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Space physiology: To Mars and beyond

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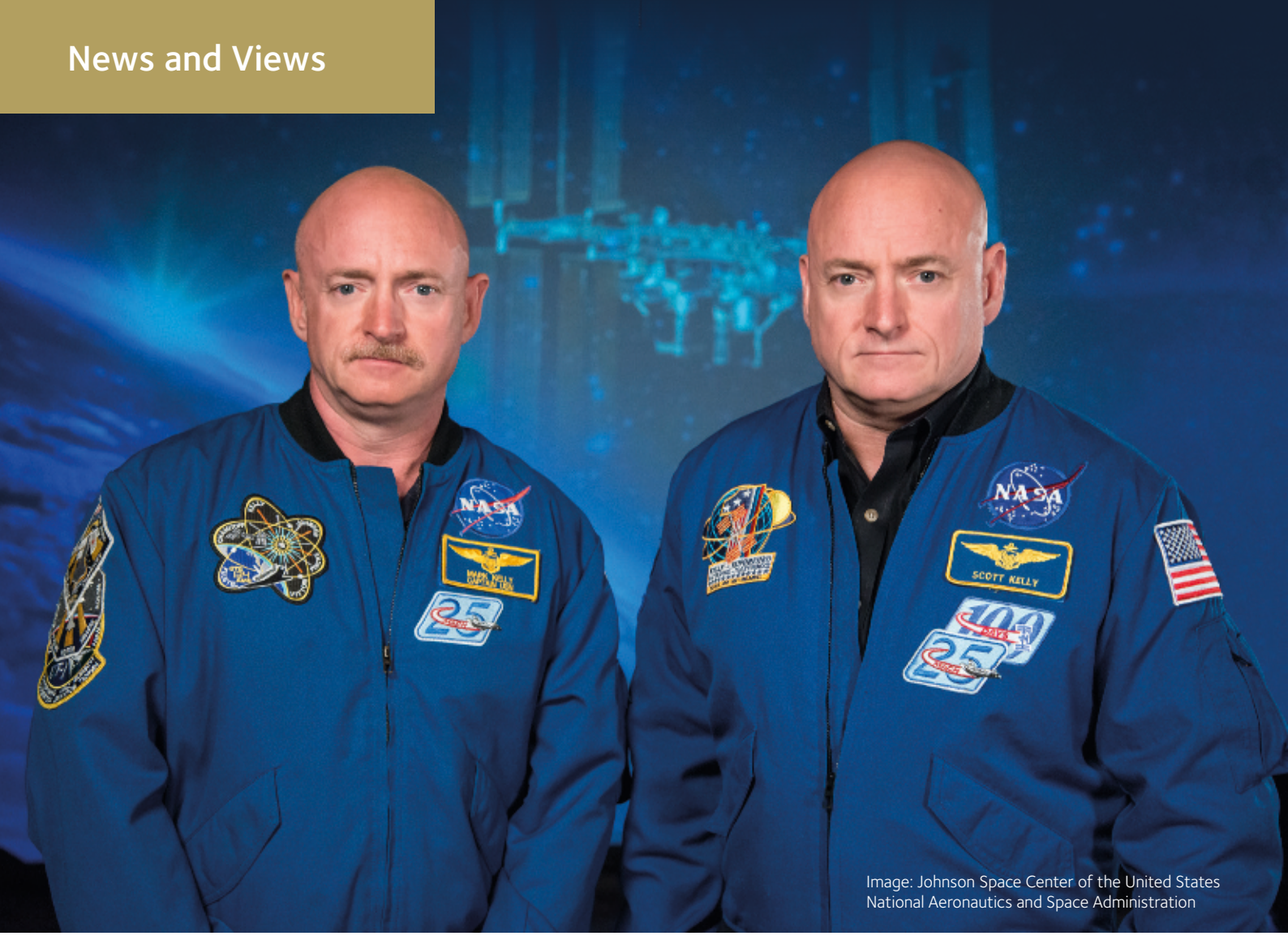

Image: Johnson Space Center of the United States National Aeronautics and Space Administration

The NASA Twins studies

The "NASA Twins" Mark (left) and Scott (right) Kelly (b. 1964). Mark served as an astronaut at NASA from 1996 to 2011 and logged a total of 54 days in orbit. Scott served as an astronaut at NASA from 1996 to 2016, and over the course of four missions, he spent a total of 520 days in orbit. The NASA studies were a unique opportunity to study the effects of long-term spaceflight on the human body, by comparing Scott who was on board the ISS for a year, to Mark who remained on Earth during that time.

The microbiome in space, from the Apollo missions to present

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Fifty years ago, when the Apollo 11 astronauts Neil Armstrong, Buzz Aldrin, and Michael Collins returned to Earth, they were quarantined for 21 days, including some days in a converted Airstream trailer where they celebrated Neil Armstrong's 39th birthday. This was a precautionary measure against the possibility of contagious potential pathogens ("moon germs"), a risk considered as unlikely but uncertain at the time. Following Apollo 14, this quarantine is not required, nor is testing astronauts' blood by injecting it into mice. Over the past 50 years, research approaches towards astronaut health, the physiological effects of space, and microorganisms have dramatically shifted, reflective of the explosive advancements in biomedical research during this time. These are clearly illustrated by the NASA Twins Study, and studies of the gut microbiome in space, in which we have had the privilege to be involved.

Nearly 50 years after the Apollo 11 mission, the scientific investigation of another landmark human space expedition, astronaut Scott Kelly's 342 days on board the International Space Station (ISS), culminated in a publication earlier this year in the journal *Science*[1]. This year-long mission marked the longest human spaceflight of a US astronaut, and was even more unique because Scott Kelly's identical twin brother, retired astronaut Mark Kelly, agreed to participate in the study. This provided an unprecedented opportunity to study the effects of long-term spaceflight on the human body. NASA assembled a consortium of 10 investigator teams to study the twins

in preparation for future long-term missions such as those around the Moon, asteroids and ultimately Mars.

The Twins Study exemplifies how scientific approaches have evolved over the years. Rather than ten discrete investigations, the study was designed to develop a cross-disciplinary picture of how various systems, from cognition to physiological and molecular processes, may respond, in concert with one another, to the challenge of spaceflight. The result is a rich and intriguing data set. The study was further strengthened by the ability to obtain comparable data from the "ground twin" so that an assessment of expected variability over time in a genetically matched individual with a busy, varied life on Earth could be made. In this manner, variance outside that range seen in the "space twin" could be more confidently attributed to space flight.

Spaceflight-induced microbiome changes seen in the Twins Study were modest, and quickly diminished after the astronaut returned to Earth. Nonetheless, these changes were beyond the day-to-day fluctuations in the gut microbiome composition in the ground twin during the same period of time. Many of our colleagues' other measures exhibited parallel spaceflight response profiles; future studies can test hypothesised links among these mirrored responses. Neither the integrated, multi-system approach nor the computational and molecular analyses were methods available 50 years ago.

"The Twins Study exemplifies how scientific approaches have evolved over the years"

Inclusion of the gut microbiome as a topic for this kind of integrated evaluation of the adaptation to spaceflight also would not have been imagined 50 years ago, when bacteria were primarily viewed with suspicion of pathogenicity. Now, modern high-throughput sequencing approaches reveal a dynamic, diverse and complex "ecosystem" of microorganisms inhabiting the gastrointestinal tract and interacting with mammalian physiology, that change in response to spaceflight and might in fact have the potential to help astronauts to adapt to spaceflight.

From a high-level view, some alterations in the gut microbiome in response to spaceflight are consistent across studies: an increase in the microbial diversity and a shifted microbial community structure have been identified in the NASA Twins Study[1] as well as another, subsequent astronaut study[2], and even in mice that have flown on the ISS[3]. Development of a new analytic strategy, a tool called STARMAPs, revealed that the consistencies of the effects of spaceflight on the gut microbiome go far deeper: the overall patterns of the microbial composition changes are reproducible when comparing mice flown on the space shuttle to those flown on the ISS[3]. Now, studies to integrate these spaceflight-specific effects with other, related systems can lead to an understanding of the role of the microbiome in adaptation to spaceflight. Such "small steps" of scientific progress, continuing into the next few decades, can advance health in space as well as on Earth, so that we are ready for future giant leaps for mankind.

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Telomeres and genomic instability during long-duration spaceflight

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The first astronauts for the American (1960) and Russian (1959) space programs demonstrated that spaceflight was physiologically possible for the human body, while also inaugurating a new era of human exploration of space. This led to current-day plans for missions to the Moon and Mars, which include new commercial and government agencies (e.g. China, India, Israel), and will involve months to years of exposure to spaceflight conditions. Yet, even as we commemorate 50 years since the first landing on the Moon, very little biomedical data exist on the health effects of long-duration spaceflight, since very few missions (n=8) have lasted for greater than 300 days. Indeed, only four individuals have ever participated in spaceflight missions lasting longer than one year. Thus, while the effects of short-duration spaceflight on the human body have been well documented, there is a paucity of data on the effects of long-duration spaceflight, and almost no data using modern methods in molecular biology. These fundamental limitations in our knowledge, concomitant with the emergence of several new, active spaceflight agencies, helped to inspire the NASA Twins Study: a multi-dimensional characterisation of the effects of a year-long spaceflight on the body[1].

The NASA Twins Study is clearly limited in the sample size (n=1 per group), and thus the experiments were designed to create benchmarks that can be used in later missions to optimise crew health, improve in-flight collections, and to compare measures of acute or long-term flight risks. Data were generated across a myriad of modalities of human and microbial biology, including stool, saliva, skin, urine, and blood samples. The Study used a wide battery of measures, which included various whole-genome, RNA, and metagenome sequencing techniques, as well as proteomics and metabolomics, and numerous other techniques.

Blood analyses elucidated several physiological changes that long-term spaceflight has on the genome, including the impact on telomeres. Telomeres are repetitive nucleotide sequences found at the physical ends of eukaryotic chromosomes, which are critical for maintaining genome stability. During each cycle of cell division, telomeres

shorten due to the end-replication problem, which results in the inability to replicate to the very end of newly synthesised lagging strands. As such, telomeres serve as "buffer zones", preserving the genes that lie medial to them. However, because telomeres shorten with ageing, as well as with a variety of lifestyle factors and stresses, they eventually become so short that cells enter a state of senescence and stop dividing. This process is also associated with ageing-related pathologies such as cardiovascular disease and cancer. Interestingly, and contrary to expectation, the NASA Twins Study found that long-duration spaceflight resulted in a significant increase (~15%) in telomere length in the "space twin" as compared with his pre-flight and post-flight telomere lengths, as well as those of the "ground twin". Given that the space twin's telomeres dramatically shortened within 48 hours of returning to Earth, it is postulated that spaceflight-specific telomere elongation may have occurred in response to galactic cosmic radiation exposure; results are consistent with one other ISS study in *C. elegans*[2]. It is also worth noting, however, that while the space twin's average telomere length stabilised to approximately his pre-flight levels, cell-by-cell FISH analysis revealed an increased number of short telomeres post-flight, potentially suggestive of ongoing damage, instability, accelerated ageing and/or future adverse health effects.

Indeed, and as expected with any significant physiological stress, thousands of genes changed their expression levels during spaceflight, including pathways related to telomere maintenance, immune (T-cell) activation, and DNA repair. Moreover, of the genes that changed expression in spaceflight, ~91% returned to normal ranges within 6 months post-flight. While the overwhelming majority of transcriptional changes returned to pre-flight levels, a distinct subset of 811 genes involved in either immunity or DNA damage remained altered post-flight, which has provided insight into candidate genes that are more susceptible to extended spaceflight and which may be driving the continued re-adaptation to gravity.

DNA repair pathways and re-acclimation mechanisms are likely confounded as part of the normal response of returning to gravity, but evidence of DNA damage was observed. In the analysis of genomic instability, the space twin demonstrated increased frequencies of chromosome aberrations, particularly inversions (i.e. rearrangements within chromosomes), during spaceflight: a finding consistent with exposure to ionising radiation, particularly high linear energy transfer (LET) cosmic radiation[3,4]. Moreover, the space twin's chromosomal inversion frequencies remained elevated post-flight, suggesting continued instability and possibly radiation-induced DNA damage to multipotent haematopoietic stem cells of the bone marrow, which could have a long-term impact on the genetic health of both myeloid and lymphoid fractions. Thus, even longer spaceflight missions should involve an expanded focus on the haematopoietic systems.

"It should be possible to survive the transit to Mars and then return back to Earth"

DNA methylation has also become a widely used proxy for assessing the ageing process, with methylation "clocks" being used successfully to predict age and mortality in several different species. The addition of a methyl group to a DNA strand typically results in local cessation of transcription. Thus, methylation in a gene's promoter region is related to reduced expression of that gene, and is a common epigenetic marker of gene expression regulation. While genome-wide methylation changes in the space twin were within the range of variation of those of the ground twin, gene ontology enrichment analysis revealed enrichment of epigenetic discordance in several genes indicative of immune stress. Thus, as with previous studies demonstrating changes in the immune system of astronauts, this is an area for continued surveillance and focus on future long-duration missions.

While a full discussion of the results presented in the Twins Study is beyond the scope of this article, it is worth noting that the Study evaluated the effects of long-duration spaceflight on several other physiological areas as well. For example, in its assessment of the immunome, the Twins Study revealed that inoculation with an annual flu vaccine in space, as well as subsequent inoculation on Earth the following year, were both successful in initiating an appropriate T cell-mediated response in the space twin, thereby suggesting that the immune system's defenses are not functionally impaired by microgravity. A cognitive assessment revealed no dramatic changes in the space twin's higher cortical functions during his time in space with respect to the ground twin; upon landing, however, the space twin exhibited a pronounced decrease in speed and accuracy while performing cognitive tasks which persisted for six months upon re-acclimation. Additional analyses demonstrated that the space twin's spaceflight resulted in a 7% decrease in body mass, a dynamic osteocyte turnover rate which first increased and then stabilised during the latter six months of flight, increased inflight folate and urinary lactic acid levels, increased inflight mitochondrial DNA and ATP-dependent respiration, and increased carotid artery intima-media thickening that persisted into the post-flight period, among other findings. Additionally, the NASA Twins Study determined that cephalad fluid shifts observed in the space twin corresponded to retinal oedema, as well as elevations in urinary aquaporin-2 (AQP2): a protein involved in regulation of water resorption that might be implicated in the pathogenesis of ophthalmologic disorders observed during and after spaceflight.

In summary, the Twins Study demonstrated that the human body is extraordinarily adaptive to the changes incurred during a one-year mission, and that it should be possible to survive the transit to Mars and then return back to Earth. However, the long-term health effects of long-duration spaceflight are exceedingly difficult to assess, and more work must be done to examine whether physiological effects, such as post-flight telomere shortening, stem cell alterations, and/or genomic instability have detectable long-term adverse health effects on individuals exposed to the space environment for prolonged periods of time.

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