A Longitudinal Epigenetic Aging and Leukocyte Analysis of Simulated Space Travel: The Mars-500 Mission

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SUMMARY

Astronauts undertaking long-duration space missions may be vulnerable to unique stressors that can impact human aging. Nevertheless, few studies have examined the relationship of mission duration with DNA-methylation-based biomarkers of aging in astronauts. Using data from the six participants of the Mars-500 mission, a high-fidelity 520-day ground simulation experiment, we tested relationships of mission duration with five longitudinally measured blood DNA-methylation-based metrics: DNAmGrimAge, DNAmPhenoAge, DNA-methylation-based estimator of telomere length (DNAmTL), mitotic divisions (epigenetic mitotic clock [epiTOC2]), and pace of aging (PoA). We provide evidence that, relative to baseline, mission duration was associated with significant decreases in epigenetic aging. However, only decreases in DNAmPhenoAge remained significant 7 days post-mission. We also observed significant changes in estimated proportions of plasmablasts, CD4T, CD8 naive, and natural killer (NK) cells. Only decreases in NK cells remained significant post-mission. If confirmed more broadly, these findings contribute insights to improve the understanding of the biological aging implications for individuals experiencing long-duration space travel.

Graphical Abstract

In Brief

Long-duration space travel is marked by a unique combination of stressors known to impact human aging. Using data from six participants of the Mars-500 mission, a high-fidelity 520-day ground simulation experiment, Nwanaji-Enwerem et al. report significant associations of mission duration with decreased biological aging measured via blood DNA methylation.

INTRODUCTION

Researchers have long made efforts to study the impact of space travel on human aging (Mohler, 1985). Crew members on a space mission and astronauts living on the International Space Station (ISS) can remain in space for many months at a time, where they experience a unique combination of stressors known to impact aging processes, including social isolation, confinement, microgravity, and cosmic radiation (Giovanetti et al., 2020; Welsh et al., 2019; Kehler et al., 2019; Pantell et al., 2013; Tanskanen and Anttila, 2016). Although the potential negative impact of these stressors is well appreciated, the financial and physical demands of studying human aging biology in outer space have resigned some aspects of aging research to animal models or cell cultures (Demontis et al., 2017; Patel et al., 2019). Nevertheless, research reporting mixed or unexpected relationships of space exposures with aging-related outcomes in human subjects provides continued motivation for research efforts that explicitly focus on studying humans (Strollo et al., 2018).

For instance, one study of seven astronauts living on the ISS for more than 100 days demonstrated that magnetic fields encountered in space were associated with improved study

participant heart rate variability (HRV)—beat-to-beat alterations in the heart rhythm, where high HRV is associated with good health and longevity (Otsuka et al., 2019). Another study of 15 astronauts who completed ~30- or ~200-day space shuttle missions reported brain white matter changes in study subjects beyond what would be expected with normal aging during the same time periods (Lee et al., 2019). Relationships of space travel have also been reported with telomere length (Garrett-Bakelman et al., 2019). Telomeres are nucleoprotein structures at the ends of DNA that contribute to the integrity of the genetic material (Turner et al., 2019). With each round of DNA replication, telomeres shorten. Hence, many studies have utilized telomere length as a marker of cellular aging, where aging is usually associated with decreased telomere length (Lulkiewicz et al., 2020). The National Aeronautics and Space Administration (NASA) Twin Study—where one identical twin astronaut was monitored before, during, and after a 1-year mission onboard the ISS, with his twin serving as a genetically matched ground control—showed associations of long periods in space with lengthened telomeres (Garrett-Bakelman et al., 2019). Interestingly, telomere length rapidly shortened upon return to earth, leaving the space-travel twin with a greater number of shorter telomeres than he previously had.

The mixed or counterintuitive results demonstrated with markers such as telomere length and HRV have surfaced the need for new studies with novel aging biomarkers that demonstrate more pervasive relationships with human aging. Recent studies have demonstrated that DNA-methylation-based aging biomarkers are substantially more robust predictors of chronological age, morbidity, and mortality (Vetter et al., 2019). DNA methylation is an epigenetic process wherein DNA expression is altered by the addition of methyl groups to cytosine residues on DNA (Moore et al., 2013). Genome-wide changes in DNA methylation have been reported with space travel in the NASA Twin Study and with long-term isolation during the Mars-500 mission simulation (Garrett-Bakelman et al., 2019; Liang et al., 2019). Nevertheless, to the best of our knowledge, relationships of long-duration space travel with DNA-methylation-based measures of biological aging remain uncharacterized.

In this study, we address this gap by examining longitudinal changes in five of the most studied and biologically significant DNA-methylation-based aging biomarkers—DNAmPhenoAge, DNAmGrimAge, pace of aging (PoA), the epigenetic mitotic clock (epiTOC2), and a DNA-methylation-based estimator of telomere length (DNAmTL) (Lu et al., 2019a)—measured in the six Mars-500 mission crewmembers over the 520-day ground simulation of interplanetary travel. DNAmPhenoAge was built using nine clinical variables (i.e., albumin, creatinine, glucose, C-reactive protein, lymphocyte percent, mean cell volume, red cell distribution width, alkaline phosphatase, and white blood cell count). DNAmPhenoAge is a robust marker of disease risk that is strongly correlated with chronological age (Levine et al., 2018). DNAmGrimAge was created using a DNA methylation surrogate of cigarette pack-years, and DNA methylation surrogates for seven plasma protein markers (adrenomedullin [ADM], beta-2-microglobulim [B2M], cystatin C, growth differentiation factor-15 [GDF-15], leptin, plasminogen activator inhibitor-1 [PAI-1], and tissue inhibitor metalloproteinases-1 [TIMP-1]) (Lu et al., 2019b). DNAmGrimAge is a predictor of mortality risk that is also highly correlated with chronological age. PoA is a novel measure of morbidity-associated pace of biological aging and was built by comparing

longitudinal changes in 18 biomarkers of organ-system function in persons of the same chronological age. For this reason, it reflects how fast aging is occurring (i.e., pace) (Belsky et al., 2020). The epiTOC2 is reported to estimate the number of stem cell divisions per stem cell per year in normal tissues and has been shown to be universally accelerated in cancer, including pre-cancerous lesions (Teschendorff, 2020).

We hypothesized that simulated long-duration space travel would be an adverse stressor that accelerated the epigenetic aging reflected by each of these five measures in the Mars-500 mission participants.

RESULTS

Study Sample Descriptive Statistics

Table 1 presents the baseline (pre-mission) characteristics of the study participants. All participants were males and at baseline, 7 days pre-mission (−7), had a mean (SD) DNAmAgeSkinBloodClock, DNAmGrimAge, DNAmPhenoAge, and PoA of 35.7 (6.5), 41.5 (4.6), 28.6 (7.6), and 0.9 (0.06) years, respectively. DNAmAgeSkinBloodClock, which is primarily viewed as a robust predictor of chronological age (Horvath et al., 2018), at baseline ranged from 30.0 to 44.1 years. DNAmGrimAge ranged from 35.6 to 48.2 years. DNAmPhenoAge ranged from 19.7 to 38.8 years. PoA ranged from 0.86 to 1.03 years. epiTOC2 had a mean (SD; range) of 2,056.91 (559.17; 1,495.24–2,911.96) divisions per stem cell per year at baseline. DNAmTL had a mean (SD; range) of 7.3 (0.15; 7.02–7.44) kb at baseline. Figure 1 presents a heatmap of Spearman correlation coefficients for all age variables and white blood cell proportions at baseline (Figure 1A) and at the end of the mission (Figure 1B). Figure 1C presents the change in Spearman coefficients (baseline values subtracted from end-of-mission values) for the study period. At baseline, the majority of the DNA methylation age metrics were positively correlated. Relationships between white blood cells and aging metrics were more variable but trended toward negative correlations.

Associations of Mission Duration with Aging Biomarkers and White Blood Cell Proportions

Table 2 describes the results from fully adjusted linear models examining the relationships of mission day with aging biomarkers and white blood cell proportions. Time points for DNAmGrimAge, DNAmPhenoAge, and epiTOC2 met Bonferroni thresholds for statistical significance. Compared with baseline, mission day 168 was associated with a 4.50-year decrease in DNAmGrimAge (95% confidence interval [CI]: −6.79 to −2.20, p = 0.001). This difference was no longer significant post-mission (day 527) (β = 0.06, 95% CI: −2.35 to 2.47, p = 0.96). Compared with baseline, mission day 168 was associated with a 5.90-year decrease in DNAmPhenoAge (95% CI: −9.03 to −2.77, p = 0.001). This difference remained significant post-mission (day 527) (β = −5.41, 95% CI: −8.70 to −2.12, p = 0.003). Compared with baseline, mission day 168 was associated with ~481 fewer divisions per stem cell per year (epiTOC2) (95% CI: −806.91 to −156.16, p = 0.006). This difference was not significant post-mission (day 527) (β = −216.67, 95% CI: −557.20 to 123.87, p = 0.20).

DNAmADM, a surrogate epigenetic measure of the protein ADM, was the only DNAmGrimAge component that significantly changed over the course of the study. Compared with baseline, mission day 168 was associated with a lower DNAmADM (β = −22.66 pg/mL, 95% CI: −32.60 to −12.72, p = 0.0003). Like the change in DNAmGrimAge, this difference was no longer significant post-mission (day 527) (β = −2.53, 95% CI: −12.99 to 7.93, p = 0.64). Notably, there were no significant changes in DNAmPackyears over the course of the experiment.

With respect to the white blood cell proportions, CD4T cells, CD8 naive cells, natural killer (NK) cells, and plasmablasts demonstrated significant changes during the study period. Although the changes did not persist post-mission (β = −0.001, 95% CI: −0.03 to 0.03, p = 0.93), CD4 T cell proportions at day 300 (β = 0.06, 95% CI: 0.03 to 0.09, p = 0.0008) and day 512 (β = 0.05, 95% CI: 0.02 to 0.09, p = 0.003) were significantly higher than pre-mission levels. Similar trends were observed for CD8 naive cells at 512 days (β = 37.95, 95% CI: 18.63 to 57.27, p = 0.0004). Compared with baseline, NK cells at 300 days (β = −0.05, 95% CI: −0.08 to −0.02, p = 0.002) and plasmablasts at 300 days (β = −0.17, 95% CI: −0.27 to −0.06, p = 0.003) and 512 days (β = −0.17, 95% CI: −0.28 to −0.07, p = 0.003) were significantly lower than their baseline levels.

Figure 2 plots the trends/change in all statistically significant aging biomarkers and white blood cell proportions across the entire Mars-500 mission. Unadjusted results for all analyses demonstrated similar trends and are presented in Table S1.

DISCUSSION

In this longitudinal study using data from the Mars-500 mission, we observed statistically significant changes in both DNA methylation aging biomarkers and white blood cell proportions. Particularly, we observed statistically significant decreases in DNAmGrimAge, DNAmPhenoAge, epiTOC2, and DNAmADM. These changes occurred at the 168- and 300-day time points of the 520-day mission for three aging metrics and at the 60- and 168-day time points for the fourth aging metric. Moreover, only the changes in DNAmPhenoAge remained statistically significant post-mission. We also observed statistically significant changes in CD4 T cells, CD8 naive T cells, NK cells, and plasmablasts. Mission duration was associated with increases in CD4 T cells at the 300-day time point and increases in both CD4 T cells and CD8 naive T cells at the 512-day time point. Mission duration was also associated with decreases in NK cells and plasmablasts at the 300- and 500-day time points. Among all white blood cells, only the changes in NK cells remained statistically significant post-mission.

We initially hypothesized that mission duration would adversely impact biological aging and would be associated with increases in our DNA methylation age measures. However, we consistently observed the opposite across all of the statistically significant aging metrics. This consistency from metrics produced by the Horvath calculator (DNAmGrimAge, DNAmPhenoAge, and DNAmADM) as well as a metric from an independent epigenetic clock (i.e., epiTOC2) increased our confidence in the biological significance of the detected phenomenon. Still, it is notable that changes in epiTOC2 happened much earlier than the

changes in the other three measures. This difference may be due to what each of these measures represents. Specifically, epiTOC2 is calculated from DNA methylation probes and is known to reflect the specific "micro" or cellular process of mitosis, a type of cell division. Increases in epiTOC2 have been reported in multiple cancers, pre-invasive cancer lesions, and normal buccal tissue exposed to cigarette smoke—a known carcinogen (Teschendorff, 2020). Notably, findings from epiTOC2 are tissue specific and when calculated from blood cells can help predict participant's risk of hematological cancer (Teschendorff, 2020).

On the other hand, DNAmGrimAge, DNAmPhenoAge, and DNAmADM are more established and robust predictors of the "macro" outcomes of health and lifespan. DNAmGrimAge is computed using DNA methylation of individual sites as well as a DNA methylation surrogate of cigarette smoke in pack-years and DNA methylation surrogates for seven plasma protein markers (ADM, B2M, cystatin C, GDF-15, leptin, PAI-1, and TIMP-1). Moreover, DNAmGrimAge has demonstrated tremendous accuracy in its ability to predict all-cause time-to-death (Cox regression $p = 2.0 \times 10^{-75}$) (Lu et al., 2019b). As noted, DNAmADM is a component of DNAmGrimAge and represents a DNA methylation surrogate for ADM—a 52-amino-acid peptide that is highly expressed in endothelial cells (Acelajado et al., 2013). High circulating ADM levels have been reported in heart failure, hypertension, and renal failure (Acelajado et al., 2013). Elevated plasma ADM levels are also associated with a worse prognosis in a host of cancers (Cuttitta and Martínez, 2013). DNAmPhenoAge is principally viewed as a predictor of disease risk (Arpón et al., 2019; Hillary et al., 2019; Rezwan et al., 2020) and was built utilizing nine clinical variables (i.e., albumin, alkaline phosphatase, creatinine, C-reactive protein, glucose, lymphocyte percent, mean cell volume, red cell distribution width, and white blood cell count) (Levine et al., 2018). Essentially, one hypothesis for why changes in epiTOC2 are first observed is because one would expect changes in a "micro" process such as mitosis to be evident before more grossly observable changes in disease or mortality. Further research is necessary to confirm this characterization.

Ultimately, it remains unclear why simulated space travel would result in decreased epigenetic aging, especially when some mission exposures (e.g., microgravity and cosmic radiation) were not replicated in the simulation. Nonetheless, our paradoxical findings and the transient nature of these findings are in line with anti-aging results from studies including telomeres in the NASA Twin Study and the astronaut study on HRV (Garrett-Bakelman et al., 2019; Otsuka et al., 2019). Our findings suggest that the simulated portions of space travel (e.g., confinement, diet, changes in circadian rhythms) may be the reason for these lapsing anti-aging periods. Alternatively, pre-mission stress levels could partly be attributed to the apparent decrease after mission initiation.

Importantly, unlike the NASA Twin Study, we observe no significant changes in telomere length (Garrett-Bakelman et al., 2019). Since DNAmTL—calculated from individual DNA methylation sites—is modestly correlated with telomere length measured via traditional methods such as quantitative polymerase chain reaction (qPCR) (r = ~0.4), this null finding could be due to differences in assessment method (Lu et al., 2019a). It is also possible that any telomere-specific changes were driven by the physical aspects of the space-travel

experience that were not fully replicable in the Mars-500 ground mission (e.g., radiation exposure and microgravity).

Of the white blood cells examined in our study, we observed increases in subject T cells near the end of mission that may mirror the proinflammatory findings reported in the return phase of the NASA Twin Study (Garrett-Bakelman et al., 2019). One important difference is that our T cell differences were no longer statistically significant 7 days post-mission. In fact, only the decreases in NK cells remained statistically significant post-mission. This finding is in line with previous studies that demonstrate increased cell death and decreased cytotoxicity in NK cells following long-duration space-travel exposures (Bigley et al., 2019; Li et al., 2013). Given the role that NK cells play in the prevention of latent virus reactivation and tumor surveillance, these findings could be concerning for the long-term well-being of astronauts (Bi and Wang, 2020). Although the decreased hematologic cancer risk suggested by our epiTOC2 results seems to be at odds with our NK cell findings, it is important to highlight that the epiTOC2 changes were no longer significant post-mission, but the NK findings were. The transient nature of some of our results—especially those whose changes peak around mission day 168—suggests that there may be an acute initial stress response to space travel; however, a state of adaptation is ultimately achieved in the setting of chronic stress. Such phenomena of stress adaptation and resilience have been previously described in contexts including hypothalamic-pituitary-adrenal physiology (Jones et al., 2016). Still, studies in these contexts demonstrate that adaptive responses may be beneficial in the short term but may come with physiological costs that are less advantageous when dealing with future stressors. These physiological costs are further correlated with the development of pathological conditions (e.g., cardiovascular disease, cancer, and diabetes) (Jones et al., 2016; Rao and Androulakis, 2019). Furthermore, this hypothesis of physiological costs is in line with previous genome-wide DNA methylation findings in the Mars-500 participants. Specifically, mission duration was associated with an over-representation of differentially methylated genes involved in platelet activation and glucose metabolism pathways (Liang et al., 2019).

This study has a number of strengths including the utilization of data among the same individuals sampled multiple times and the calculation of biological aging rates using novel DNA methylation markers. Given that study participants were not allowed to smoke during the mission, our study also utilized a negative DNA methylation control: DNAmPackyears. Appropriately, in fully adjusted linear models, DNAmPackyears did not demonstrate any statistically significant associations with mission duration. Still, we do have some limitations. We had limited information on important covariates such as chronological age and lifestyle factors. Thus, we cannot rule out the impact of residual confounding and the aforementioned unknowns in our analyses. Furthermore, space travel—even when simulated —is a complex exposure potentially involving changes in circadian rhythm, sleep patterns, light levels, physical activity, etc. Lacking lifestyle data limits our ability to identify the most important psychosocial and physical drivers of our observed relationships. Additionally, generalizability and statistical power are limited given that we only had information on six male participants from a limited number of ethnicities.

In conclusion, our study describes novel relationships of simulated long-term space travel, biological aging measured via DNA methylation biomarkers, and white blood cell proportions. These findings will be valuable for understanding the health implications for future human interplanetary space travel.

STAR★METHODS

RESOURCE AVAILABILITY

Lead Contact—Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Jamaji Nwanaji-Enwerem (jamaji_nwanaji-enwerem@hms.harvard.edu).

Materials Availability—This study did not generate unique reagents.

Data and Code Availability—This study did not generate any unique datasets or code.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

This repeated-measures, longitudinal analysis was conducted using publicly available DNA methylation data from the Mars-500 mission conducted by the Institute of Biomedical Problems (IBMP) of the Russian Academy of Sciences (RAS). From June 2010 to November 2011, six male volunteers (three Russian, one French, one Italian, and one Chinese) lived in a mockup spacecraft in Moscow, Russia for 520 days to simulate a full interplanetary mission from Earth to Mars. The overall goal of the mission was to study the technological, psychological, and physiological challenges associated with long-duration space travel. Participants only had personal contact with each other. They had voice contact with a simulated control center as well as friends/family in a manner similar to a true spaceflight mission. Participants also had access to films, books, and laptops. Their diets mirrored those provided to astronauts in the International Space Station (The European Space Agency, 2020).

Prior to participation, all participants completed a thorough physical examination and informed consent forms for the simulation experiment. All study protocols and experiments, including the collection of DNA methylation data, were approved by the IBMP committee on bioethics (Liang et al., 2019). Importantly, chronological ages for participants were not provided in the publicly available metadata.

METHOD DETAILS

Whole blood samples for this study were collected at 6 time points: 7 days prior to the start of the mission (day −7); on days 60, 168, 300, and 512 of the mission; and 7 days after the end of the mission (day 527) (Liang et al., 2019; Xiong et al., 2015). Full descriptions of whole blood cell DNA collection protocols, isolation, and methylation measurements are described in the original article (Liang et al., 2019). Raw data files were downloaded from the NASA GeneLab Repository (https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-140/) (Xiong et al., 2015). Raw HumanMethylation450 BeadChip (450K) data were normalized with functional normalization and basic quality control benchmarks (e.g., sex,

intensity, and detection) were carried out (Fortin et al., 2014). In short, IDAT files were processed in R using the *minfi* package and normalization was performed with functional normalization (FunNorm). Probes with a bead-count < 3 in at least 5% of samples, probes with SNPs, non-cg probes, and probes aligning to multiple locations were removed. X and Y chromosome probes were kept given that all subjects were male (Fortin et al., 2014). Normalized DNA methylation beta values were uploaded to Horvath's publicly available online calculator (http://dnamage.genetics.ucla.edu). The online calculator provided values for a DNA-methylation-based estimator of telomere length (DNAmTL), DNAmGrimAge, DNAmPhenoAge, DNAmAgeSkinBloodClock, and the white blood cell proportions (Lu et al., 2019a). Pace of Aging (PoA) was calculated using R code available at https://github.com/danbelsky/DunedinPoAm38. The mitotic clock (epiTOC2) was calculated using R code available at https://zenodo.org/record/2632938 (Teschendorff, 2020).

QUANTIFICATION AND STATISTICAL ANALYSIS

In the first phase of the analysis, we used linear regression models to examine the relationships of Mars-500 mission day (categorical; day −7, 60, 168, 300, 512, and 527) with each of the five DNA-methylation-based age biomarkers (DNAmGrimAge, DNAmPhenoAge, DNAmTL, PoA, and epiTOC2). Importantly, given that chronological age was not provided in the metadata and might be an important confounder in these analyses, we adjusted for it using the chronological age predictor, DNAmAgeSkinBloodClock at baseline (continuous) (Horvath et al., 2018). Models were additionally adjusted for estimated white blood cell proportions (continuous; plasmablasts, monocytes, granulocytes, CD8T, naive CD8T, CD4T, naive CD4T, CD8+CD28-CD45RA- T, NK, and B cells). White blood cell proportions were estimated using Houseman and Horvath DNA-methylation-based methods and were provided as downloadable data along with the other online DNA methylation age calculator results (Horvath, 2013; Houseman et al., 2012). All covariates were determined *a priori* based on available data and the previous study using this dataset (Liang et al., 2019). Models also included a random intercept for participants to account for repeated-measures per participant. For this phase of the analysis, five independent tests were performed so any aging metric with time points that had a p value less than the Bonferroni-corrected threshold of 0.01 were considered to be statistically significant.

In the second phase of the analysis, we used linear regression models to examine the relationships of Mars-500 mission day with each of the eight DNA methylation components of DNAmGrimAge: DNAm cigarette pack-years and DNAm protein markers [adrenomedullin (ADM), beta-2-microglobulim (B2M), cystatin C, growth differentiation factor-15 (GDF-15), leptin, plasminogen activator inhibitor-1 (PAI-1), and tissue inhibitor metalloproteinases-1 (TIMP-1)]. DNAm pack-years was included as a negative control as smoking was not permitted during the Mars-500 mission. Models were again adjusted for DNAmAgeSkinBloodClock at baseline and white blood cell proportions. For this phase of the analysis, eight independent tests were performed so any markers with time points with a p value less than the Bonferroni-corrected threshold of 0.006 were considered to be statistically significant.

The final phase of the analysis used linear regression models to examine the relationships of Mars-500 mission day with each of the ten previously mentioned white blood cell proportions. These models were only adjusted for DNAmAgeSkinBloodClock at baseline. Ten independent tests were performed so any leukocytes with time points with a p value less than the Bonferroni-corrected threshold of 0.005 were considered to be statistically significant.

All statistical analyses were performed using R Version 3.6.3 (R Core Team, Vienna, Austria). Linear mixed effects models were executed using the lme function from the nlme package (Pinheiro et al., 2020).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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Highlights

- Mission duration was associated with significant decreases in epigenetic aging

- Decreases in PhenoAge, a morbidity biomarker, remained significant post-mission

- There were significant changes in estimated proportions of some white blood cells

- Only decreases in NK cells remained significant post-mission
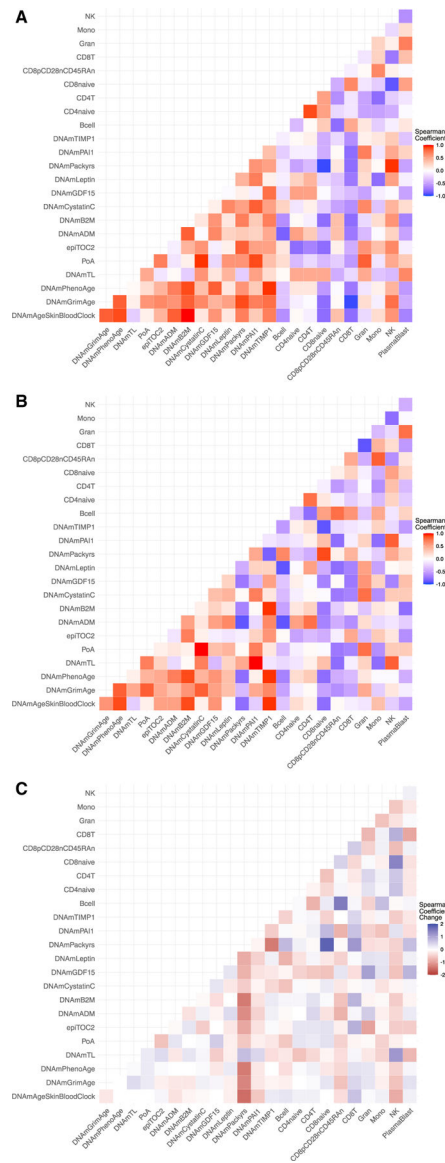
Figure 1. Heatmap of Spearman Correlation Coefficients for Aging Biomarkers and White Blood Cell Proportions

The figure depicts a heatmap of Spearman correlation coefficients for the age biomarkers and white blood cell proportions of Mars-500 mission participants at baseline (−7 days pre-mission) and post-mission (day 527). All correlations are based on the full cohort (n = 6). (A) Reflects relationships at baseline, (B) reflects relationships at the end of the mission, and (C) reflects the change in Spearman coefficients derived by subtracting pre-mission coefficients from post-mission coefficients.
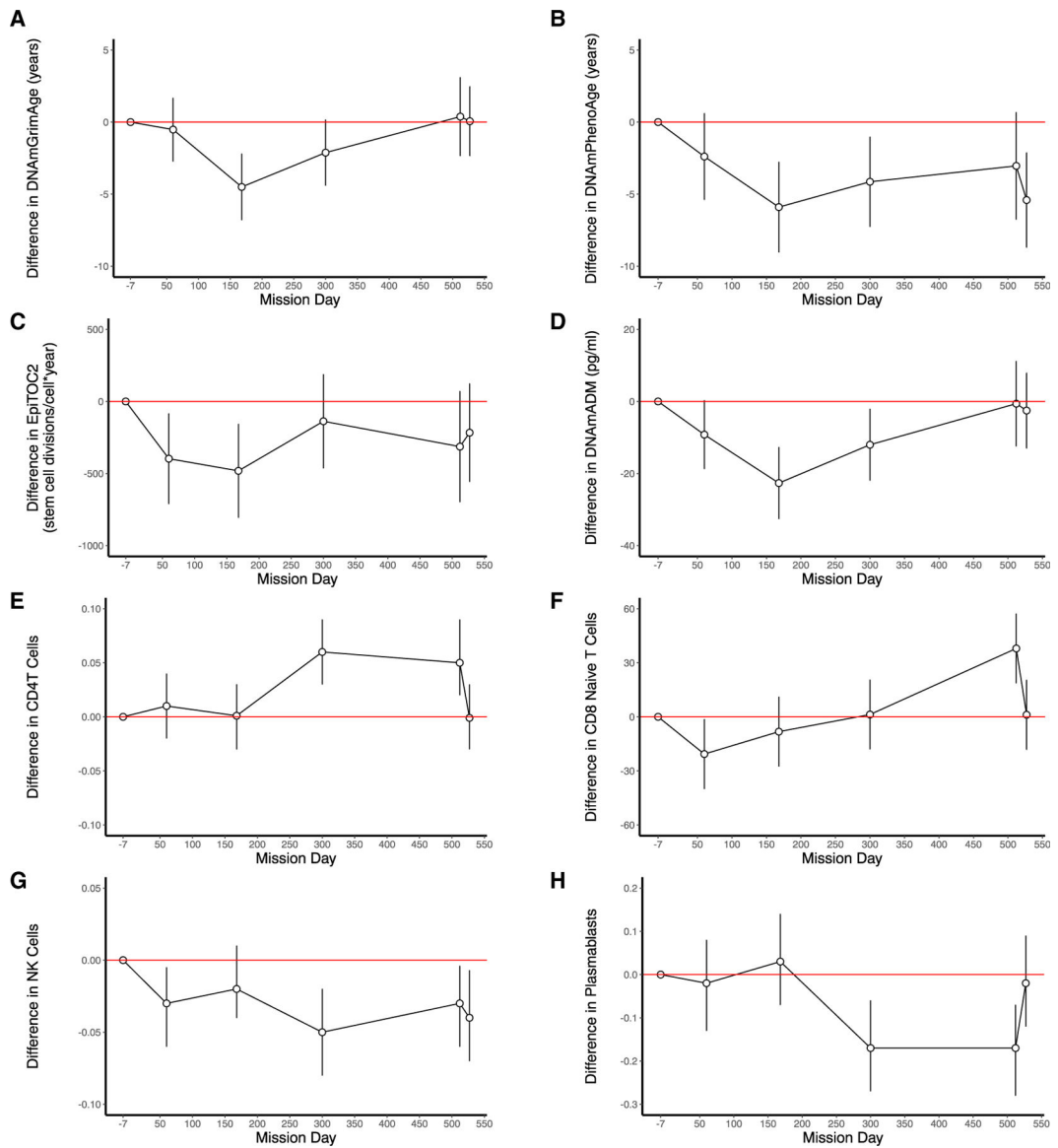
Figure 2. Statistically Significant Trends in Aging Biomarkers and White Blood Cell Proportions
This figure depicts longitudinal estimated changes and 95% CIs for DNAmGrimAge (A), DNAmPhenoAge (B), epiTOC2 (C), DNAmADM (D), CD4 T cells (E), CD8 naive T cells (F), NK cells (G), and plasmablasts (H) over the Mars-500 mission duration. Differences provided are from adjusted linear regression models where measures on days 60, 168, 300, 512, and post-mission (day 527) are compared with baseline measurements made pre-mission on day −7.

Table 1.

Study Participant Baseline (Pre-Mission) Characteristics (n = 6)

Baseline Characteristic	Mean (SD) [Range]
DNAmAgeSkin BloodClock (years)	35.7 (6.5) [30.0–44.1]
DNAmGrimAge (years)	41.5 (4.6) [35.6–48.2]
DNAmPhenoAge (years)	28.6 (7.6) [19.7–38.8]
Pace of Aging/PoA (years)	0.9 (0.06) [0.86–1.03]
Mitotic Divisions/epiTOC2 (divisions per stem cell per year)	2,056.91 (559.17) [1,495.24–2,911.96]
DNAmTL (kb)	7.3 (0.15) [7.02–7.44]

Table 2.

Relationships of Mission Duration with Aging Biomarkers and Leukocyte Proportions

	Difference in DNA Methylation Marker (95% CI) Aging Biomarker Model[a]	p[b]
DNAmGrimAge (Years)		
Baseline (−7 days)	reference	ND
60 days	−0.52 (−2.72 to 1.67)	0.65
168 days	−4.50 (−6.79 to 2.20)	*0.001*
300 days	−2.12 (−4.41 to 0.17)	0.09
512 days	0.38 (−2.35 to 3.10)	0.79
Post-mission (527 days)	0.06 (−2.35 to 2.47)	0.96
DNAmPhenoAge (Years)		
Baseline (−7 days)	reference	ND
60 days	−2.39 (−5.38 to 0.61)	0.11
168 days	−5.90 (−9.03 to −2.77)	*0.001*
300 days	−4.14 (−7.27 to −1.02)	*0.01*
512 days	−3.04 (−6.76 to 0.68)	0.10
Post-mission (527 days)	−5.41 (−8.70 to −2.12)	*0.003*
DNAmTL (kb)		
Baseline (−7 days)	reference	ND
60 days	0.03 (−0.07 to 0.14)	0.48
168 days	0.02 (−0.08 to 0.12)	0.67
300 days	0.02 (−0.09 to 0.13)	0.70
512 days	0.03 (−0.08 to 0.14)	0.58
Post-mission (527 days)	−0.01 (−0.11 to 0.08)	0.76
Pace of Aging (PoA) (Years)		
Baseline (−7 days)	reference	ND
60 days	0.03 (−0.02 to 0.09)	0.19
168 days	0.01 (−0.04 to 0.07)	0.60
300 days	−0.01 (−0.07 to 0.05)	0.65
512 days	0.04 (−0.02 to 0.10)	0.15
Post-mission (527 days)	0.002 (−0.05 to 0.05)	0.94
Mitotic Age (epiTOC2) (Divisions per Stem Cell per Year)		
Baseline (−7 days)	reference	ND
60 days	−397.21 (−710.69 to −83.73)	0.02
168 days	−481.53 (−806.91 to −156.16)	0.006
300 days	−137.62 (−463.80 to 188.56)	0.39
512 days	−313.55 (−698.51 to 71.40)	0.10
Post-mission (527 days)	−216.67 (−557.20 to 123.87)	0.20
DNAmGrimAge Component Model[a]		

	Difference in DNA Methylation Marker (95% CI) Aging Biomarker Model[a]	p[b]
DNAm ADM (pg/mL)		
Baseline (−7 days)	reference	ND
60 days	−9.21 (−18.72 to 0.31)	0.07
168 days	−22.66 (−32.60 to −12.72)	*0.0003*
300 days	−12.02 (−21.95 to −2.08)	0.03
512 days	−0.62 (−12.44 to 11.19)	0.92
Post-mission (527 days)	−2.53 (−12.99 to 7.93)	0.64
DNAm B2M (pg/mL)		
Baseline (−7 days)	reference	ND
60 days	−11,537.7 (−54,851.00 to 31,775.68)	0.58
168 days	−46,459.3 (−90,099.30 to −28,19.31)	0.04
300 days	−42,559.8 (−87,307.27 to 2,187.72)	0.06
512 days	17,348.3 (−31,813.55 to 66,510.23)	0.47
Post-mission (527 days)	21,684.3 (21,852.57 to 65,221.21)	0.31
DNAm Cystatin C (pg/mL)		
Baseline (−7 days)	reference	ND
60 days	8,025.9 (−11,508.78 to 27,560.51)	0.40
168 days	−4,456.8 (−24,120.16 to 15,206.56)	0.64
300 days	−5,041.1 (−25,701.20 to 15,619.04)	0.61
512 days	−5,248.7 (−26,859.24 to 16,361.76)	0.62
Post-mission (527 days)	7,124.1 (−12,024.12 to 26,272.35)	0.44
DNAm GDF-15 (pg/mL)		
Baseline (−7 days)	reference	ND
60 days	−36.51 (−103.14 to 30.12)	0.26
168 days	−96.62 (−164.04 to −29.20)	0.008
300 days	−39.12 (−111.96 to 33.71)	0.27
512 days	10.92 (−61.50 to 83.35)	0.76
Post-mission (527 days)	−19.67 (−83.85 to 44.51)	0.53
DNAm Leptin (pg/mL)		
Baseline (−7 days)	reference	ND
60 days	1,225.78 (−57.88 to 2,509.45)	0.06
168 days	−168.65 (−1,463.03 to 1,125.73)	0.79
300 days	188.22 (−1,135.56 to 1,512.00)	0.77
512 days	−306.50 (−1,769.60 to 1,156.60)	0.67
Post-mission (527 days)	360.37 (−935.18 to 1,655.92)	0.57
DNAm Packyears (Pack-Years)		
Baseline (−7 days)	reference	ND
60 days	0.36 (−3.11 to 3.84)	0.83
168 days	0.14 (−3.40 to 3.67)	0.94

	Difference in DNA Methylation Marker (95% CI) Aging Biomarker Model[a]	p[b]
300 days	0.70 (−2.87 to 4.28)	0.68
512 days	1.21 (−2.87 to 5.29)	0.54
Post-mission (527 days)	0.77 (−2.84 to 4.38)	0.66
DNAm PAI-1 (pg/mL)		
Baseline (−7 days)	reference	ND
60 days	1,160.27 (−383.77 to 2,704.31)	0.13
168 days	−1,364.61 (−2,920.97 to 191.75)	0.08
300 days	261.24 (−1,390.39 to 1,912.88)	0.74
512 days	−218.50 (−1,914.57 to 1,477.58)	0.79
Post-mission (527 days)	−132.06 (−1,635.02 to 1,370.91)	0.86
DNAm TIMP-1 (pg/mL)		
Baseline (−7 days)	Reference	ND
60 days	−170.00 (−683.06 to 343.07)	0.50
168 days	−681.05 (−1,198.05 to−164.04)	0.01
300 days	−280.04 (−809.90 to 249.82)	0.28
512 days	71.26 (−511.54 to 654.06)	0.80
Post-mission (527 days)	57.91 (−458.20 to 574.01)	0.82
Leukocyte Model[c]		
B Cells		
Baseline (−7 days)	reference	ND
60 days	−0.003 (−0.02 to 0.01)	0.65
168 days	−0.00005 (−0.02 to 0.01)	0.99
300 days	0.02 (0.001 to 0.03)	0.03
512 days	0.02 (0.002 to 0.03)	0.03
Post-mission (527 days)	−0.003 (−0.02 to 0.01)	0.73
CD4 Naive Cells		
Baseline (−7 days)	reference	
60 days	−53.30 (−111.04 to 4.44)	0.07
168 days	−84.27 (−142.01 to −26.54)	0.01
300 days	−11.06 (−68.80 to 46.67)	0.70
512 days	41.34 (−16.39 to 99.08)	0.15
Post-mission (527 days)	−36.39 (−94.12 to 21.35)	0.21
CD4 T Cells		
Baseline (−7 days)	reference	ND
60 days	0.01 (−0.02 to 0.04)	0.54
168 days	0.001 (−0.03 to 0.03)	0.96
300 days	0.06 (0.03 to 0.09)	*0.0008*
512 days	0.05 (0.02 to 0.09)	*0.003*
Post-mission (527 days)	−0.001 (−0.03 to 0.03)	0.93

	Difference in DNA Methylation Marker (95% CI) Aging Biomarker Model[a]	p[b]
CD8 Naive Cells		
Baseline (−7 days)	reference	ND
60 days	−20.67 (−40.00 to −1.36)	0.04
168 days	−8.23 (−27.55 to 11.09)	0.39
300 days	1.27 (−18.05 to 20.59)	0.89
512 days	37.95 (18.63 to 57.27)	*0.0004*
Post-mission (527 days)	1.13 (−18.19 to 20.45)	0.91
CD8pCD28nCD45Ran T Cells		
Baseline (−7 days)	reference	ND
60 days	1.36 (−1.06 to 3.79)	0.26
168 days	1.76 (−0.67 to 4.18)	0.15
300 days	−1.39 (−3.81 to 1.03)	0.25
512 days	0.03 (−2.39 to 2.45)	0.98
Post-mission (527 days)	1.76 (−0.66 to 4.18)	0.15
CD8 T Cells		
Baseline (−7 days)	reference	ND
60 days	−0.01 (−0.04 to 0.01)	0.31
168 days	−0.03 (−0.06 to −0.003)	0.03
300 days	−0.002 (−0.03 to 0.02)	0.86
512 days	0.02 (−0.007 to 0.05)	0.14
Post-mission (527 days)	−0.02 (−0.04 to 0.008)	0.17
Granulocytes		
Baseline (−7 days)	reference	ND
60 days	0.01 (−0.04 to 0.06)	0.70
168 days	0.04 (−0.008 to 0.09)	0.10
300 days	0.0008 (−0.05 to 0.05)	0.98
512 days	−0.05 (−0.10 to 0.002)	0.06
Post-mission (527 days)	0.05 (0.0009 to 0.10)	0.05
Monocytes		
Baseline (−7 days)	reference	ND
60 days	0.02 (0.002 to 0.03)	0.03
168 days	0.01 (−0.004 to 0.03)	0.15
300 days	−0.01 (−0.03 to 0.0002)	0.05
512 days	−0.01 (−0.02 to 0.007)	0.30
Post-mission (527 days)	0.002 (−0.01 to 0.02)	0.74
NK Cells		
Baseline (−7 days)	reference	ND
60 days	−0.03 (−0.06 to −0.005)	0.02
168 days	−0.02 (−0.04 to 0.01)	0.29

	Difference in DNA Methylation Marker (95% CI) Aging Biomarker Model[a]	p[b]
300 days	−0.05 (−0.08 to −0.02)	*0.002*
512 days	−0.03 (−0.06 to −0.004)	0.03
Post-mission (527 days)	−0.04 (−0.07 to −0.007)	0.02
Plasmablasts		
Baseline (−7 days)	reference	ND
60 days	−0.02 (−0.13 to 0.08)	0.64
168 days	0.03 (−0.07 to 0.14)	0.53
300 days	−0.17 (−0.27 to −0.06)	*0.003*
512 days	−0.17 (−0.28 to −0.07)	*0.003*
Post-mission (527 days)	−0.02 (−0.12 to 0.09)	0.71

[a]Models adjusted for baseline DNAmAgeSkinBloodClock and white blood cell proportions.

[b]Italicized p values are considered statistically significant in these analyses. ND, no data.

[c]Models adjusted for baseline DNAmAgeSkinBloodClock.

KEY RESOURCES TABLE

RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Raw DNA Methylation 450K data	Xiong et al., 2015	GLDS-140, https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-140/
Software and Algorithms		
R	https://www.r-project.org	Version 3.6.3
DNAmGrimAge, DNAmPhenoAge, DNAmTL, and white blood cell proportion algorithm	Lu et al., 2019b	http://dnamage.genetics.ucla.edu
EpiTOC2 algorithm	Teschendorff, 2020	https://zenodo.org/record/2632938
Pace of Aging algorithm	Belsky et al., 2020	https://github.com/danbelsky/DunedinPoAm38