Editorial

BioTechniques

Is space the final frontier for mitochondrial study?

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BioTechniques 76: 47–51 (February 2023) 10.2144/btn-2023-0071

First draft submitted: 14 August 2023; Accepted for publication: 30 November 2023; Published online: 12 December 2023

TWEETABLE ABSTRACT

This perspective considers several avenues for future research on mitochondrial dynamics, stress, and DNA in outer space.

KEYWORDS:

metabolism • mitochondria • mitochondrial structure • space travel

The effects of space travel on mitochondrial biology, including mitochondrial ultrastructure, oxidative stress, DNA and organelle contact sites, are becoming of greater interest as the possibility of commercial space travel becomes closer to reality. Studies of the effects of space travel on biology, including mitochondria, are not new: in fact, *in vitro* studies of such effects have been performed since the 1990s [1]. Some common space-linked diseases that currently constrain prolonged space travel, beyond those caused by isolation and neurological conditions, include muscle atrophy, bone demineralization, immune dysfunction, cardiovascular deconditioning, cancer, and vestibular and sensory imbalance, all of which are reminiscent of advanced aging [2,3]. Mitochondrial dysfunction is strongly associated with many of these outcomes, highlighting the need for a better understanding of the effects of space on mitochondrial biology. Here, we discuss our current understanding of mitochondria in space, the feasibility of long-term space travel, and potential experiments to further study mitochondrial functions, structure and adaptive mechanisms in space.

Space as a unique environment & known space-related changes

As previously reviewed in National Aeronautics and Space Administration (NASA) Briefings, the principal stressors of and barriers to long-term spaceflight are exposure to microgravity and radiation [4]. Furthermore, research has found an increasing number of environmental pressures and age-related diseases that are strongly associated with mitochondrial dysfunction, including ultrastructural defects, damage caused by reactive oxygen species (ROS), mitochondrial DNA (mtDNA) mutations, and changes in inter-organelle contact sites [5–7]. Together, these findings demonstrate the necessity of studying space to gain a deeper understanding of these mitochondrial processes, both as they relate to space and as they more commonly result from aging or environmental stressors.

Microgravity

On Earth, gravity places constant pressure on the body. In microgravity, that gravitational pull is reduced, and bones weaken because they are no longer subjected to the same level of stress [3,8]. The same effects may concomitantly impact mitochondrial metabolism and function. Studies have indicated that mitochondrial function is altered in microgravity conditions, which may significantly impact gene regulation, innate immunity, and lipid metabolism [2]. Interestingly, microgravity is associated with lower metabolic demand and may therefore reduce the expression of proteins associated with oxidative phosphorylation [3]. In specific tissues, such as skeletal muscle, researchers have found that mitochondrial respiration decreases after extended periods of spaceflight, associated with muscle atrophy and weakness [3,9]. In this process, known as the unloading of skeletal muscle, a glycolytic phenotype of skeletal muscle is prioritized as muscle mass is lost [10], which parallels the changes seen in age-dependent sarcopenia on Earth [11]. One of the most comprehensive studies on mitochondria in space to date [2] indicated that space-related mitochondrial stress occurs through several pathways, including vascular remodeling although mitochondrial function is conserved, and causes metabolomic changes that are evolutionarily conserved across numerous organisms.

Radiation

Space is an irradiated environment with highly charged particles that can increase one's risk of developing deleterious mutations, oxidative stress, and other mitochondrial changes [2,12,13]. Although mitochondrial function mainly depends on their cytosolic environment, these external stimuli may still affect mitochondria, either directly or by altering the cytosol [4]. Some circumstances, such as exposure to ionizing radiation from cosmic rays, can directly impact mitochondrial metabolism [14]. Similarly, research has increasingly shown that extravehicular activity and ionizing radiation, among other factors associated with spaceflight, result in the generation of additional

ROS [4]. The mitochondrial outcomes of other space-related stressors, such as microgravity, are less clear; for example, microgravity can cause a sex-dependent increase in insulin resistance [15], which indicates a space-induced microenvironment alteration that may ultimately cause mitochondrial changes. However, mechanistic insights into these changes are currently lacking.

Cellular stress

Though the human body is not adapted to space environments [4], the cellular stress induced by space travel remains unclear. This cellular stress can result from factors such as psychological isolation, which can influence mitochondrial function [16], and environmental stressors, including extravehicular activity and pre-extravehicular activity breathing [4]. The best existing study on space travel–related cellular stress was the NASA Twins Study, which comprehensively showed changes in microbiota, immune pathways, DNA methylation, and telomere lengthening during spaceflight [17]. The study also noted an increased quantity of mitochondrial DNA (mtDNA), altered oxidative capacity and an associated increase in lactic acid buildup [17], and wide genomic changes in mitochondrial pathways during spaceflight. Past *in vitro* studies have indicated that both Schneider S-1 and Jurkat cells exhibit changes in mitochondrial organization in space, including microtubule-mediated clustering and disorganization of cristae (the folds of the inner mitochondrial membrane) [18], suggesting that space can affect mitochondrial structure. Moreover, spaceflight-dependent ROS generation can have various negative effects, including DNA damage and apoptosis [12,19]. Together, these findings indicate that these pluralistic cellular stresses may affect mitochondria through many different mechanisms. Importantly, NASA's Twins Study [17] allows for important inferences about adaptive mitochondrial mechanisms in response to prolonged periods of stress, which can aid in the development of future therapies and further our understanding of mitochondrial regulation and homeostasis.

Future directions

The following sections discuss some characteristics of mitochondria that may be considered in space and the questions that remain to be answered in future research.

ROS: An avenue for lipid metabolism

There is little conclusive information on the effects of oxidative stress during spaceflight and their potential interactions with lipid-fueled peroxidation. Oxidative damage appears to increase in space given the upregulation in ROS and other markers of oxidative stress seen in humans and other model organisms [2,3,20]. NASA's Twins Study further suggests that although mitochondria have pathways to resist oxidative stress during a prolonged time in space [17], prolonged space travel resulted in reduced antioxidant defenses, increased ROS, and modified mtDNA, which impaired mitochondrial function [2,17]. Other studies have shown that simulated microgravity conditions produce NADPH oxidase and ROS in Hodgkin's lymphoma cells, toward an end pathway of autophagy [21]. The relative contributions of various factors to this oxidative stress require further elucidation. However, we do know that ROS may be generated by extravehicular activity, pre-extravehicular activity breathing, ionizing radiation, increased air pressure and hyperoxic conditions, and altered iron loading [4].

Lipid peroxidation may be an important pathology-driving consequence of oxidative stress. Eye problems are exceedingly common in astronauts returning from Space Shuttle missions [12]. Eyes have high concentrations of lipids, and studies of C57BL/6 mice in space have illustrated that vision problems may develop due to lipid peroxidation, which occurs due to overexpression of certain genes, such as NADPH oxidase 1 [12]. The consequences of space-induced lipid peroxidation and antecedent oxidative stress in other lipid-rich regions of the body, such as the brain [22], require further study. We currently know that mitochondria make physical contact with lipid droplets to fulfill a variety of biochemical roles [23], including assisting in lipid droplet formation and degradation [24]. Still, it remains unclear whether lipids sequestered on these contact sites in space serve the same roles as on Earth, including as antioxidants.

These lipid droplet contact sites may offer valuable potential avenues for future therapeutic tools. However, ROS may be more readily targeted by activating antioxidant defenses, for example, through the delivery of therapeutics that mimic MnSOD to reduce oxidative stress [25] or MitoQ, which reduces oxidative stress specifically caused by space-originating iron ion ^{56}Fe radiation while increasing mitochondrial fusion proteins [26]. In the future, *in-vivo* studies should examine whether these therapeutics can prevent certain pathologies when delivered in space to help build a mechanistic understanding of whether oxidative stress and associated lipid peroxidation can be ameliorated in this environment.

Mitochondrial dynamics & biogenesis

Mitochondria undergo normal cycles of fusion and fission in addition to regular cycles of biogenesis (creation of new mitochondria), which may be altered in spaceflight. Mitochondrial membrane fusion is generally regulated by a group of dynamin-related proteins (DRP) in the presence of guanosine triphosphatase [27]. Inner membrane mitochondrial fusion is controlled by OPA1 with the help of the tethering GTPase Mgm1 and outer mitochondrial membrane fusion and fission are controlled by GTPase DRPs, which principally include DRP1, DNM1, Fzo1, FSI1 and two types of mitofusins (MFN1 and MFN2) [27–30]. Research on the impacts of space on mitochondrial dynamics proteins is ongoing. A study comparing *Drosophila* exposed to spaceflight showed increased DRP1 and OPA1, indicating increased dynamics without a shift toward fusion or fission [20], whereas another study showed that prolonged bed use (which simulates microgravity) resulted in reduced OPA1 and fusion proteins with a concomitant increase in DRP1, indicating pro-fission states [31]. These

findings indicate that microgravity alone generally increases fission, which may functionally result in more fragmented mitochondria and impaired energy production; however, spaceflight in totality increases dynamics in general, suggesting that mitochondria can quickly adjust their morphology and function to meet specific cellular needs. Nevertheless, these studies remain limited and do not offer adequate mechanistic insight into the modulation of dynamics. Because decreased OPA1 can cause age-related muscle atrophy [32], future studies must further consider how alterations in mitochondrial dynamics proteins impact the effects observed in long-term space travel.

Mitochondrial biogenesis is mainly regulated in a PGC-1α-dependent manner (reviewed in [33]). Prolonged bed rest (simulating microgravity) reduces PGC-1α levels, indicating impaired mitochondrial biogenesis [34,35]. By contrast, the NASA Twins Study reported increased PGC-1α [2] and biogenesis in microgravity, suggesting a mechanism independent of microgravity-induced changes. These elevated PGC-1α levels in spaceflight, despite microgravity, may be due to astronauts regularly engaging in exercise [36]. However, past studies in stimulated microgravity reported that PGC-1α levels were not affected by exercise, though other metabolic effectors were [34]. These conflicting findings indicate a need to optimize exercise regimes to support mitochondrial dynamics and biogenesis [37]. Dysregulation of mitochondrial fusion, fission, or biogenesis can lead to degenerative effects across the tissue system [33,38]; thus, a deeper understanding of how specific proteins associated with these pathways change with prolonged spaceflight is essential.

Mitochondrial structure

Although some studies have examined the ultrastructure of mitochondria in space or simulated space environments [8,39], they have primarily used two-dimensional imaging methods. The ultrastructural findings in Rhesus monkeys that underwent 14 days of spaceflight [39] and male rats in spaceflight for 12.5 days [1] generally showed that soleus subsarcolemmal mitochondria decreased in count but increased in cross-sectional area. However, the findings therein may be limited in interpretation as mitochondria exist in complex three-dimensional (3D) shapes that depend on their environment, tissue, and cell type [40–42]. Given the rise of 3D ultrastructural imaging techniques, some of which enable specific protein identification, it is worthwhile to revisit the previous findings with this new technology.

Some 3D structural phenotypes are more prevalent in response to disease states, such as mitochondrial donuts, which commonly form in the brains of individuals with Alzheimer's disease [43], and nanotunnels, which form in mitochondrial diseases [44]. It is unclear whether unique structural phenotypes also arise in spaceflight; for example, there may be a 3D mitochondrial phenotype in certain tissues or cell types that appears in space more commonly than on Earth and helps the mitochondria retain their function despite chronic environmental stress. Studying the effects of space travel on mitochondrial ultrastructure and morphology may provide essential insight for the development of novel therapies, highlighting the need for studies using 3D reconstruction [45,46]. However, as there are currently no 3D microscopes in space, future research must first develop new mechanisms to study mitochondria in low gravity, such as methods of collecting and fixing tissue in space, without hypoxia-induced artifacts [47], for analysis on Earth.

Mitochondria–endoplasmic reticulum contact sites

Beyond lipid droplets (as discussed in the ROS section), other mitochondrial contact sites may play a role in modulating the mitochondrial response to space. For instance, it may be helpful to examine mitochondria–endoplasmic reticulum contact sites (MERCs) in samples experiencing microgravity and the effects of space-induced ROS generation. MERCs play a crucial role in calcium transfer, lipid metabolism, and the coordination of various cellular processes by coordinating mitochondrial and endoplasmic reticulum (ER) processes [48,49]. The limited studies on mitochondria in space have shown that activation of the mitochondrial unfolded protein response, which is associated with ER stress, may confer muscle unloading in spaceflight [50]. MERCs may also play an adaptive role in ER stress [48] and undergo 3D structural rearrangement in response to ER stress [46]. Notably, NASA's Twins Study reported upregulation of ER stress proteins, including CHOP and ATF5 [17], which is hypothesized to implicate the integrated stress response pathway in space-dependent mitochondrial dysfunction [2] and may also alter MERCs. Furthermore, ER stress may alter calcium dynamics, but further studies are needed to understand whether changes in gravity affect mitochondrial calcium uptake and release and whether MERCs mediate this interaction [49].

mtDNA

Wide variability has been observed in extracellular mtDNA following space travel [51], indicating mtDNA degradation, which is known to accompany novel alterations in mitochondrial structure [44]. Past spaceflight studies have shown heterogeneity in the circulating mtDNA levels of individuals who underwent long-term spaceflight, with upticks immediately after return but varied levels in subsequent weeks [52]. Although the trend was not significant, it requires further investigation, especially because the study predominately considered mtDNA changes related to inflammatory responses [52]. Similarly, the NASA Twins Study showed that mtDNA activity increases in response to decreased mitochondrial function, suggesting a compensatory mechanism in which mtDNA is replicated alongside ROS generation [2]. Nevertheless, the long-term changes in mtDNA following space travel are still poorly elucidated; future studies might consider comparing mutation rates following the return to Earth.

Furthermore, mitochondria contain DNA to repair dysfunctional pathways and mechanisms to repair this very mtDNA, which form a comprehensive system to prevent the pathologies that large-scale mtDNA deletions may cause [53]. However, we have little information on the rate at which mtDNA deletions occur in space, how space may alter the pathways associated with mtDNA repair, and whether mtDNA repair pathways can sufficiently combat the level of damage from radiation encountered in space. Future studies should

strengthen our understanding of how mitochondria may adapt to extreme environments to support the future development of clinical interventions.

Exploring cellular-specific responses & new imaging techniques

Space's impact on mitochondria must be investigated beyond basic "dysfunctional" and "functional" binary categories to consider pluralistic quality control mechanisms of mitochondria [5]. Beyond the principal quality control mechanisms, several other quality control factors have yet to be explored comprehensively in the context of space, including dynamics and mtDNA which we have outlined here.

Past studies have indicated changes in overall gene expression following space travel, but there is a lack of research on tissue-dependent responses [54]. For example, a study on human-induced pluripotent stem cell–derived cardiomyocytes in microgravity environments found that mitochondria metabolism was altered following the return to normal gravity but failed to clarify whether this response occurred only in cardiomyocytes, which would suggest the possibility of cell-specific responses [54]. Furthermore, murine studies examining specific regions, including subsarcolemmal and intermyofibrillar skeletal muscle, have indicated that the distribution of oxidative metabolism enzymes and the functional capacity of mitochondria may respond to space travel in a region-specific manner [1]. This further highlights the need for *in-vivo* studies of whether evolutionarily conserved tissue-dependent mechanisms of mitochondria modulation in space differ from those on Earth. One avenue would be to explore the relationship between radiation-sensitive species and mitochondrial dysfunction. Some tissue types may be radiation-resistant or associated with specific mitochondrial adaptations [13].

Moreover, microgravity conditions may influence the activity of metabolite transport systems, affecting the import of essential molecules and disrupting mitochondrial function. Previous studies have mainly considered gross cell metabolomics; subcellular imaging mass spectrometry [31] and organelle-specific metabolomics [55] may provide additional insight into how specific mitochondrial metabolomics may be altered. Similarly, although few studies have examined how space travel affects specific cellular signaling pathways and protein localization, new technologies, such as the compact fluorescence microscope, allow for live-cell imaging in space [56]. Thus, future research must utilize these new technologies to provide greater mechanistic insight into space-induced mitochondrial alterations.

Conclusion

Studying mitochondria in space promises several important benefits. First, these studies can provide greater reassurance of humans' ability to endure prolonged space travel and, therefore, to colonize the wider space system. Second, a greater understanding of how spaceflight alters mitochondrial ultrastructure and specific activities may reveal novel mechanisms applicable to mitochondrial dysfunction on Earth. Current studies show pluralistic roles of mitochondrial functions in space-induced pathologies and cellular stress responses. These findings highlight the crucial and central role of mitochondria in the metabolic response to spaceflight. However, despite the many studies described here, our understanding of mitochondria in space remains limited.

In future, studies must be expanded to improve their rigor and significance in several key areas. First, these studies must define how changes in specific mitochondrial structures and organelle contacts depend on other factors within the cell. Though past studies have focused on *in-vivo* models, additional human or primate studies on mitochondrial structures and function are needed. Second, given that physiological conditions alter mitochondria [16], *in-vivo* studies should examine whether phenotypical changes arise due to physiological or physical changes and how the unique physiological conditions of space affect mitochondria. Third, although the NASA Twins Study is one of the strongest existing studies to describe the effects of long-term spaceflight on various tissue systems and metabolic processes [17], its limited sample size and the potential lifestyle differences of the participants (e.g., in exercise regimens) limits its mechanistic insight. Finally, novel therapies to mitigate mitochondrial damage during spaceflight must be further explored. For example, some therapies have targeted the gut microbiome composition [17], which is known to be affected during spaceflight. Notably, C57BL/6J mice in space supplemented with fructo-oligosaccharide, a non-digestible carbohydrate that interacts with the gut microbiome, have shown reduced oxidative stress, indicating a potential therapy [57]. As studies increasingly investigate space-induced alterations, they should also consider whether known mitochondrial therapies function effectively in space.

Future research on pathway activation following space travel may offer insight into mechanisms that modulate and protect mitochondrial activity. Given the relatively fast recovery of mitochondria following space travel, the body may activate a form of antioxidative protection. In addition, future studies can determine whether certain mitochondrial phenotypes are more energetically favorable for spaceflight than others and whether the environment can modulate these phenotypes. Space's impacts on mitochondrial efficiency—especially across types of fission and fusion—should also be further explored. By addressing these questions, future studies can contribute to our long-term survival on Earth and in space.

Author contributions

All authors contributed to the researching, writing, and editing for this manuscript. Kit Neikirk: literature review, writing - original draft preparation, writing – review and editing. HK Beasley: literature review, writing – review and editing. DC Stephens: literature review, writing – review and editing. AG Marshall: literature review, writing – review and editing. JA Gaddy: literature review, writing – review and editing. SM Damo: literature review, writing – review and editing. A Hinton: funding, conceptualization, supervision, writing – review and editing.

Financial disclosure

The UNCF/Bristol-Myers Squibb E.E. Just Faculty Fund, Career Award at the Scientific Interface (CASI Award) from Burroughs Welcome Fund (BWF) ID # 1021868.01, BWF Ad-hoc Award, NIH Small Research Pilot Subaward to 5R25HL106365-12 from the National Institutes of Health PRIDE Program, DK020593, Vanderbilt Diabetes and Research Training Center for DRTC, CZI Science Diversity Leadership grant number 2022- 253529 from the Chan Zuckerberg Initiative DAF, an advised fund of Silicon Valley Community Foundation (AHJ). NSF EES2112556, NSF EES1817282, NSF MCB1955975, and CZI Science Diversity Leadership grant number 2022-253614 from the Chan Zuckerberg Initiative DAF, an advised fund of Silicon Valley Community Foundation (to SM Damo). Additional support was provided by NIH R01HD090061 and I01 BX005352 from the Department of Veterans Affairs Office of Research (to JA Gaddy). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Competing interests disclosure

The authors have no competing interests or relevant affiliations with any organization or entity with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Writing disclosure

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Bell GJ, Martin TP, Ilyina-Kakueva EI, Oganov VS, Edgerton VR. Altered distribution of mitochondria in rat soleus muscle fibers after spaceflight. *J. Appl. Physiol.* 73(2), 493–497 (1992).
2. da Silveira WA, Fazelinia H, Rosenthal SB *et al.* Comprehensive multi-omics analysis reveals mitochondrial stress as a central biological hub for spaceflight impact. *Cell* 183(5), 1185–1201.e20 (2020).
3. Nguyen HP, Tran PH, Kim K-S, Yang S-G. The effects of real and simulated microgravity on cellular mitochondrial function. *MNPJ Microgravity* 7(1), 1–11 (2021).
4. Smith SM. *Human Adaptation to Spaceflight: The Role of Food and Nutrition*. National Aeronautics and Space Administration, Lyndon B. Johnson Space Center (2021).
5. Monzel AS, Enriquez JA, Picard M. Multifaceted mitochondria: moving mitochondrial science beyond function and dysfunction. *Nat Metab.* 5(4), 546–562 (2023).
6. Blajszczak C, Bonini MG. Mitochondria targeting by environmental stressors: implications for redox cellular signaling. *Toxicology* 391, 84–89 (2017).
7. Bratic A, Larsson N-G. The role of mitochondria in aging. *The Journal of clinical investigation* 123(3), 951–957 (2013).
8. Ji G, Chang H, Yang M *et al.* The mitochondrial proteomic changes of rat hippocampus induced by 28-day simulated microgravity. *PLOS ONE* 17(3), e0265108 (2022).
9. Qaisar R, Karim A, Elmoselhi AB. Muscle unloading: a comparison between spaceflight and ground-based models. *Acta Physiol.* 228(3), (2020).
10. Mazin MG, Kiseleva EV, Nemirovskaia TL, Shenkman BS. Ultrastructure of skeletal muscle fibers in monkeys after space flight. *Tsitologiia* 42(1), 96–103 (2000).

• Focuses on specifically how muscle fibers and ultrastructure alter in space flight, a relevant area which remains underexplored.

11. Coen PM, Musci RV, Hinkley JM, Miller BF. Mitochondria as a target for mitigating sarcopenia. *Front. Physiol.* 9, 1883 (2019).
12. Mao XW, Pecaut MJ, Stodieck LS *et al.* Spaceflight environment induces mitochondrial oxidative damage in ocular tissue. *Radiat. Res.* 180(4), 340–350 (2013).

• Key article that discusses the oxidative stress associated with space.

13. Averbeck D, Rodriguez-Lafrasse C. Role of mitochondria in radiation responses: epigenetic, metabolic, and signaling impacts. *Int. J. Mol. Sci.* 22(20), 11047 (2021).
14. Kim H-N, Richardson KK, Krager KJ *et al.* Simulated galactic cosmic rays modify mitochondrial metabolism in osteoclasts, increase osteoclastogenesis and cause trabecular bone loss in mice. *Int. J. Mol. Sci.* 22(21), 11711 (2021).
15. Kenny HC, Rudwill F, Breen L *et al.* Bed rest and resistive vibration exercise unveil novel links between skeletal muscle mitochondrial function and insulin resistance. *Diabetologia* 60(8), 1491–1501 (2017).
16. Picard M, McEwen BS. Psychological stress and mitochondria: a systematic review. *Psychosom. Med.* 80(2), 141–153 (2018).
17. Garrett-Bakelman FE, Darshi M, Green SJ *et al.* The NASA Twins Study: a multidimensional analysis of a year-long human spaceflight. *Science* 364(6436), eaau8650 (2019).

•• One of the most comprehensive articles to date, which details the pluralistic effects of space travel.

18. Schatten H, Lewis ML, Chakrabarti A. Spaceflight and clinorotation cause cytoskeleton and mitochondria changes and increases in apoptosis in cultured cells. *Acta Astronautica* 49(3), 399–418 (2001).

• Considers the specific *in vitro* changes to mitochondria that occur alongside space travel.

19. Singh R, Rajput M, Singh RP. Simulated microgravity triggers DNA damage and mitochondria-mediated apoptosis through ROS generation in human promyelocytic leukemic cells. *Mitochondrion* 61, 114–124 (2021).
20. Mhatre SD, Iyer J, Petereit J *et al.* Artificial gravity partially protects space-induced neurological deficits in *Drosophila* melanogaster. *Cell Reports* 40(10), (2022).
21. Jeong AJ, Kim YJ, Lim MH *et al.* Microgravity induces autophagy via mitochondrial dysfunction in human Hodgkin's lymphoma cells. *Sci. Rep.* 8(1), 14646 (2018).
22. O'Brien JS, Sampson EL. Lipid composition of the normal human brain: gray matter, white matter, and myelin*. *J. Lipid Res.* 6(4), 537–544 (1965).
23. Neikirk K, Vue Z, Katti P *et al.* Systematic transmission electron microscopy-based identification and 3D reconstruction of cellular degradation machinery. *Adv. Biol.* 7(6), 2200221 (2023).
24. Jarc E, Petan T. Lipid droplets and the management of cellular stress. *Yale J. Biol. Med.* 92(3), 435–452 (2019).
25. Miriyala S, Spasojevic I, Tovmasyan A *et al.* Manganese superoxide dismutase, MnSOD and its mimics. *Biochim. Biophys. Acta* 1822(5), 794 (2012).
26. Gan L, Wang Z, Si J *et al.* Protective effect of mitochondrial-targeted antioxidant MitoQ against iron ion 56Fe radiation induced brain injury in mice. *Toxicol. Appl. Pharmacol.* 341, 1–7 (2018).
27. Abel ED. Mitochondrial dynamics and metabolic regulation in cardiac and skeletal muscle (2019). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6116613/
28. Meeusen S, DeVay R, Block J *et al.* Mitochondrial inner-membrane fusion and crista maintenance requires the dynamin-related GTPase Mgm1. *Cell* 127(2), 383–395 (2006).
29. Darshi M, Mendiola VL, Mackey MR *et al.* ChChd3, an inner mitochondrial membrane protein, is essential for maintaining crista integrity and mitochondrial function. *J. Biol. Chem.* 286(4), 2918–2932 (2011).

30. Escobar-Henriques M, Anton F. Mechanistic perspective of mitochondrial fusion: tubulation vs. fragmentation. *Biochimica et Biophysica Acta – Mol. Cell Res.* 1833(1), 162–175 (2013).
31. Murgia M, Ciciliot S, Nagaraj N *et al.* Signatures of muscle disuse in spaceflight and bed rest revealed by single muscle fiber proteomics. *PNAS Nexus* 1(3), pgac086 (2022).
32. Tezze C, Romanello V, Desbats MA *et al.* Age-associated loss of OPA1 in muscle impacts muscle mass, metabolic homeostasis, systemic inflammation, and epithelial senescence. *Cell Metab* 25(6), 1374–1389.e6 (2017).
33. Jornayvaz FR, Shulman GI. Regulation of mitochondrial biogenesis. *Essays in Biochemistry* 47, 69–84 (2010).
34. Irimia JM, Guerrero M, Rodriguez-Miguelez P *et al.* Metabolic adaptations in skeletal muscle after 84 days of bed rest with and without concurrent flywheel resistance exercise. *J. Appl. Physiol.* 122(1), 96–103 (2017).
35. Rosa-Caldwell ME, Brown JL, Perry RA *et al.* Regulation of mitochondrial quality following repeated bouts of hindlimb unloading. *Appl. Physiol. Nutr. Metab.* 45(3), 264–274 (2020).
36. Lira VA, Benton CR, Yan Z, Bonen A. PGC-1α regulation by exercise training and its influences on muscle function and insulin sensitivity. *Am. J. Physiol. Endocrinol. Metab.* 299(2), E145–E161 (2010).
37. Gao T, Huang J, Zhang X, Gao F. Exercise counteracts vascular aging in long-term spaceflight: challenges and perspective. *Curr. Opin. Physiol.* 31, 100628 (2023).
38. Chan DC. Fusion and fission: interlinked processes critical for mitochondrial health. *Annu. Rev. Genet.* 46, 265–287 (2012).
39. Shenkman BS, Nemirovskaya TL, Belozerova IN, Mazin MG, Matveeva OA. Mitochondrial adaptations in skeletal muscle cells in mammals exposed to gravitational unloading. 23, 35–38 (2002).
40. Vue Z, Neikirk K, Vang L *et al.* Three-dimensional mitochondria reconstructions of murine cardiac muscle changes in size across aging. *Am. J. Physiol. – Heart Circulat. Physiol.* (2023).
41. Vue Z, Garza-Lopez E, Neikirk K *et al.* Mouse skeletal muscle decrease in the MICOS complex and altered mitochondrial networks with age. *bioRxiv* 2022–03 (2022).
42. Glancy B, Kim Y, Katti P, Willingham TB. The functional impact of mitochondrial structure across subcellular scales. *Front. Physiol.* 11 (2020).
43. Long Q, Zhao D, Fan W *et al.* Modeling of mitochondrial donut formation. *Biophys. J.* 109, 892–899 (2015).
44. Vincent AE, White K, Davey T *et al.* Quantitative 3D mapping of the human skeletal muscle mitochondrial network. *Cell Reports* 26(4), 996–1009.e4 (2019).
45. Glancy B. Visualizing mitochondrial form and function within the cell. *Trend. Mol. Med.* 26(1), 58–70 (2020).
46. Garza-Lopez E, Vue Z, Katti P *et al.* Protocols for generating surfaces and measuring 3D organelle morphology using amira. *Cells* 11(1), 65 (2022).
47. Hinton A, Katti P, Christensen TA *et al.* A comprehensive approach to sample preparation for electron microscopy and the assessment of mitochondrial morphology in tissue and cultured cells. *Adv. Biol. (Weinh.)* e2200202 (2023).
48. Resende R, Fernandes T, Pereira AC, Marques AP, Pereira CF. Endoplasmic reticulum-mitochondria contacts modulate reactive oxygen species-mediated signaling and oxidative stress in brain disorders: the key role of sigma-1 receptor. *Antioxid. Redox Signal.* 37(10–12), 758–780 (2022).
49. Bustos G, Cruz P, Lovy A, Cárdenas C. Endoplasmic reticulum–mitochondria calcium communication and the regulation of mitochondrial metabolism in cancer: a novel potential target. *Front. Oncol.* 7, 199 (2017).
50. Liu P, Li D, Li W, Wang D. Mitochondrial unfolded protein response to microgravity stress in nematode *Caenorhabditis elegans*. *Sci. Rep.* 9(1), 16474 (2019).
51. Bisserier M, Shanmughapriya S, Rai AK *et al.* Cell-free mitochondrial DNA as a potential biomarker for astronauts' health. *J. Am. Heart Assoc.* 10(21), e022055 (2021).
• Notable study that links mtDNA changes as a potential marker of space flight with significant changes following aforementioned flight.
52. Capri M, Morsiani C, Santoro A *et al.* Recovery from 6-month spaceflight at the International Space Station: muscle-related stress into a proinflammatory setting. *FASEB J.* 33(4), 5168–5180 (2019).
53. Fontana GA, Gahlon HL. Mechanisms of replication and repair in mitochondrial DNA deletion formation. *Nucleic Acids Res.* 48(20), 11244–11258 (2020).
54. Wnorowski A, Sharma A, Chen H *et al.* Effects of spaceflight on human induced pluripotent stem cell-derived cardiomyocyte structure and function. *Stem Cell Rep.* 13(6), 960–969 (2019).
55. Hogan KA, Zeidler JD, Beasley HK *et al.* Using mass spectrometry imaging to visualize age-related subcellular disruption. *Front. Mol. Biosci.* 10 (2023).
56. Corydon TJ, Kopp S, Wehland M *et al.* Alterations of the cytoskeleton in human cells in space proved by life-cell imaging. *Sci. Rep.* 6(1), 20043 (2016).
57. Ohira T, Ino Y, Kimura Y *et al.* Effects of microgravity exposure and fructo-oligosaccharide ingestion on the proteome of soleus and extensor digitorum longus muscles in developing mice. *NPJ Microgravity* 7(1), 1–11 (2021).