidoreductase Subunit B8.
OXPHOS= Mitochondrial Oxidative Phosphorylation System.
PBMCs= Peripheral blood mononuclear cells.
PGC-1α = Peroxisome proliferator-activated receptor-gamma coactivator-1α.
PMF= Peptide mass fingerprinting.

post-d BR= Following days of bed rest.
PRDX3= Peroxiredoxin-3.
PRDX6= Peroxiredoxin-6.
pre-BR= Before bed rest.
qRT-PCR= Quantitative real-time polymerase chain reaction.
R+ = Days after return.
RL= Reloading.
RNA-seq= RNA sequencing.
RT-PCR= Real time polymerase chain reaction.
SDH= Succinate dehydrogenase.
SM= Space Mission.
SOD1= Superoxide dismutase 1.
SOD2= Superoxide dismutase 2.
SREBP-1= Sterol regulatory element-binding protein-1.
SS= Space Simulation.
TCA cycle= Tricarboxylic acid cycle.
ToF MS= Time of fligt Mass Spectrometer.
UL= Unloading.

In their study, Garrett-Bakelman et al. (Garrett-Bakelman et al., 2019) observed increased levels of mitochondrial RNA (mtRNA) during spaceflight compared to pre-flight and post-flight in one of the twin astronauts aboard the ISS. They also found a rise in the expression of five mtDNA genes related to oxidative phosphorylation (OXPHOS), which returned to baseline levels shortly after returning to Earth (da Silveira et al., 2020). In addition, the authors reported a reduction in antioxidant capacity in astronauts who spent 4–6 months on the ISS. Another study involving a 6-month ISS mission identified reduced muscle protein concentrations of Superoxide dismutase (SOD2) and Peroxiredoxin 6 (PRDX6) in one astronaut (Capri et al., 2019). Ground-based studies, including five BR studies, showed downregulation of genes involved in mitochondrial metabolism, including electron transport, oxidative phosphorylation, redox, and the tricarboxylic acid cycle after a BR period ranging from 20 to 60 days (Brocca et al., 2012; Chopard et al., 2009; Irimia et al., 2017; Ogawa et al., 2006; Salanova et al., 2015). Additionally, just two days of unloading limb suspension (ULS) led to the downregulation of genes such as Coenzyme Q3 (COQ3), Monoamine oxidase B (MAOB), Holocytochrome c synthase (HCCS), and NADH Dehydrogenase Ubiquinone 1 alpha subcomplex 8 (NDUFS4) (Reich et al., 2010). In contrast, prolonged immobilization for 84 days resulted in an upregulation of genes associated with the mitochondrial electron transport chain (Fernandez-Gonzalo et al., 2020). Furthermore, after 24 days of BR, Sterol regulatory element-binding protein-1 (SREBP-1) mRNA levels were higher than before BR (Brocca et al., 2012). Respiratory capacity was also found to be significantly reduced after only 10 days of immobilisation, resulting in reduced expression of all five complexes of the respiratory proteins, as well as the peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) gene (Hafen et al., 2019). Similar findings were reported by Salvadego et al. (Salvadego et al., 2018), who observed significantly lower mitochondrial respiration and maximal capacity of the electron transport system after 21 days of BR, particularly in men belonging to the normobaric normoxic horizontal BR group. Furthermore, previous studies by Ogawa and colleagues (Ogawa et al., 2006) and Brocca and coworkers (Brocca et al., 2012) also reported decreased expression of mitochondrial proteins, including Malate dehydrogenase (MDH) involved in oxidative metabolism, as well as SOD1 and PRDX3, which are important in cellular defense against ROS and free radicals. These changes in mitochondrial gene expression and protein levels were evident after relatively short periods of BR ranging from 8 to 35 days.

Collectively, these findings suggest that spaceflight conditions, as well as prolonged immobilization, induce significant alterations in mitochondrial function, affecting respiratory capacity, ATP production and antioxidant defenses, leading to a consequent increase in oxidative stress. Further research, especially involving astronauts, is necessary to gain a comprehensive understanding of the complex impact of space conditions on mitochondrial pathways. This knowledge will be crucial for ensuring the health and performance of astronauts during space missions and may also shed light on potential strategies to counteract ageing-related mitochondrial dysfunction on Earth.

4.7. Spaceflight and cellular senescence

Cellular senescence refers to a stable arrest of the cell cycle and it is characterized by an heterogeneous phenotype including modifications in signaling pathways and cell morphology (Campisi and d'Adda di Fagagna, 2007; Collado et al., 2007; Hernandez-Segura et al., 2018; Kuilman et al., 2010). Variations in signaling pathways, include activation of chronic DNA damage response (DDR), and several cyclin-dependent kinase inhibitors (CDKi i.e., p16, p15, p21 and its upstream regulator p53), secretion of the "senescence-associated secretory phenotype" (SASP), including matrix metalloproteinases and pro-inflammatory cytokines, expression of antiapoptotic genes, changed metabolic rates, and endoplasmic reticulum stress. As a consequence of these alterations in signaling pathways, senescent cells exhibit structural abnormalities in size and shape, altered plasma membrane composition, an increased number of lysosomes and mitochondria and nuclear modifications (Hernandez-Segura et al., 2018).

Table 7 shows studies (n=5) found about the effect of spaceflight on cellular senescence. All are longitudinal (before and after) studies conducted in a restricted set of subjects (from n=2 to n=14). Two studies were conducted on male astronauts who were involved in ISS space-missions with different duration, from 6 months (Luxton et al., 2020a) to one-year (Garrett-Bakelman et al., 2019; Luxton et al., 2020a). Three were BR studies conducted on healthy volunteers (from n=8 to n=12) (Chopard et al., 2009; Fernandez-Gonzalo et al., 2020; Oranger et al., 2023). PBL were collected in spaceflight studies as the easiest available tissue (Garrett-Bakelman et al., 2019; Luxton et al., 2020a), while skeletal muscle biopsies were collected in BR studies (Oranger et al., 2023), including vastus lateralis muscles (Chopard et al., 2009; Fernandez-Gonzalo et al., 2020) and soleus muscles (Chopard et al., 2009). All PBL and muscles samples were collected at different time points spanning the missions and ground-based simulation, always before and after (Chopard et al., 2009; Fernandez-Gonzalo et al., 2020; Garrett-Bakelman et al., 2019; Luxton et al., 2020a; Oranger et al., 2023). Different techniques of markers of cellular senescence were used, including qRT-PCR (Garrett-Bakelman et al., 2019; Luxton et al., 2020a; Oranger et al., 2023) and microarray (Chopard et al., 2009; Fernandez-Gonzalo et al., 2020), but also Telo- FISH and dGH (Garrett-Bakelman et al., 2019; Luxton et al., 2020a).

Expression of genes involved in pathways related to DDR were significantly altered inflight (one year mission) (Garrett-Bakelman et al., 2019), and signatures of this persistent DDRs, including mitochondrial and oxidative stress, inflammation, and telomeric and chromosomal aberrations were detected (Garrett-Bakelman et al., 2019; Luxton et al.,

Table 7
Spaceflight and cellular senescence.

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Chopard et al., 2009	SS	N=8 healthy young European women	60 days of BR	Multiple time points: 2 days before reambulation and on day 59 of the BR period	Soleus and vastus lateralis muscle	Microarray	↓ CASP3 after BR.
Fernandez-Gonzalo et al., 2020	SS	N=12 healthy males	84 days of BR	Multiple time points: before and post-84d BR	Vastus lateralis muscle	Microarray	↑ NF-kB post-84d BR.
Garrett-Bakelman et al., 2019	SM	NASA twin astronauts (male monozygotic): one twin in space (TW) and one twin on Earth (HR)	A 340-day mission	Multiple time points: before (preflight), during (inflight), and after flight (postflight), for a total of 25 months	PBMCs	qRT-PCR	Genes, whose expression was altered inflight, were significantly enriched in pathways related to DDR.
Luxton et al., 2020a	SM	N=3 NASA astronauts (aged 35–55 year) N=11 healthy age- and sex-matched ground control subjects (NASA volunteers)	One-year mission (n=1) and six-month mission (n=2)	Multiple time points: before (L-270, L-180, L-60), during (Flight Day ~45 or 90, 140 or 260), after (L+1–7, L+60, L+180, L+270) spaceflight	PBMCs	qRT-PCR Telo-FISH	Signatures of persistent DDR were detected, including mitochondrial and oxidative stress, telomeric and chromosomal aberrations.
Oranger et al., 2023	SS	N=10 young volunteer healthy males	10 days of horizontal BR	Multiple time points: first day of BR and post-10d BR	Skeletal muscle biopsies	RT-PCR	↔ Senescence marker gene expression: p16, p53 and p21, between post-10d and first day of BR. Negative correlation between p21 mRNA and irisin serum levels post-10d BR, but not p16 and p53.

Abbreviations:
BR= Bed rest.
CASP3= Caspase 3.
DDR= DNA damage response.
L- = Days before launch.
L+ = Days after launch.
NASA= National Aeronautics and Space Administration.
NF-kB= Nuclear factor-kappa B.
PBMCs= Peripheral blood mononuclear cells.
post-d BR= Following days of bed rest.
qRT-PCR= Quantitative real-time polymerase chain reaction.
RT-PCR= Real time polymerase chain reaction.
SM= Space Mission.
SS= Space Simulation.
Telo-FISH= telomere-fluorescence in situ hybridization.

2020a), as reported in the corresponding paragraphs. Alterations in signaling DDR pathways, are primarily related to inflight IR chronic exposure, measured by a physical dosimeter recording a dose of 76.18 milligrays with an effective dose of 146.34 millisieverts (Garrett-Bakelman et al., 2019). This suggests that protecting astronauts from this radiation injury remains one of the most important steps during spaceflight in order to prevent cellular senescence. No BR studies, which mostly mimic the microgravity typical of the space environment, were performed on alterations in signaling DDR pathways. Results from BR studies report no variation in senescence marker gene expression including, p16, p21 and p53, in young individuals after 10 days of BR (Oranger et al., 2023). After 60 days of BR, Chopard et al. (Chopard et al., 2009) instead reported: a downregulation in Caspace 3 (CASP3), a protease enzyme that plays essential role in cell death and an upregulation of genes associated with cell death such as the molecule of programmed cell death 4 (PDCD4), that is able to inhibit the translational initiation of multiple genes, including tumor suppressor gene p53 and apoptosis-related gene pro-caspase 3 (Jiang et al., 2017). Fernandez-Gonzalo et al. (Fernandez-Gonzalo et al., 2020) found an upregulation of genes encoding nuclear factor-kappa B (NF-kB) proteins, a main mediator of the SASP (Salminen et al., 2008), after 84 days of BR.

Further investigations are warranted to ascertain whether the modifications observed in the signaling pathways associated with DDR are unique to the context of IR exposure as opposed to the effects induced by the conditions of spaceflight. Conversely, there is a pressing need for dedicated studies conducted during space missions to corroborate the findings obtained in terrestrial-based study regarding the perturbations evident in the regulatory mechanisms governing SASP and to try to find out how best to counteract them.

4.8. Spaceflight and stem cell exhaustion

One of the most obvious signs of ageing is the decline in the ability of tissues to regenerate. Stem cells (SCs) are undifferentiated cells in the body with the ability to differentiate any type of cells, replacing degenerating cells to preserve normal tissue function and healing damaged tissues (Research, 2002). In particular, adult stem cells are essential for avoiding organ and tissue degeneration and can delay the ageing process. Stem cell exhaustion, including exhaustion of hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), satellite cells, and intestinal epithelial stem cells (IESCs) (López-Otín et al., 2023), other than a loss of function associated with ageing, explains why older people are more susceptible to immunological suppression, anemia, sarcopenia, osteoporosis, and intestinal dysfunction (Sameri et al., 2020).

There are no studies that directly investigated the effect of spaceflight on stem cell outcomes, because in our review we considered only human studies, so during spaceflight it is difficult to isolate stem cells.

Table 8
Spaceflight and stem cells exhaustion.

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissues	Method	Results
Arentson-Lantz et al., 2016	SS	N = 7 healthy middle-aged adults (4 male, 3 female)	14 days of BR	Multiple time points: pre-BR and post-14d BR	Vastus lateralis muscle	Immunohistochemistry	↓ Satellite cells associated with both type 1 and type 2 fibers. ↓ Total satellite cells content in the muscle following BR period.
Brooks et al., 2010	SS	N= 7 healthy males	49 days: 7 days of baseline, 28 days of BR, 14 days of active recovery	Multiple time points: baseline prior to randomization, post-28d BR, and after 14 days of active recovery	Vastus lateralis muscle	Immunohistochemistry	↔ Satellite cells during BR or recovery.

Abbreviations:
BR= Bed rest.
post-d BR= Following days of bed rest.
pre-BR= Before bed rest.
SS= Space simulation.

Table 8 shows two ground-based studies on BR, conducted on a restricted set of subjects (n=7) exploring the effects of 14 (Arentson-Lantz et al., 2016) and 28 days of BR (Brooks et al., 2010). The immunochemical analyses were conducted on vastus lateralis muscle samples collected before and after BR (Arentson-Lantz et al., 2016; Brooks et al., 2010). These studies reported inconsistent results. The first study showed that 28 days of BR had no significant effect on SCs (Brooks et al., 2010), while a later study reported that 14 days of BR reduced total SCs content in muscle, with a decrease in SCs associated with both type I and type II myofibres (Arentson-Lantz et al., 2016).

Future studies are necessary to shed light on the effect of spaceflight and analog studies on stem cell exhaustion.

4.9. Spaceflight and altered intercellular communication

Ageing process elicits alterations in intercellular communication at the endocrine, neuroendocrine or neuronal levels. The neurohormonal signalling, including renin-angiotensin, adrenergic, insulin-IGF1 signalling, is impaired/disrupted with ageing, as the composition of the peri- and extracellular environment changes, the immunosurveillance decreases and the inflammatory reactions increase (López-Otín et al., 2023). These alterations in intercellular communication, that principally regards blood-borne systemic factors with pro-ageing properties and protein components of extracellular matrix disruption, are cell intrinsic but they sum up to meta-cellular hallmarks including chronic inflammation and alterations in the communication between human genome and microbiome that leds to dysbiosis, both considered as novel

Table 9
Spaceflight and altered intercellular communication.

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Biolo et al., 2008	SS	N=19 healthy male volunteers (Study A N=10; Study B N=9)	5 weeks of BR	Pre and post BR periods	Blood samples (plasma)	ELISA	↑ Leptin levels after BR, greater in subjects with higher energy balance than lower one.
Hughson et al., 2016	SM	N=9 astronauts (4 female)	Six-month spaceflight mission	Multiple time points: pre-fight (24–105 days), inflight (99 and 159 days)	Blood samples	ELISA	↓ Levels of MMP-2 during spaceflight, while MMP-1 and MMP-9 were not significantly changed (↔).
Stein et al., 1999	SM	N= 9 astronauts (3 for SLS; 6 for SLS2)	2 shuttle missions: SLS1 of 9.5 days; SLS2 of 15 days	Multiple time points: 10 days pre-flight, inflight period, 7 days post-flight	Urine samples	EIA	↓ PGE2 and PGF2 inflight and post-flight vs pre-flight
Strollo et al., 2018	SS	N=6 male subjects	520-day ground-based space simulation "Mars-500 project"	Multiple time points: 60, 120, 168, 249, 300, 360, 418, 510 and 7 days after the confinement	Blood samples	ELISA	No changes (↔) of plasma leptin and adiponectin over the entire observation period. ↓ Plasma levels of adiponectin in the first 120 days of mission, which returned to baseline levels around the end of the isolation period.

Abbreviations:
BR= Bed Rest.
EIA= Enzyme immunoassays.
ELISA= Enzyme-linked immunosorbent assay.
MMP-1= matrix metalloproteinase-1.
MMP-2= matrix metalloproteinase-2.
MMP-9= Matrix metallopeptidase 9.
PGE2= Prostaglandin E2.
PGF2= Prostaglandin F2.
SLS= Shuttle life sciences mission.
SLS2= Shuttle life sciences mission 2.
SM= Space mission.
SS= Space Simulation.

and independent integrative hallmarks of ageing (López-Otín et al., 2023). Chronic inflammation and dysbiosis are treated separately in the paragraphs 3.11 and 3.12. Notably, intercellular communication also involves the interaction of short-lived extracellular molecules (including ROS, nitric oxide, nucleic acids, prostaglandins), soluble factors from various tissues including white and brown adipose tissues (adipokines, baptokines), heart (cardiokines), liver (hepatokines), and skeletal muscles (myokine). All of these communication systems are being investigated for their potential pro- and anti-ageing features (Fafián-Labora and O'Loghlen, 2020).

Table 9 reports studies (n=4) showing results concerning the effect of spaceflight on these classical (i.e., soluble factors, growth factors, and matrix remodeling enzymes released in the classical soluble SASP model) and non classical or not secreted intercellular communication systems not driven by released factors but mediated via receptor interaction (Fafián-Labora and O'Loghlen, 2020).

All are longitudinal studies conducted in a restricted set of subjects (from n=6 to n=19). Two studies were conducted on astronauts involved in short-term space shuttle missions, ranging between 9 and 15 days (Stein et al., 1999), and in six-months ISS space-missions (Hughson et al., 2016). Two studies were ground based, including one study on BR (Biolo et al., 2008) and other one study on ICC analog (Strollo et al., 2018). Blood (Biolo et al., 2008; Hughson et al., 2016; Strollo et al., 2018) and urine samples (Stein et al., 1999) were collected in spaceflight and ground-based studies. Secrete matrix metalloproteases, prostaglandins and adipokines, were analysed by enzyme immunoassay (EIA) (Stein et al., 1999) and ELISA (Biolo et al., 2008; Hughson et al., 2016; Strollo et al., 2018), as intercellular communication molecules.

Astronauts involved in short-term shuttle missions showed a decrease in plasma prostaglandins, i.e. PGE2 and PGF2α, during spaceflight, suggesting protein loss by muscle (Stein et al., 1999). Astronauts involved in a six-months ISS mission presented decreased levels of matrix metalloproteinase-2 (MMP-2) inflight, which might reflect less repair of vascular wall extracellular matrix during spaceflight (Hughson et al., 2016).

Ground-based studies reported divergent results about adipokines, short-lived extracellular molecules, showing an increase in leptin after 5 weeks of BR, in particular in subjects with greater energy balance than lower one (Biolo et al., 2008). Instead, no changes of plasma leptin were observed over the 52 days of ICC analog (Strollo et al., 2018). The latter study also reported a decline in plasma levels of adiponectin in the first 120 days, which progressively returned to baseline levels around the end of the isolation period, supporting the impact of environmental stress upon metabolic adaptations (Strollo et al., 2018).

In summary, the protein components of extracellular matrix disruption and the short-lived extracellular molecules are the most investigated systems of intercellular communication in both space and ground-based studies. A decreasing trend of these molecules inflight and in the first period of ICC analog, probably due to the influence of environmental stress on metabolic adaptations was observed. An appropriate energy balance as shown in the BR study could counteract the altered increase in leptin. Further studies are needed to investigate the effect of spaceflight on the multiple features of intercellular communication and its interconnection with meta-cellular hallmarks including chronic inflammation and dysbiosis.

4.10. Spaceflight and disabled macroautophagy

Disabled macroautophagy was originally considered as a special case of loss of proteostasis because both of them are characterized by a divergence from the young equilibrium state with an accumulation of waste products, as result of several age-associated alterations, and a simultaneously compromised waste removal (López-Otín et al., 2023). However, macroautophagy targets entire organelles, as well as non-proteinaceous macromolecules and invading pathogens (Levine and Kroemer, 2019), and it is now considered a new primary hallmark of ageing (López-Otín et al., 2023).

Literature search has not produced any results about the effect of spaceflight on macroautophagy, remaining an unexplored field.

4.11. Spaceflight and dysbiosis

Over the last years, the gut microbiome has emerged as a crucial feature in multiple physiological processes, including ageing. Dysbiosis, which is an imbalance in the microbiota composition, is associated to many human illnesses and age-related diseases, including cardiovascular diseases, cancer, respiratory diseases, diabetes, inflammatory Bowel Disease (IBD), brain disorders, chronic kidney diseases, and liver diseases (Hou et al., 2022). This association makes dysbiosis a central concept for understanding how the human microbiota contributes to health and disease (Tiffany and Bäumler, 2019), and to multiple pathological conditions ageing-associated (López-Otín et al., 2023). The human gut composition of host-associated microbial communities (microbiota) significantly changes during ageing, finally leading to a general decrease in ecological diversity.

Table 10 shows studies (n=11) exploring the effects of spaceflight and ground-based stimulations on dysbiosis. All are longitudinal (before and after) studies conducted in a restricted set of subjects (from n=2 to n=9). Five studies were conducted on astronauts during space mission with different duration, ranging between 15 and 35 days during short-term spaceflight mission (Liu et al., 2020) and one-year in the ISS mission (Garrett-Bakelman et al., 2019; Morrison et al., 2021; Voorhies et al., 2019). Six studies are ground-based stimulations, in particular ICC analogs, with different duration, ranging between 56 days (Holdeman et al., 1976) to 520 days (Mardanov et al., 2013; Turroni et al., 2017). Faecal samples were collected at different time points spanning the missions and ground-based simulations (Garrett-Bakelman et al., 2019; Hao et al., 2018; Holdeman et al., 1976; Li et al., 2016; Liu et al., 2020; Mardanov et al., 2013; Turroni et al., 2017). Personal microbiome swabs were also taken by Voorhies and colleagues (Voorhies et al., 2019) and Morrison and collaborators (Morrison et al., 2021), while saliva samples were also collected by Urbaniak et al. (Urbaniak et al., 2020) and Morrison (Morrison et al., 2021) to compare different body compartments. Similarly swab samplings were taken from skin surfaces (Mahnert et al., 2021). High-throughput sequencing technology, e.g. Illumina Mise sequencing, was used to detect and measure previously not cultivable bacterial microbiome by comparing 16S ribosomal RNA sequences (Hao et al., 2018; Li et al., 2016; Liu et al., 2020; Mahnert et al., 2021; Turroni et al., 2017; Urbaniak et al., 2020; Voorhies et al., 2019), as well as amplification and pyrosequencing (Mardanov et al., 2013); while metagenomic sequencing was performed in two different studies (Garrett-Bakelman et al., 2019; Morrison et al., 2021). Cultural and microscopic counts (Holdeman et al., 1976) and liquid chromatography–mass spectrometry (LC–MS) for metabolomic data (Garrett-Bakelman et al., 2019) were also utilized to analyse dysbiosis.

Changes in composition and functions of gut microbiome were observed during spaceflight, irrespective of mission duration (Garrett--Bakelman et al., 2019; Liu et al., 2020; Voorhies et al., 2019), including an increase in Bacteroides abundance and a decrease in Lactobacillus and Bifidobacterium abundances (Liu et al., 2020), i.e. an increase in opportunistic pathogens and a decrease of defense microorganisms (Ragonnaud and Biragyn, 2021), a typical pro-ageing condition. Metabolomic data reveals a decrease of bacterial intestinal 3-indole propionic acid, with anti-inflammatory propriety, observed in the astronaut twin in space (Garrett-Bakelman et al., 2019; Liu et al., 2020; Voorhies et al., 2019). However, most of these microbiome changes reverted to pre-fight levels after return to Earth (Garrett-Bakelman et al., 2019; Voorhies et al., 2019). Also, microbial communities of the skin, nose and tongue changed inflight (Voorhies et al., 2019), as well as microbial communities in saliva samples (Urbaniak et al., 2020). Anyway, one study did not find relevant changes in the number or relative abundance of taxa for four astronauts during ISS mission (Morrison

Table 10
Spaceflight and dysbiosis.

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Garrett-Bakelman et al., 2019	SM	NASA twin astronauts (male monozygotic): one twin in space (TW) and one twin on Earth (HR)	A 340-day mission	Multiple time points: before (preflight), during (inflight), and after flight (postflight), for a total of 25 months	Faecal swabs	Shotgun metagenome sequencing LC-MS for metabolomics data	TW's changes in microbiome (composition and function) inflight than in HR. ↓ levels of 3-indole propionic acid in TW throughout the duration of the study. TW's microbiome changes returned to near pre-fight levels within 6 months after return to Earth.
Hao et al., 2018	SS	N=3 Chinese crewmembers (1 man and 2 women)	105-day in LP1	Multiple time points: pre-experiment stage (from 7 to 1 day before 105d-E); early stage of 105d-E (from 0 to 30th day); middle stage of 105d-E (from 31st to 75th day); late stage of 105d-E (from 76th to 105th day); post-experiment stage (from 7 to 30 days after 105d-E)	Faecal samples	16 S rRNA gene Illumina MiSeq sequencing	Artificial ecosystem, such as a bioregenerative life-support system, impacts the gut microbiota during the 105d-E. Convergence and similar dynamic change in gut microbiota community composition of the three crewmembers during 105d-E. ↑ Phylum Firmicutes and ↓ Bacteroidetes; Firmicutes/ Bacteroidetes ratio ↑ over time during 105d-E. ↑ abundance of Lachnospira, Faecalibacterium and Blautia.
Holdeman et al., 1976	SS	N=3 male astronauts	56-day confinement SMEAT study	Multiple time points: before and on 15, 30, and 56 days of confinement	Faecal samples	Cultural and microscopic count	↑ Bacteroides species (Bacteroides fragilis subsp. thetaiotaomicron) during confinement of in the Skylab test chamber.
Li et al., 2016	SS	N=3 Chinese crewmembers (1 man and 2 women)	105-day in LP1	Multiple time points: every 2 weeks	Faecal samples	Illumina MiSeq platform	Faecalibacterium spp. had the highest abundance over the study.
Liu et al., 2020	SM	N=5 astronauts	2 short-term spaceflight missions: 15 and 35 days	Five time points, including preflight (L-7) and postflight (R+1, R+7, R+14, R+28)	Faecal samples	Illumina HiSeq sequencing	Postflight ↑ Bacteroides abundance, while ↓ Lactobacillus and Bifidobacterium abundances.
Mahnert, et al., 2021	SS	N=6 crewmembers (3 males and 3 females)	1-year HI-SEAS IV mission	Multiple time points: every other week for 1 year	Skin (swab)	Illumina MiSeq sequencing	Change in skin microbiome with ↑ Methanobrevibacter (a gut/urogenital-associated sp) between crew members within the first 200 days.
Mardanov et al., 2013	SS	N=6 crewmembers	520-day ground-based space simulation MARS500	Multiple time points: prior to entering the isolation module, then after 14, 30, 210, 363, 510 days of stay in the module and 2 weeks after exiting the module (524 days)	Faecal samples	Amplification and pyrosequencing of fragments of 16 S ribosomal RNA genes, and Sequencing of metagenomes	Changes in the compositions of the microbiomes occurred just 14–30 days after the beginning of the experiment. A tendency toward a reversion of the microbiomes to their initial composition was observed two weeks after the end of the experiment, but complete recovery was not achieved.
Morrison et al., 2021	SM	N=4 male astronauts	>4 months ISS missions	Eight time points over the study, including pre-fight, inflight and post-flight.	Body swabs and saliva samples: mouth, nasal cavity, forehead, armpits, forearms (antecubital fossa), navel region, and the back of both ears	Shotgun metagenomic sequencing Microbial Detection Array by AMA	No changes in the number or relative abundance of taxa from all four astronauts were analyzed. Individually, saliva samples showed significant changes in the relative abundance of taxa during and after spaceflight with ↑ Prevotella and ↓ Neisseria, Rothia, and Haemophilus during two astronauts' time onboard the ISS.
Turroni et al., 2017	SS	N=6 crewmembers	520-day ground-based space	Multiple time points: before, throughout the entire 520-day	Faecal samples	Illumina MiSeq sequencing	↑ Bacteroides species in all subjects in the inflight very first stage of the mission.

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Table 10 (*continued*)

Author, year	Type of study	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
			simulation MARS500	simulation experiment, and after exiting the module up to 6 months later			Maintenance of individual specificity of the microbiota compositional.
Urbaniak et al., 2020	SM	N=10 male astronauts	Spaceflight missions ranged from 2–9 months with an average ISS	Multiple time points: two times pre-flight, three times during flight, four times post-flight	Saliva samples	Illumina Mi-Seq sequencing	↓ Streptococcus and Actinobacteria during spaceflight. ↑ Proteobacteria and Fusobacteria during spaceflight. At the genus level, ↑ Catonella, Megasphera, and Actinobacillus inflight if compared with pre-fight.
Voorhies et al., 2019	SM	N=9 astronauts (7 men, 2 women)	6 months at the ISS (N=8) 1 year at the ISS (N=1)	10 time points: pre-fight (L-), inflight, post-flight (R+)	Five personal microbiome swabs: forehead, both forearms, interior nares, tongue and stool	MiSeq sequencing	Microbial communities of the gastrointestinal tract, skin, nose, and tongue changed inflight. Inflight composition of the intestinal microbiota became more similar across astronauts in space. Most of these compositional changes reverted to pre-flight levels after astronauts returned to Earth.

Abbreviations:
105d-E= 105 days experiment.
AMA= Axiom Microbiome Array.
HI-SEAS IV= Hawaii Space Exploration Analog and Simulation IV.
ISS= International Space Station.
L- = Days before launch.
LC–MS= Liquid chromatography–mass spectrometry.
LP1=Lunar Palace 1.
NASA= National Aeronautics and Space Administration.
R+ = Days after return.
SM= Space mission.
SMEAT= Skylab Medical Experiments Altitude Test.
SS= Space Simulation.

et al., 2021).

Ageing-like changes in the gut microbiome were observed in the ICC analogs, as reported in several studies (Hao et al., 2018; Holdeman et al., 1976; Li et al., 2016; Mahnert et al., 2021; Mardanov et al., 2013; Turroni et al., 2017). These alterations were particularly evident after 14–30 days into the experiment, suggesting the influence of stress factors during the initial phase (Mardanov et al., 2013; Turroni et al., 2017). The observed increase in Bacteroides species (Turroni et al., 2017) also confirmed previous findings from the Skylab's 56-day confinement study (Holdeman et al., 1976). However, a tendency towards a partial reversion to the original microbiome composition was observed two weeks after the experiment's conclusion, indicating an incomplete recovery (Mardanov et al., 2013). Similarly, the skin microbiome of crew members showed changes within the first 200 days of the one-year Hawaii Space Exploration Analog and Simulation IV mission, with an increase in Methanobrevibacter, a gut/urogenital-associated species (Mahnert et al., 2021). Interestingly, Hao et al. (Hao et al., 2018) found that the use of an artificial ecosystem, such as a bioregenerative life-support system (BLSS) with a beneficial dietary structure, during a 105-day study in the Chinese Lunar Palace 1 (LP1), positively impacted the gut microbiota. This was evidenced by an increase in the phylum Firmicutes and a simultaneous decrease in Bacteroidetes, indicating that the BLSS dietary structure and specific lifestyle were beneficial for maintaining a healthy gut microbiome. Furthermore, the three Chinese crew members showed that the higher abundance of Faecalibacterium spp. was associated with a positive mood (Li et al., 2016).

Studies, conducted to date, show that the microbiome unsafely changes with an imbalance in favour of opportunistic species, during both space missions, irrespective of mission duration, and ICC analogs. Further studies are needed to confirm BLSS dietary structure and the particular lifestyle (Hao et al., 2018; Li et al., 2016) that seem to be helpful for the maintenance of an healthy gut microbiome as countermeasure of dysbiosis during space missions. Furthermore, a prebiotic, postbiotic and symbiotic supplementation before, during and after space missions, could be ideal for astronauts, stimulating the restoration of an healthy anti-ageing gut microbiome (Arora et al., 2022).

4.12. *Spaceflight and chronic inflammation*

A chronic, sterile low-grade inflammation is a well-known ageing-associated alteration, also called "inflammaging", which plays a key role in the pathogenesis of age-related diseases (Franceschi et al., 2018). This chronic inflammation stems from multiple imbalances of all the other ageing hallmarks (López-Otín et al., 2023). Monitored by an increase in circulating concentrations of inflammatory cytokines and biomarkers, enhanced inflammation elicites an immune function declines, detected by increased myeloid and lymphoid cells (Mogilenko et al., 2021).

Table 11 shows studies (n=19) investigating the effects of spaceflight on chronic inflammation. Thirteen studies are about space missions conducted in astronauts with different duration, ranging between 5 days of space shuttle mission (Kaur et al., 2005) to one-year of ISS mission

Table 11
Spaceflight and chronic inflammation.

Author, year	Type of study and funding	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
Biolo et al., 2008	SS	N=19 healthy male volunteers (Study A N=10; Study B N=9)	5 weeks of BR.	Pre and post BR periods.	Blood samples (plasma)	ELISA	↑ CRP levels after BR, greater in subjects with higher energy balance than lower one.
Capri et al., 2019	SM	N= 2 crewmembers	6-months ISS mission	Multiple time points: preflight between L-76 and L-79, and postflight R+1 and R+15	Blood/ plasma Soleus muscle tissue samples	ELISA RT-PCR	↑ Inflamma-miR-21–5p in crewmember A at R+15, and not significant changes in crewmember B. ↑ Inflamma-miR-126–3p and inflamma-miR-146a-5p in crewmember A at R+1, ↑ in crewmember B at R+1, completely recovered at R+15. ↔ TGF-β1 in the 2 crewmembers. ↑ IL-6 at R+1 and recovered in crewmember A, ↔ in crewmember B.
Crucian et al., 2013	SM	N=19 astronauts (18 males and 1 female)	9 Space Shuttle missions, duration between 10 and 15 days	Multiple time points: preflight (L-180, L-10), in-flight (day before landing), postflight (R+0, R+14)	Blood samples	WBC ELISA	↑ WBC post-flight vs pre-flight, in particular granulocyte levels while lymphocyte and monocyte levels were unaltered. ↑ Plasma concentration of IL-1β, IL-6, IL-12, IL-4, IL-10, and IL-17 inflight, that returned to baseline levels postflight. ↑ Plasma concentration of TNFα, IFNα, and IFNγ inflight and post-flight (R+14).
Crucian et al., 2014	SM	N= 8 astronauts	6 months of ISS Missions	Multiple time points: pre-flight (L-180, L-45, L-10), inflight (15, 30, 60, 120, 180 days), post-flight (R+0, R+30)	Blood samples	Multiplex bead immunoassay	↑ Levels of TNFα, IL-8, IL-1ra, Tpo, VEGF, CCL2, CCL4, and CXCL5 inflight. No changes (↔) in levels of IL-1α, IL-1β, IL-2, IFN-γ, IL-17, IL-4, IL-5, IL-10, G-CSF, GM-CSF, FGF, CCL3.
Crucian et al., 2015	SM	N= 23 astronauts (18 male and 5 were female)	6-month spaceflight ISS mission	Multiple time points: preflight (L-180, L-45), inflight (14 days, months 2, 4 and 6), postflight (R+0, R+30)	Blood samples	Cytokine cytometric bead array Immunophenotype analysis ematology analyzer	↓ in mitogen-stimulated production of IFNγ, IL-10, IL-5, TNFα, and IL-6 inflight. ↓ in mitogen-stimulated production of IL-8 inflight. ↑ WBC and granulocyte levels inflight.
da Silveira et al., 2020	SM	N=59 crewmembers (47 male, 12 female)	Space missions of 4–6 months.	Multiple time points: preflight: (L-180; L-45 days), inflight (days 15, 30, 60, 120, and 180), postflight (R+0, R+30)	Blood samples	Multiplex bead immunoassay and Luminex 100 instrument	↑ Levels of IL-1α, IL-1β, and IL-1ra inflight in astronauts that again resolved to baseline levels upon returning to Earth.
Fernandez-Gonzalo et al., 2020	SS	N=12 healthy males	84 days of BR	Multiple time points: before and post-84d BR	Vastus lateralis muscle	Microarray	Gene of NF-kβ upregulated post-84d BR.
Garrett-Bakelman et al., (2019)	SM	NASA twin astronauts: (male monozygotic), one twin in space (TW) and one twin on Earth (HR)	A 340-day mission	Multiple time points: before (pre-flight), during (inflight), and after flight (post-flight), for a total of 25 months	Blood samples	RNA-seq qPCR qRT-PCR	↑ Cytokines levels in TW's plasma after return, continued to be increased 6 months after return: CSF2, HGF, IL10, IL17A, IL18, LEP, CD40L, LTA, VEGFD, NGF, FGF2, IFNA2, IFNB1, IFN-γ, SERPINE1, RETN, ICAM1, VCAM1, TNFSF10, VEGFA. ↑ Cytokines inflight: BDNF, EGF, ENA78, GROα, IL2, IL6, LIF, PGDF-BB, TGFA, and TNF, that were at relatively high levels in TW preflight, and that decreased after

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Table 11 (*continued*)

Author, year	Type of study and funding	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
							return. ↑ Inflammatory levels of CCL2, CRP, and IL-1ra immediately after landing (R+0 and R+4). All three measures returned to baseline levels. ↑ Lysophospholipids containing proinflammatory omega-6 fatty acid (arachidonic acid) ↓ Lysophospholipids containing anti-inflammatory omega-3 fatty acid (eicosapentaenoic acid).
Heer et al., 2014	SS	N=7 healthy males	2 campaigns of BR separated by 154 days. Each campaign: 7 days of pre-BR, 21 days of HDT BR, 6 days of recovery	Multiple time points: 4 days pre-BR, day 21 of HDT BR, day 6 of recovery, after 15 days of HDT BR	Blood samples	Clinical chemistry & immunochemistry	↑ CRP concentration after BR.
Hughson et al., 2016	SM	N=9 astronauts (4 female)	Six-month spaceflight mission	Multiple time points: prefight (24–105 days), inflight (99 and 159 days)	Blood samples	ELISA	No changes (↔) in CRP from pre-flight to inflight. ↑ IL-1ra levels inflight.
Kaur et al., 2005	SM	N=25 astronauts (17 males and 8 females)	4 space shuttle missions of 5- to 11-day	Multiple time points: pre-flight (L−10), post-flight (R+0, R+3)	Blood samples	WBC	↑ Monocyte count of all crewmembers immediately postflight (R+0) than pre-flight values, but it returned to pre-flight values 3 days after landing (R+3).
Kelsen et al., 2012	SS	N= 7 healthy young males	2 periods of 3 weeks of HD BR: 7-day adaptation phase, 21-day intervention phase, 6-day recovery phase	Multiple time points: after 7-day adaptation phase, 21-day intervention phase, and 6-day recovery phase	Blood samples	Quantiferon whole blood assay	↓ Levels of IL-2, IFN-γ, TNFα and IL-10, stimulated by phytohemagglutinin, after 21 days of HD BR compared to baseline levels, with an ↑ during recovery phase.
Krieger et al., 2021	SM	N= 13 astronauts (11 males and 2 females)	Missions between 136 and 290 days	Saliva samples: pre-flight (L- 180, L-45), 2 timepoints inflight, post-flight (R+0, R+30, R+90). Blood samples: pre-flight (L-180, L-45, L-10), inflight (days 15, 30, 60, 120, 180), post-flight (R+0, and R+30).	Blood samples Saliva samples (N=6)	Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel Premixed 30 Plex Multiplex assay (blood) Milliplex MAP Human High Sensitivity T Cell Panel Premixed 13-plex multiplex assay (saliva)	↑ Blood concentrations of IL-3, IL-7, IL-15, IL-12p40, TGF-ß1 and TGF-ß2 inflight vs pre-flight. ↓ Salivary levels of GM-CSF, IFNγ, IL-12p70, IL-6 and TNFα inflight, that returned to pre-flight levels after landing.
Linossier et al., 2017	SS	N=12 healthy male volunteers	7 days: 3 days of baseline measurements, 3 days of DI, 1 day for recovery after immersion period	Multiple time points: pre-immersion baseline data collection (72–24–0 h), daily during DI (24–48–72 h), after recovery (R+3 h and R+24 h)	Blood sample	EIA Automated clinical chemistry analyzer	↔ Inflammatory response assessed by unchanged CRP values. Undetectable levels in inflammatory cytokines: TNFα, IL-1β, IL-6, IL-17.
Meehan et al., 1992	SM	N=30 astronauts (27 males, 3 females)	6 US space shuttle flights of 4–5 days	Multiple time points: pre-flight (L-10, L-2), postflight (R+0, R+3)	Blood samples	Hematocrit WBC	23% ↑ of leucocytes number following spaceflight: - 60% ↑ of granulocytes - 52% ↑ in monocytes - 25% ↓ in lymphocytes all values returned to baseline post-flight (R+3) 3 days after landing.
Mehta et al., 2013	SM	N=17 astronauts (14 males, 3 females) N= 10 age-matched healthy control subjects (6 males, 4 females)	8 different space shuttle missions of 9–14 days	Multiple time points: preflight (L-10, L-2), postflight (R+0, R+3)	Blood samples	Luminex microbead technology	↑ IL-4 and IP-10 levels post-flight. ↑ Levels of IL-1α, IL-6, IL-8, IFNγ, IL-4, IL-10, IL-12, IL-13 inflight associated with reactivation of latent viruses. ↑ 10 plasma cytokines (IL-1α,

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Table 11 (*continued*)

Author, year	Type of study and funding	Subjects	Mission or study duration	Collection Timing	Tissue	Method	Results
							IL-6, IL-8, IFNγ, IL-4, IL-10, IL-12, IL-13, eotaxin, and IP-10) compared to the non-shedders, who only exhibited ↑ in IL-4 and IP-10.
Stowe et al., 1999	SM	N= 16 astronauts (14 male, 2 female)	3 Space shuttle missions (8, 9, and 14 days	Multiple time points: preflight (L-10, L-2), postflight (R+0, R+3)	Blood samples	Isolation and hemocytometer count.	1.5-fold ↑ in neutrophils inflight.
Voorhies et al., 2019	SM	N=9 astronauts (7 men, 2 women)	6 months at the ISS (N=8) 1 year at the ISS (N=1)	10 multiple time points pre-fight, inflight, post-flight	Blood samples (plasma)	Magnetic multiplex bead immunoassay	↑ Cytokine levels inflight: IL-1ra, MCP-1, IL-8, IL-1b and MIP-1β, TNFa, and IL-2. Cytokine concentrations reverted to pre-flight levels within 2 months of returning to Earth.
Yi et al., 2014	SS	N=6 healthy male volunteers	520-day ground-based space simulation MARS500	Multiple time points: day 360, 410, and 510	Blood samples	Luminex xMAP technology	↔ IFNγ, IL-2 and TNFα throughout the study. ↑ Lymphocyte number and percentages (~ 50%) of the total leukocyte vs pre-simulation(360th, 410th and 510th day) ↓ percentage, but not number, of neutrophils.

Abbreviations:
BDNF= Brain Derived Neurotrophic Factor.
BR= Bed rest.
CCL2= chemokine (C-C motif) ligand 2.
CCL4= chemokine (C-C motif) ligand 4.
CD40L= CD40 ligand.
CRP= C-reactive Protein.
CSF2= Colony stimulating factor 2.
DI= Dry immersion.
EGF= Epidermal Growth Factor.
EIA= Enzyme immunoassays.
ELISA= Enzyme-linked immunosorbent assay.
ENA78= Epithelial Neutrophil-Activating Protein 78.
FGF2= Fibroblast growth factor-2.
GM-CSF= Granulocyte macrophage colony-stimulating factor.
GROα= Chemokine growth-regulated protein alpha.
HD BR= Head-down bed rest.
HDT BR= Head-down tilt bed rest.
HGF= Hepatocyte growth factor.
ICAM1= Intercellular adhesion molecule 1.
IFNα= Interferon Alpha.
IFNγ= Interferon-gamma.
IFNA2= Interferon Alpha 2.
IFNB1= Interferon Beta 1.
IL= Interleukins.
IL-1ra= Interleukin 1 receptor antagonist.
IP-10= Interferon-gamma-induced protein 10.
ISS= International Space Station.
L- = Days before launch.
LEP= Leptin.
LIF= Leukemia Inhibitory Factor.
LTA= Lymphotoxin-alpha.
MCP-1= Monocyte chemoattractant protein-1.
MIP-1β= Macrophage inflammatory protein-1 beta.
NASA= National Aeronautics and Space Administration.
NF-kβ= Nuclear factor-kappa B.
NGF= Nerve growth factor.
PDGF-BB= Platelet-derived growth factor-BB.
post-d BR= Following days of bed rest.
pre-BR= Before bed rest.
qPCR= Quantitative real-time polymerase chain reaction.
qRT-PCR= Quantitative real-time polymerase chain reaction.
R+ = days after return.

RETN= Resistin.
RNA-seq= RNA sequencing.
RT-PCR= Real time polymerase chain reaction.
SM= Space mission.
SS= Space Simulation.
TGFA= Transforming growth factor alpha.
TGF-β1= Transforming growth factor beta 1.
TGF-β2= Transforming growth factor beta 2.
TNF= Tumor necrosis factor.
TNFα= Tumor necrosis factor alpha.
TNFSF10= Tumor necrosis factor ligand superfamily member 10.
Tpo= Thrombopoietin.
VCAM1= Vascular cell adhesion molecule 1.
VEGF= Vascular endothelial growth factor.
VEGFA= Vascular endothelial growth factor A.
VEGFD= Vascular Endothelial Growth Factor D.
WBC= White blood cells count.

(Garrett-Bakelman et al., 2019; Voorhies et al., 2019). Seven studies are ground-based, which include five studies on BR (Brooks et al., 2014; Capri et al., 2019; Fernandez-Gonzalo et al., 2020; Linossier et al., 2017), one study on dry-immersion (Linossier et al., 2017), and one study on ICC analog (Yi et al., 2014). All studies are longitudinal and conducted on a restricted set of subjects (from n=2 to n=59). All samples, including blood samples (Biolo et al., 2008; Brooks et al., 2014; Capri et al., 2019; Crucian et al., 2015, 2013, 2014; da Silveira et al., 2020; Garrett-Bakelman et al., 2019; Heer et al., 2014; Hughson et al., 2016; Kaur et al., 2005; Kelsen et al., 2012; Krieger et al., 2021; Linossier et al., 2017; Meehan et al., 1992; Mehta et al., 2013; Stowe et al., 1999; Voorhies et al., 2019; Yi et al., 2014), soleus and vastus lateralis muscle samples (Capri et al., 2019; Fernandez-Gonzalo et al., 2020), and saliva samples (Krieger et al., 2021) were collected before, after and at multiple time points spanning the missions and ground-based simulations. Biomarkers of inflammaging were analysed using different technique, including molecular (i.e., qPCR, microarray, RNA-seq (Capri et al., 2019; Fernandez-Gonzalo et al., 2020; Garrett-Bakelman et al., 2019), as well as ELISA, EIA and Automated clinical chemistry analyzer, quantiferon (Brooks et al., 2014; Capri et al., 2019; Kelsen et al., 2012; Linossier et al., 2017) and multiplex bead immunoassay using Luminex instrument (Voorhies et al., 2019; Yi et al., 2014) and haemocytometer for white blood cells (WBC) count (Crucian et al., 2013; Kaur et al., 2005; Meehan et al., 1992; Stowe et al., 1999).

In space mission studies, a pro-inflammatory framework, during and after, short- and long-term missions was detected (Capri et al., 2019; Garrett-Bakelman et al., 2019; Voorhies et al., 2019). In particular, in the "twin study", the astronaut twin in space, presented an increase in cytokines levels inflight and post-flight, as well as, an increase in lysophospholipids, containing the proinflammatory omega-6, and a decrease in lysophospholipids, containing the anti-inflammatory omega-3 (Garrett-Bakelman et al., 2019). Increased levels of several blood cytokines (e.g., Interleukin –IL-1α, IL-1β, and Interleukin 1 receptor antagonist –IL-1ra, Monocyte chemoattractant protein-1 –MCP-1, IL-8, Macrophage inflammatory protein-1 beta –MIP-1β, Tumor necrosis factors –TNFa, IL-2, IL-6, IL-12, IL-4, IL-10, and IL-17, IL-3, IL-7, IL-15, Transforming growth factor beta –TGFβ1, TGFβ2) were also detected during spaceflight in a larger groups of astronauts, that again, reached the baseline levels upon returning to Earth (Crucian et al., 2013; da Silveira et al., 2020; Hughson et al., 2016; Krieger et al., 2021; Voorhies et al., 2019). By contrast, salivary levels of several cytokines decreased inflight and, after landing, returned to pre-flight levels (Krieger et al., 2021). Furthermore, Crucian et colleagues (Crucian et al., 2014), analysing 22 different plasma cytokines in 28 astronauts, reported an increase in the plasma concentration of TNFα, IL-8, IL-1ra, thrombopoietin (Tpo), vascular endothelial growth factor (VEGF), C-C motif chemokine ligand 2 (CCL2), chemokine ligand 4/macrophage inhibitory protein 1b (CCL4), and C-X-C motif chemokine 5/epithelial neutrophil-activating protein 78 (CXCL5). A decrease in mitogen-stimulated production of cytokines inflight was also described (Crucian et al., 2013). Metha and co-workers (Mehta et al., 2013) reported elevated levels of blood cytokines inflight associated with reactivation of latent herpes virus. Relevant inter-individual differences in the pro-inflammatory setting were observed between 2 crewmembers after six-months of ISS mission, in terms of inflamma-miRs (c-miRs-21–5p, −126 to 3p, and −146a-5p), muscle specific (myo)-miR-206, c-proteasome and IL-6/leptin (Capri et al., 2019). The increased inflamma-miR may be due to an augmented exocytosis due to an increased cell injury (Fleshner and Crane, 2017). No alterations were instead observed during or following spaceflight for the inflammatory or adaptive/T-regulatory cytokines (IL-1α, IL-1β, IL-2, Interferon-gamma −IFN-γ, IL-17, IL-4, IL-5, IL-10, Granulocyte macrophage colony-stimulating factor −GM-CSF, Fibroblast growth factor −FGF, CCL3, or CCL5); as well as for C reactive protein (CRP) (Hughson et al., 2016).

Furthermore, circulating leukocytes alterations with increased neutrophils were described in astronauts after short-term shuttle missions (Stowe et al., 1999). This cell redistribution is similar to that produced by stress hormones, highligthing the role of physical and mental stress during spaceflight (Stowe et al., 1999). This result is in line with other studies that described an increase in leukocytes immediately post-flight, in particular with a rise in monocytes (Kaur et al., 2005; Meehan et al., 1992) and granulocytes (Crucian et al., 2013; Meehan et al., 1992), which was also confirmed in astronauts during long-term ISS mission (Crucian et al., 2015).

With regard to ground-based studies differences were observed between local and systemic inflammation. Localized vastus lateralis NF-kB expression was upregulated after 84 days of BR (Fernandez-Gonzalo et al., 2020). By contrast, no significant changes were detected on systemic inflammation cytokines after 3 days of dry immersion (Linossier et al., 2017) and 28 days BR (Brooks et al., 2014). Divergent results were reported for CRP, with decreased levels after 28 days BR (Brooks et al., 2014) and increased levels after 5 weeks and more of BR (Biolo et al., 2008; Heer et al., 2014), in particular in subjects with higher energy balance (Biolo et al., 2008). A weakening of cell-mediated immunity, with a decrease in the phytohemagglutinin-stimulated production of IL-2, IFN-γ and TNF-α, was observed after 21 days of hat head-down BR, however, immunological changes were less apparent in the second period of hat head-down BR, indicating some degree of adaptation to the challenges for the healthy volunteers (Kelsen et al., 2012). Although no changes in level of IFN-γ, IL-2 and TNF-α were detected throughout the 520-day ICC analog MARS500, Yi and colleagues (Yi et al., 2014) observed changes in immune function with a rise in lymphocyte number and percentage, up to the 50% of the total leukocyte baseline pre-simulation level, and, simultaneously, a reduction in the percentage, but not in number, of neutrophils. The heightened immune response, mainly due to the increase of lymphocyte numbers during the isolation period, suggests that prolonged exposure to stressors are able to trigger leukocyte phenotype alterations and poorly controlled immune

responses (Yi et al., 2014).

Overall, these data indicate that spaceflight mission elicits a pro-inflammatory context, acting through both circulating concentrations of inflammatory cytokines and immune alterations, confirming what Capri et al., (Capri et al., 2023) reported. In contrast, BR and dry-immersion elicit local inflammation in the skeletal muscle and do not appear to have effect at systemic level. The immune function is instead affected by prolonged stressors, in particular isolation, as demonstrated in ICC analogs. This suggests that factors other than the microgravity, can actively affect the inflammation during spaceflight. However, even for this hallmark of ageing, further studies are needed to fully understand the connection between chronic inflammation and spaceflight, as well as its interconnection with others ageing hallmarks. As already mentioned, the beneficial effect of meditation practices (Pavanello et al., 2019), by evoking RR (Lazar et al., 2000), could reduce the levels of stress hormones, inflammation and oxidative stress (Black and Slavich, 2016; Kaliman et al., 2014; Paul-Labrador et al., 2006), and could be used during the flight and after landing to alleviate chronic inflammation. On the other hand, the introduction of meals rich in bioactive compounds (polyphenols, vitamins and mineral salts) and nutraceuticals supplements with high anti-inflammatory properties, could be another excellent countermeasure.

4.13. Work on the opposite data that spaceflight slows ageing

While most studies presented valuable insights into a pro-ageing effect of spaceflight, there are studies on the opposite data that spaceflight and terrestrial analogs slow ageing.

The only BR study on genomic instability, which simulates microgravity in absence of radiation exposure, shows the activation of pathways that protect muscle cells from DNA damage caused by ROS (Chopard et al., 2009). This observation suggests an adaptive response of muscle to long-term atrophy, challenging the conventional understanding of atrophy.

Furthermore, contrary to the expected telomere shortening associated with ageing, spaceflight induces a specific telomere dynamics characterized by elongation during the mission and rapid shortening upon return to Earth (Garrett-Bakelman et al., 2019; Luxton et al., 2020a, 2020b; Luxton and Bailey, 2021). The elongation of TL observed inflight seems to indicate a slowing down of mitotic biological ageing as a form of specific adaptive response to the stressors of spaceflight, which, however, is immediately followed by an acceleration of mitotic biological ageing upon return to Earth. This unique pattern challenges the conventional understanding of telomere dynamics in ageing and warrants further investigation.

Another unique pattern that needs to be further investigated is related to the epigenetic alterations. A decrease in epigenetic ageing biomarkers, in particular DNAmPhenoAge, during and after a simulated interplanetary mission is reported by Nwanaji-Enwerem et al. (Nwanaji-Enwerem et al., 2020). It remains unclear why such a simulation of space travel would cause a reduction in epigenetic ageing, especially considering that microgravity and cosmic radiation were not replicated in the simulation. However, the authors suggest that the simulated aspects of space travel (e.g. confinement, diet, changes in circadian rhythms) may be the cause of the slowing of ageing over time, or alternatively, that high levels of pre-mission stress may be partly responsible for the apparent decrease after mission initiation.

Furthermore, a BR study (Fernandez-Gonzalo et al., 2020) mentioned in the deregulated nutrient sensing section, reported an upregulation of FoxO3 in vastus lateralis muscle samples after 84 days of BR. FoxO3 is a downstream intracellular effector of the IIS pathway, which plays a major role in slowing ageing. Similarly, another BR study (Chopard et al., 2009) reported a downregulation of CASP3, a protease enzyme that plays an essential role in cell death and cellular senescence, after 60 days of BR. Hao et al. (Hao et al., 2018) described that the use of an artificial ecosystem, such as a BLSS with a beneficial dietary structure, positively impacts the gut microbiota preventing dysbiosis, a typical sign of biological ageing.

4.14. Limitations

While our investigation provides valuable insights, it's crucial to recognise certain constraints that may influence the interpretation and generalizability of findings. Firstly, the complexity of the space environment introduces confounding variables —cosmic radiation, gravitational forces, and psychological stressors during missions. These factors intricately intertwine, potentially influencing observed effects on biological ageing. Secondly, the predominantly small sample sizes in both space missions and ground-based analogs may compromise statistical power and generalizability. The limited number of participants poses challenges in drawing definitive conclusions. As we navigate through the review, the prospective nature of the studies brings inherent limitations. Although prospective studies are ideal to better control for all potential variables, they do not assess the influence of ageing over time. The reliance on easily accessible tissues like blood and saliva, especially during space missions, introduces a potential bias. A limited focus on muscle tissue during ground-based studies underscores the importance of studying less accessible tissues for a holistic understanding. The use of different ground-based analogs, from BR to dry immersion, introduces heterogeneity. Furthermore, the inclusion of ground-based analogs funded exclusively by space agencies may introduce selection bias, limiting the generalizability of findings. The emphasis on agency-funded research, while essential, prompts us to approach our conclusions with a discerning eye. This variability calls for cautious interpretation, recognizing the diverse stressors presented by each analog. Moreover, the specific conditions of space missions and analogs may not perfectly replicate the complexities of extended space travel. This cautions us against overgeneralization and emphasizes the need for context-aware interpretation. Certain aspects, such as the effect of spaceflight on macroautophagy, remain unexplored, unveiling gaps in our understanding of specific ageing mechanisms influenced by space conditions. In the midst of these challenges, the synthesized literature forms a crucial foundation for future research in understanding the intricacies of ageing in the space environment. Recognizing and addressing these limitations will enhance the robustness and applicability of future studies aimed at deciphering the complexities of biological ageing in space.

5. Conclusions and future perspectives

In this comprehensive review, we have synthesized the literature investigating the impact of spaceflight on human biological ageing. Studies reviewed encompass both space missions and ground-based analogs, such as BR, dry immersion, and ICC simulations (Cromwell et al., 2021). The data analyzed include studies on space flights (n=30) and ground research analogs (n=30), specifically focusing on BR (n=20) and dry immersion (n=1) for altered microgravity, and ICC analogs (n=9) that simulate the major human health risks in the space environment. It's worth noting that ground-based analogs were only included if funded by space agencies.

All the studies reviewed were longitudinal and mainly conducted on a limited number of subjects, with a maximum of 59 individuals in one study. During these investigations, samples were collected before, after, and at multiple time points spanning the missions and ground-based simulations. Blood and saliva were the most commonly collected tissues, as they are more easily accessible, particularly during space missions. Conversely, muscle samples were mainly examined during ground-based studies on BR and dry immersion. It is crucial to conduct studies that compare the hallmarks of biological ageing in readily available tissues with those that are more challenging to collect, such as muscle tissue. Establishing the degree of similarity between these tissues would allow the potential use of blood or saliva as proxies

for assessing the effects of space missions, even during long-term space voyages.

Among the hallmarks of biological ageing that have been investigated, genomic instability, chronic inflammation, and deregulated nutrient sensing have been the most extensively studied, with over 14 articles dedicated to their examination in space environments and analogs. Genomic instability, largely associated with spaceflight, is closely related to the consistent chronic exposure to IR during missions. Further research on BR could be helpful in disentangling the contribution of microgravity to this hallmark of ageing. In addition, spaceflight has been found to induce a pro-inflammatory state, as evidenced by elevated levels of circulating inflammatory cytokines and immune alterations, which are also observed during prolonged isolation in controlled simulations, i.e., ICC analogs. Conversely, BR studies revealed a more localized inflammatory response rather than a systemic one. Nutrient sensing pathways have mainly been investigated in ground-based studies, with increased systemic IGF-1 levels consistently found, while specific indications regarding systemic insulin levels remain inconclusive. A few studies have explored the intracellular signaling cascade involving the PIK3-AKT pathway. Further research is needed to differentiate DDR pathway changes due to radiation from those resulting from spaceflight conditions. Dedicated in-space studies are crucial to validate terrestrial findings on the SASP regulation and develop effective countermeasures. Noteworthy, studies on microbiome showed unsafely changes with an imbalance in favour of opportunistic species, during both space missions, irrespective of mission duration, and ICC analogs. Research on stem cell exhaustion, intercellular communication, epigenetic alterations, and telomere length in the context of spaceflight and ageing is relatively scarce. Nonetheless, the available data suggest specific dynamics, particularly regarding telomere length. Notably, telomere lengthening has been observed during spaceflight, even during short-term missions, followed by rapid telomere shortening upon return to Earth. The literature search did not yield any results on the effect of spaceflight on macroautophagy, which remains an unexplored field.

While the majority of studies suggest a pro-ageing effect of spaceflight, some intriguing findings propose the opposite. Unique patterns, such as protective mechanisms in muscle cells and unconventional telomere dynamics, challenge established beliefs. Surprising reductions in ageing biomarkers during simulated interplanetary missions and BR studies hint at the influence of space-related factors. These contrasting data highlight the complex interplay between space-related stressors and ageing, necessitating further exploration for insights into space exploration and terrestrial health. Overall, these studies, although not yet conclusive, provide a necessary and fundamental basis for guiding future research into the dynamics of ageing mechanisms in the space environment. Importantly, the countless interconnections among these twelve characteristics of ageing should be taken into account, and multidisciplinary studies should be undertaken to examine these aspects holistically, as demonstrated by the NASA Twin Study (Garrett-Bakelman et al., 2019).

Promising countermeasures against the hallmarks of biological ageing could encompass a spectrum of approaches. Within the space environment, strategies such as optimizing dietary patterns, integrating novel prebiotics, postbiotics, and symbiotics, along with tailored physical exercise regimens, have shown potential. Additionally, drawing from terrestrial practices for diverse purposes, techniques like meditation to induce RR could be harnessed. The inclusion of nutritionally rich meals containing bioactive compounds and the incorporation of nutraceutical supplements possessing potent anti-inflammatory and antioxidant properties may offer further avenues to combat ageing's effects. These multifaceted interventions, combining space-tested and Earth-derived methods, hold promise in addressing the intricate facets of biological ageing.

The space environment appears to act as an accelerator of the biological ageing process, making it imperative to explore the interconnectedness between the biological features of spaceflight and the hallmarks of ageing. Identifying the key ageing pathways affected by spaceflight, and consequently identifying potential targets to slow down biological ageing, is essential to ensure the well-being and health of astronauts during future missions. As space travel continues to expand, the understanding and management of age-related diseases on Earth will undoubtedly benefit from the invaluable insights gained through the study of spaceflight and ageing. By unraveling the enigmatic link between spaceflight and ageing, we can ensure humanity's safe voyage beyond the stars and simultaneously discover new strategies to combat age-related ailments on our home planet.

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Manuela Campisi: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. Luana Cannella: Methodology, Investigation, Writing – review & editing. Sofia Pavanello: Conceptualization, Writing – original draft, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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Data availability

Data will be made available on request.

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