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Variations in Heart Rate Variability and Physiological Responses during Analog Space Missions: An Exploratory Study

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Abstract: This exploratory study investigates changes in the autonomic cardiac system of young analog astronauts in a hostile, confined, and isolated environment. It uses linear and nonlinear indices of heart rate variability (HRV) during a Mars analog mission to assess how HRV varies under day and night stressors. This study is guided by the hypothesis that significant HRV changes occur based on adaptation days, aiming to offer insights into autonomic nervous system (ANS) adaptation to environmental stressors. Over five days in August 2022, five analog astronauts faced adverse conditions in the Mojave Desert, simulating Martian conditions. Electrocardiograms were recorded daily for five minutes during morning and evening sessions to extract short-term RR time series. HRV parameters were analyzed using both time- and frequency-domain indices and nonlinear measures. Significant differences in HRV parameters across days highlight the mission environment's impact on autonomic cardiac function. Morning measurements showed significant changes in average RR intervals and heart rate, indicating ANS adaptation. Nonlinear indices such as detrended fluctuation analysis and approximate entropy also showed significant differences, reflecting shifts in autonomic function. The Borg scale indicated reduced perceived exertion over time, aligning with HRV changes. Increased vagal activity during Mars analog adaptation under confinement/isolation may be crucial for cardiovascular adaptation and survival in future space flights.

Keywords: HRV; electrocardiogram; analog mission; mars; autonomic adaptation to space

1. Introduction

Outer space is a highly inhospitable environment incompatible with human life. During long-term space missions, challenges such as microgravity and increased exposure to radiation pose significant threats to the crew's health [1], including dizziness, dehydration, urinary tract infections, musculoskeletal issues, and cardiovascular events [2]. The cardiovascular system has been a primary focus of space health research because it is one of the most severely affected systems and is probably the most resilient and adaptable under microgravity conditions [3].

Alterations to the cardiovascular system due to spaceflight encompass specific manifestations such as a 35–41% increase in cardiac output after 3–6 months [4], a significant rise in heart rate and diastolic blood pressure within the first 24 h, and a noticeable reduction in cardiac muscle mass by 12% during ten days in space [5]. Therefore, continuous health monitoring and the development of countermeasures to mitigate the effects of the space environment on humans represent immediate research necessity.

Additionally, psychological stressors play a crucial role in the overall health and performance of astronauts during space missions. Prolonged isolation, confinement, and exposure to extreme conditions can lead to significant mental health challenges, including anxiety, depression, and sleep disorders. These psychological stressors can exacerbate physiological issues, creating a feedback loop that impacts cardiovascular health. Stress has been shown to affect the autonomic nervous system (ANS), leading to variations in heart rate variability (HRV), which is a key indicator of ANS activity and overall cardiovascular health [6].

The National Aeronautics and Space Administration (NASA) has crafted an extensive strategy for space exploration that highlights the advancement of biomedical knowledge and technology to ensure the health and performance of astronauts during missions. This initiative is central to the Human Exploration and Development of Space (HEDS) Enterprise, which aims to push the boundaries of space exploration and improve the human experience in space. A key goal of HEDS is to prepare for human exploration of planetary bodies, particularly Mars. To achieve this, NASA is developing capabilities to safeguard astronaut health and implementing next-generation life support and environmental monitoring systems [7].

NASA continuously monitors its astronauts to understand how human bodies adapt to microgravity and to maintain their physical and mental health. Currently, biometric sensors are being developed to track vital signs for health monitoring [8]. One critical measure is HRV, which serves as an indirect metric for assessing the activity of the ANS under stressful and extreme conditions [9]. HRV is the variation in heart rate from beat to beat over a specified time interval [10]. Space flight deeply affects the dynamics of the cardiovascular system; a decrease in HRV during controlled breathing (six breaths/min) has been documented [11], indicating a compromised parasympathetic reserve, potentially attributable to acute physiological and psychological stresses encountered in the early stages of space flight. This decrease may influence subsequent physiological changes after the flight [12].

The International Space Station (ISS) provides an invaluable experimental platform for medical research. However, inherent logistical limitations and operational challenges aboard the ISS can delay access to relevant data. Therefore, it is essential to study Earth analog environments. In this manuscript, we use the term "analog" to describe our experimental setup, which is a form of ground-based simulation designed to replicate the conditions of a manned space mission as several space agencies and private training companies [13–15]. These analog environments are locations on Earth that replicate extreme space conditions, such as prolonged isolation and resource constraints, as closely as possible. Examples of these environments include arid deserts, polar regions, underground caves, and underwater habitats, where physical conditions like extreme temperatures, low humidity, and lack of sunlight simulate the challenges astronauts would face during long-duration space missions. During astronaut recruitment and training phases, these analog environments allow scientists and trainers to observe how "analog astronauts"—civilians with the appropriate competencies to be considered potential candidates for off-planet missions and training in these analog settings [16]—respond to potential clinical scenarios that may affect the physical and mental health of astronauts in space. Participants subjected to these analog conditions may experience typical symptoms ranging from neurocognitive changes and fatigue to circadian rhythm misalignment, sleep disorders, variations in stress hormone levels, disruptions in daily routines, and immunomodulatory modifications [17,18].

Additionally, these conditions provide a deeper understanding of human interaction, adaptability to psychological stress, and the impact of confinement and isolation in outer space [19]. Moreover, they enable the exploration of workflows; crew time management in plant cultivation systems; and the evaluation of instruments, risks, and challenges for future missions on planetary surfaces, all within a representative terrestrial environment [20,21].

This exploratory study aims to compare the changes in the autonomic cardiac system of a crew of young analog astronauts subjected to stress in a hostile environment of confinement and isolation. Using both linear and nonlinear indices of HRV during a Mars analog mission, this study seeks to explore how HRV, as an indicator of ANS activity, varies under the unique stressors of a simulated space environment during daytime and nighttime. This study is specifically designed to highlight HRV as a non-invasive tool for assessing physiological adaptation in environments where non-invasive technologies are essential, demonstrating HRV's utility, especially in challenging contexts like space missions, where invasive techniques such as blood analyses present significant logistical challenges. We hypothesize that HRV metrics will exhibit significant changes depending on the days of adaptation to a hostile environment. Additionally, analyzing HRV separately for day and night is crucial to avoid the influences of circadian rhythms. These variations in HRV are expected to provide insights into the ANS adaptation to environmental stressors, with nighttime recovery patterns playing a crucial role in the crew's overall well-being and mission performance.

2. Materials and Methods

2.1. Subjects

Over five days in August 2022, five analog astronauts (three men and two women, n = 5) with an average age of 24 ± 4 years, a height of 1.73 ± 0.09 m, and weight of 66.2 ± 11.8 kg were subjected to adverse and adaptive conditions in the Mojave Desert, California, USA. This analog space mission was dedicated to exploration and investigation, simulating the hypothetical arrival of human civilization on Mars. The Mojave Desert has been considered a potential analog ecosystem for Mars on Earth [22].

Various activities were carried out during this isolation period. Figure 1 presents a timeline of the mission divided into five key phases: the beginning with the arrival and integration of the team, four mission days (sol 1 to sol 4) with both daytime and nighttime activities, including morning and evening ECG measurements, as well as medical, robotic, and engineering tasks, and finally, the conclusion with a final area inspection before the team's return to their homes.

Each stage is illustrated with images showing participants performing activities in the desert, using analog space suits and medical equipment, highlighting the daily structure of the simulation and the technical and scientific operations. Additionally, a specific schedule based on the activities and individual roles of each participant has been attached and can be accessed directly through the URL provided in the Data Availability Statement section. The first file, "*General Data*", contains detailed information such as the pseudonyms of the participants; their roles, ages, and nationality; Borg scale data; as well as environmental variables like temperature and humidity throughout the mission days. The second file, "*Analog_Space_Mission_Schedule*", establishes the schedule for the four mission days, divided into individual activities according to the assigned roles of each participant.

Most of the activities were scheduled, and a fixed timetable was implemented for the astronauts due to several obligations during the mission. The experimental protocol was registered in advance by the local committee of the Mars-Moon Astronautics Academy & Research Science (MAAARS; assigned number: MMAARS001100). Each subject provided written informed consent before participating. This study complied with the Declaration of Helsinki.

Figure 1. Summary of the analog mission over the days. The timeline illustrates the key activities and events during each Martian day (sol) of the Mars analog mission. The mission starts with initial preparations and continues through a series of activities such as simulation of extravehicular activity, scientific experiments, and medical simulations. Each sol (from sol 1 to sol 4) represents a day in the mission, highlighting the progression and various tasks undertaken by the crew, culminating in the end of the simulation.

2.2. Data Collection and Preprocessing

For the recording of electrocardiograms (ECG), a bioelectric device was used (OpenBCI Ganglion Board with four channels, OpenBCI, Brooklyn, NY, USA). These recordings were conducted daily for five minutes over four days (or sols) during both morning and evening sessions, with a sampling frequency of 200 Hz. Specifically, the morning recordings were conducted between 7:00 a.m. and 9:00 a.m., and the evening sessions were carried out after the analog astronauts had completed their scheduled activities and before beginning their nightly rest routines (8:00 p.m. and 10:00 p.m.). It is essential to mention that technical difficulties precluded obtaining five continuous recordings during the evening sessions, resulting in the capture of only three recordings.

Figure 2 provides details of the precise positioning of the electrodes. Before placing the electrodes, the application area was thoroughly cleaned using swabs and alcohol to remove the stratum corneum and prevent potential signal interference. Additionally, all measurements were performed with participants in a supine position, ensuring no metallic objects were in contact with their bodies.

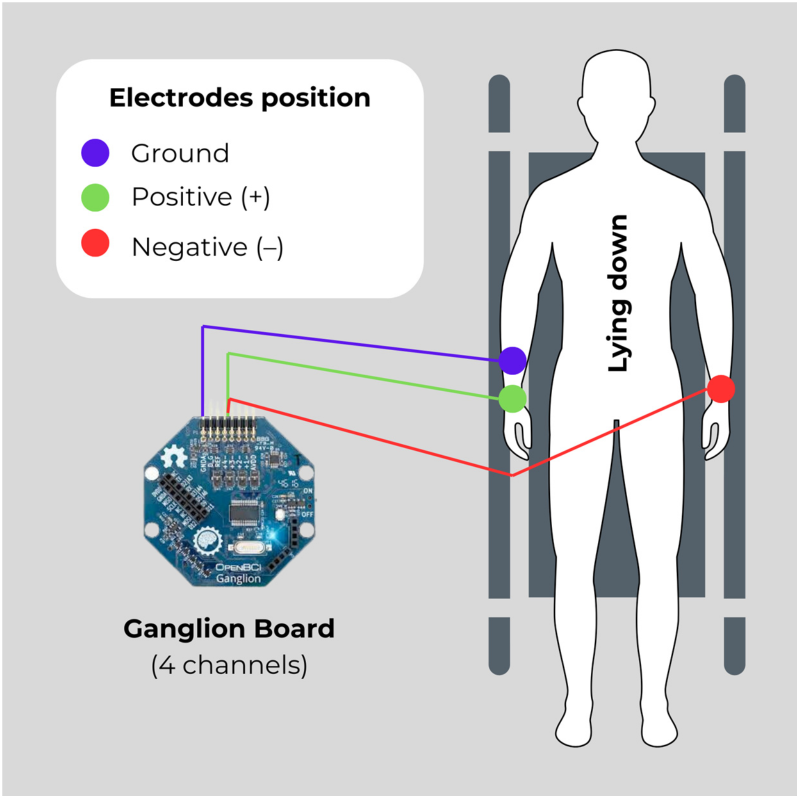

Figure 2. The figure illustrates the setup for recording an electrocardiogram (ECG) using a 4-channel Ganglion Board, with electrodes placed on an individual in a lying down position. A green positive electrode is located on the right arm, 3 cm above the radiocarpal joint, a purple ground electrode is positioned 5 cm above the green electrode, and a red negative electrode is placed on the left arm at the same height as the green electrode. This placement enables the recording of the lead I (DI). The wires connect the electrodes to the Ganglion Board, illustrating the wire configuration for cardiac signal acquisition.

At the conclusion of the analog mission, the collected data for each astronaut were organized into individual folders, segmented by day with corresponding annotations, and manually reviewed. Subsequently, the time series of RR intervals were calculated for each ECG segment using the MATLAB® R2017b platform (The MathWorks, Inc., Natick, MA, USA). The implementation of the Pan–Tompkins algorithm was crucial for detecting the R peak in each ECG segment for each sol [23].

Likewise, the RR signals for each participant can be downloaded from the URL provided in the Data Availability Statement section. The folder titled “RR-Signals” contains four subfolders labeled “SOL-1”, “SOL-2”, “SOL-3”, and “SOL-4”, which group the records for each mission day.

2.3. HRV Parameters

Employing the Kubios HRV Standard® analysis software, version 3.5 (2022), developed by the University of Kuopio in Finland, temporal, spectral, and nonlinear indices of HRV from short-term RR interval time series were computed.

2.4. Linear HRV Parameters

As time-domain measures, the mean RR values (AvRR), the standard deviation of NN intervals (SDNN), the root mean square of successive RR interval differences (RMSSD), and the percentage of successive RR intervals that differ by more than 50 ms (pNN50) were calculated. The following frequency-domain indices were also computed: the high-frequency (HF) power, the low-frequency (LF) power, the LF peak, and the HF peak.

The low-to-high-frequency power ratio was also calculated to reflect the sympathovagal balance (LF/HF) [24]. These classical indices were selected according to relevant literature on HRV analysis, which emphasizes their relevance in evaluating autonomic function and cardiovascular health [25–28].

2.5. Nonlinear HRV Parameters

The application of nonlinear measures provides the ability to quantify the unpredictability present in a time series, which emerges from the intricate complexity inherent in the regulatory mechanisms of HRV [29]. This approach enables a quantitative assessment of unpredictability and a deeper understanding of cardiac dynamics. In this study, the most used nonlinear parameters were computed: the standard deviation of the Poincaré plot perpendicular to the line of identity (SD1), the standard deviation of the Poincaré plot along the line of identity (SD2), and the ratio SD1/SD2. To address the irregularity of the RR signals, approximate entropy (ApEn) and sample entropy (SampEn) were calculated. Detrended fluctuation analysis (DFA) was also performed, providing the scaling exponent α_1 and α_2, which describe short-term and long-term fluctuations, respectively.

2.6. Borg Scale

The Borg scale has been implemented in various crews during space flights in the Space Shuttle program, such as on STS 51-L and STS 61-B [30], to assess muscular and cardiovascular aspects. To enhance the subjective dimension of this study, each participant daily evaluated their perceived exertion using an adapted version of this scale, which ranges from 0 to 10 points. These assessments were conducted explicitly between 9:30 p.m. and 10:30 p.m., allowing for collecting personal evaluations of fatigue and effort after a day filled with simulated activities. This self-assessment method was crucial because it provides a perceived measure of the psychophysical impact of the tasks performed [31]. The Borg scale data are only available from sol 1 to sol 4 because the evaluations were conducted explicitly during the initial four days of the analog mission. This limitation was due to logistical constraints and the need to focus on the participants' early adaptation to the simulated isolation and stress conditions.

2.7. Statistical Analysis

Statistical analyses were performed using GraphPad Prism® 8.02 (GraphPad Software, Boston, MA, USA) with a significance level set at $\alpha = 0.05$. A nonparametric Friedman test for repeated measures was applied to each HRV parameter to compare the autonomic cardiac activity among sols. Following this, uncorrected post hoc Dunn tests were conducted to identify specific differences between days. Nonparametric tests were chosen due to the small sample size [32].

3. Results

Tables 1 and 2 present the median (75th—25th percentile) results of the morning measurements taken during the analog space mission period. The Friedman test identified significant differences across different sols for various HRV parameters.

For the AvRR, the Friedman test indicated significant differences ($F_r = 12.84$, $p = 0.0003$). Specifically, there were significant differences between sol 1 and sol 2 (806.72 ms vs. 1041.25 ms, $p = 0.0006$) and between sol 1 and sol 3 (806.72 ms vs. 1003.96 ms, $p = 0.0143$).

According to the Friedman test, the pNN50 parameter displayed significant differences ($F_r = 8.280$, $p = 0.0313$). Individual comparisons revealed significant differences between sol 1 and sol 3 (11.47% vs. 17.34%, $p = 0.0143$) and between sol 2 and sol 3 (4.21% vs. 17.34%, $p = 0.0275$). In the frequency domain, the low-frequency peak (LF peak) showed significant differences ($F_r = 8.386$, $p = 0.0260$). There was a significant difference between sol 3 and sol 4 (0.11 Hz vs. 0.05 Hz, $p = 0.0071$).

Table 1. Median (75th—25th) percentile values of linear HRV indices over five days or sols during morning measure.

Linear HRV Indices					
Time domain					
Index	sol 1	sol 2	sol 3	sol 4	*p*-Value
RMSSD (ms)	31.39 (74.36–20.76)	24.76 (71.72–20.96)	41.53 (75.26–32.25)	32.72 (47.75–22.76)	0.2982
AvRR (ms)	806.72 (966.54–762.57)	1041.25 (1173.7–887.75) $^{+}$	1003.96 (1137.90–873.70) $^{\#}$	901.89 (1101.87–801.65)	0.0003
SDNN (ms)	34.74 (65.64–23.75)	27.35 (60–21.22)	37.69 (62.35–28.94)	34.27 (39.99–23.13)	0.6522
pNN50 (%)	11.47 (13.23–2.63)	4.21 (13.80–2.31)	17.34 (19.84–9.99) $^{\#,\%}$	10.73 (20.28–2.91)	0.0313
Frequency domain					
LF peak (Hz)	0.06 (0.1–0.05)	0.07 (0.1–0.06)	0.11 (0.13–0.06)	0.05 (0.06–0.04) $^{\circ}$	0.0260
HF peak (Hz)	0.18 (0.28–0.16)	0.21 (0.31–0.16)	0.19 (0.26–0.16)	0.3 (0.32–0.22)	0.8566
LF HF ratio	1.29 (3.06–0.79)	1.11 (2.99–0.66)	1.16 (1.44–0.45)	1.13 (3.97–0.74)	0.5206
LF (ms2)	543.26 (1812.4–366.8)	575.36 (935.47–184.69)	801.84 (2660.9–344.82)	396.49 (820.97–273.6)	0.2982
HF (ms2)	406.9 (2190.9–157.45)	222.14 (1789–116.31)	489.22 (4870.9–392.79)	388.76 (683.3–135.1)	0.3720

$^{+}$ between sol 1 and sol 2, $p < 0.05$. $^{\#}$ between sol 1 and sol 3, $p < 0.05$. $^{\%}$ between sol 2 and sol 3, $p < 0.05$. $^{\circ}$ between sol 3 and sol 4, $p < 0.05$.

Table 2. Median (75th—25th) percentile values of nonlinear HRV indices over five days during morning measures.

Nonlinear HRV Indices					
Index	**sol 1**	**sol 2**	**sol 3**	**sol 4**	***p*-Value**
SD1	22.23 (52.68–14.7)	17.54 (50.83–14.85)	29.42 (37.94–22.84)	23.18 (33.83–16.12)	0.9438
SD2	43.85 (76.53–29.91)	35.69 (67.94–25.3)	44.46 (61.34–33.79)	43.51 (46.09–26.57)	0.9999
SD1/SD2	1.53 (2.33–1.44)	1.55 (1.98–1.34)	1.22 (1.63–1.15)	1.39 (2.13–1.25)	0.2096
ApEn	1.11 (1.14–1.03)	1.01 (1.04–0.97)	1.02 (1.09–0.93)	1.06 (1.07–1.01)	0.1232
SampEn	1.79 (1.91–1.69)	1.80 (1.95–1.64)	1.84 (2.14–1.58)	2.01 (2.19–1.62)	0.7090
DFA $\alpha 1$	1.10 (1.33–0.82)	0.94 (1.19–0.73)	0.81 (1.09–0.67)	0.84 (1.2–0.77)	0.3720
DFA $\alpha 2$	0.40 (0.44–0.26)	0.33 (0.4–0.21)	0.19 (0.36–0.11) $^{\#}$	0.44 (0.7–0.27) $^{\circ}$	0.0313

$^{\#}$ between sol 1 and sol 3, $p < 0.05$. $^{\circ}$ between sol 3 and sol 4, $p < 0.05$.

For nonlinear indices (Table 2), the DFA α_2 parameter exhibited significant differences (Fr = 8.280, $p = 0.0313$). Significant differences were found between sol 1 and sol 3 (0.40 vs. 0.19, $p = 0.0143$) and between sol 3 and sol 4 (0.19 vs. 0.44, $p = 0.0275$).

In the second part of this study, which involved nighttime measurements, significant statistical differences were found only in the nonlinear index ApEn between sol 1 and sol 3 (1.02 vs. 1.15, $p = 0.0044$). The Friedman test indicated significant differences for ApEn ($F_r = 8.400$, $p = 0.0085$). The results, presented as median (75th—25th percentile) values of the measurements taken at night during the analog space mission period, are summarized in Tables 3 and 4.

Table 3. Median (75th—25th) values of HRV indices over three days during night measure.

Linear HRV Indices				
Time domain				
Index	sol 1	sol 2	sol 3	*p*-Value
RMSSD	43.83 (60.69–22.95)	42.42 (61.94–18.92)	34.82 (63.89–22.32)	0.9537
AvRR	862.03 (917.93–839.66)	946.46 (1048.40–728.94)	814.50 (906.34–745.64)	0.9537
SDNN	36.97 (51.96–20.6)	38.81 (55.38–22.03)	23.36 (56.73–19.97)	0.3673
HR	69.60 (71.5–65.46)	63.39 (82.35–57.58)	73.66 (80.96–66.76)	0.3673
pNN50	18.38 (24.86–1.91)	15.17 (20.34–1.19)	10.19 (12.95–1.83)	0.3673
Frequency domain				
LF peak (Hz)	0.07 (0.11–0.07)	0.09 (0.12–0.07)	0.06 (0.09–0.05)	0.5216
HF peak (Hz)	0.25 (0.33–0.17)	0.27 (0.36–0.16)	0.35 (0.37–0.21)	0.4228
LF HF ratio	1.05 (3.42–0.69)	1.50 (3.84–0.92)	1.34 (2.01–0.95)	0.6914
LF (ms^2)	559.9 (3858.89–204.96)	659.65 (1728.5–354.86)	698.97 (1609.59–237.25)	0.6914
HF (ms^2)	530.35 (3789.17–127.5)	557.37 (1468.38–89.23)	610.85 (1427.69–117.05)	0.5216

Table 4. Median (75th—25th) values of nonlinear HRV indices over three days during the night measure.

Nonlinear Indices				
Index	**sol 1**	**sol 2**	**sol 3**	***p*-Value**
SD1	31.05 (42.57–16.25)	30.05 (43.89–13.39)	25.51 (45.26–15.81)	0.9537
SD2	42.17 (60.16–24.15)	45.99 (64.95–28.03)	45.62 (66.73–23.35)	0.9999
SD1/SD2	1.35 (1.64–1.18)	1.57 (2.03–1.44)	1.53 (1.59–1.36)	0.6914
ApEn	1.02 (1.08–0.91)	1.07 (1.18–0.98)	1.15 (1.28–1.05) [#]	0.0085
SampEn	1.83 (2.07–1.36)	1.93 (2.16–1.8)	2.11 (2.25–1.86)	0.5216
DFA $\alpha1$	0.94 (1.09–0.89)	0.97 (1.19–0.86)	1.04 (1.1–0.86)	0.5216
DFA $\alpha2$	0.36 (0.54–0.28)	0.31 (0.44–0.21)	0.36 (0.43–0.23)	0.9537

[#] between sol 1 and sol 3, $p < 0.05$.

Moreover, significant changes were observed in the Borg Scale and the crew's median weight throughout the analog mission period. The Friedman test for the Borg Scale indicated significant differences across the states of load ($F_r = 11.47$, $p = 0.0022$). Specifically, as shown in Table 5, the Borg Scale ratings between sol 1 and sol 2 decreased significantly from a median of 6 points (7.0–5.0) to 3 points (3.5–2.5) ($p = 0.0015$).

Table 5. Median (75th—25th) values of Borg Scale during the analog mission.

Borg Scale					
Data	**sol 1**	**sol 2**	**sol 3**	**sol 4**	***p*-Value**
Borg Scale (points)	6 (7–5)	3 (3.5–2.5) [+]	5 (6–4)	5 (6–4)	0.0022

[+] between sol 1 and sol 2, $p < 0.05$.

Significant changes were found in the crew's median weight, which decreased from 66.18 kg (75.45–56.21 kg) in sol 1 to 64.71 kg (73.72–55.25 kg) in sol 5. This represents a median weight reduction of 1.44 kg (2.04–0.96 kg) by the end of the study period.

Additionally, complementary data were collected (Table 6) regarding the environment where the analog space mission occurred over four days. In the morning (7:30 a.m.), the average temperature was 21.2 °C, with an average humidity of 7.7%. For the night (7:30 p.m.), the average temperature was 31.2 °C, with an average humidity of 10.5%

Table 6. Weather values during the analog mission.

Habitat Data				
Data	**sol 1**	**sol 2**	**sol 3**	**sol 4**
Temperature at 7:30 a.m. (°C)	20.5	21.1	22.7	20.5
Humidity at 7:30 a.m. (%)	7%	7%	8%	9%
Temperature at 7:30 p.m. (°C)	31.6	31.1	31.6	30.5
Humidity at 7:30 p.m. (%)	9%	10%	11%	12%

4. Discussion

This study investigated heart rate fluctuations in five analog astronauts over a four-day period within a confined and isolated environment simulating Martian conditions. Short-term HRV measurements were utilized to examine changes in the autonomic nervous system attributable to isolation and confinement. Significant differences in HRV parameters were observed among different sols, underscoring the impact of the mission environment on autonomic cardiac function.

Significant changes in morning median AvRR were observed during this study, suggesting an adaptation of the autonomic nervous system. Specifically, AvRR showed significant differences between sol 1 and sol 2 and between sol 1 and sol 3. These findings may indicate a reduction in heart rate and an increase in vagal activity over time, potentially reflecting cardiovascular adaptation and improved autonomic regulation in response to the simulated space environment. Shaffer and Ginsberg [29] documented that the observation schedule is a relevant external factor due to a circadian rhythm in HRV. In contrast, no significant changes in nocturnal linear HRV were observed among the different sols.

The median values of the scaling exponent α_2 of the analog crew decreased significantly during the analog mission. In recent observational studies, Otsuka [27] suggests that some indices describing the nonlinear dynamics of HRV, such as the short-term fractal scaling exponent (α), measured by DFA, may constitute important predictors of adverse cardiovascular events.

The only nonlinear index that detected differences in nocturnal HRV was ApEn. Interestingly, this increase in sol 3 could be linked to an increase in HRV complexity and the combined activity of the sympathetic and parasympathetic systems. Beckers [33] indicates that ApEn is strongly associated with indices describing heart rate vagal modulation. Therefore, we can infer that there was distinct autonomic modulation during the progression of the analog mission.

The LF peak, although known to be amplified by vagal activity, primarily serves as an index of sympathetic modulation and its changes [34]. Changes in spectral analysis, particularly in the LF peak towards the end of the mission, suggest potential alterations in both sympathetic and parasympathetic activity. DeBoer [35] indicated that the LF peak in HRV was related to the relatively slow baroreceptor response to beat-to-beat changes in arterial pressure. Psychological stress, physical activity, and moderate exercise in healthy subjects are some factors that typically increase LF [25].

The modified Borg scale (0 to 10) was used to assess the work intensity after each day's activities. Scores on this scale are subjective. At the beginning of the mission, crew members reported a level of 6 on the Borg scale, indicating a perception of "heavier" exertion. However, on sol 2, this perception decreased to level 3, indicating an effort perceived as "light." This reduction in perceived exertion aligns with the observed decrease in heart rate, suggesting an adaptation to the environment. For the remainder of the mission, the perception of effort returned to a level 5, indicating ongoing "heavy" exertion. Our findings show significant differences compared to the survey conducted by Kordi et al. [36], who studied crew performance following chest compressions in simulated hypogravity and microgravity. The use of the Borg scale, combined with HRV, is significant as it can help detect and prevent overtraining in the future. Overtraining leads to a hormonal response that alters the sympathetic–vagal balance, and early detection through these measures could mitigate such effects.

The results of this preliminary analog mission, involving a crew exposed to extreme conditions, high workloads, and isolation, suggest the need for further studies in similar environments. This aligns with Smith's findings on weight loss in a simulated lunar gravity workload study and Heinicke's observations on group-living habits during three long-duration Mars analog missions at Hi-Seas in Hawaii, USA. Smith's study revealed significant differences in metabolic expenditures and cognitive tasks during a 60 min walking trial at reduced body weight, while Heinicke's research provided insights into crew self-organization and social dynamics in long-duration missions [37,38].

The activities conducted during this mission align with the previous research carried out by Mall et al. [39] at the Mars Desert Research Station (MDRS). Like Mall's study, our mission encompassed a variety of critical tasks, ranging from extravehicular activities (EVA) to drone operation and the use of advanced telecommunication systems. In addition, key aspects of human adaptation to hostile environments were assessed, rigorously analyzing the participants' ability to perform complex tasks under adverse environmental conditions. Field tests of 3D printing were also integrated, exploring its potential as a tool for producing emergency parts and structural components during long-duration missions. This alignment strengthens the validity of our study and highlights the importance of developing and comparing operational procedures across multiple analog environments to advance research in human adaptation and applied technologies for space exploration.

Vigo's research during Mars500 [40] reported an increase in parasympathetic activity over the days in the crew, identified by a notable increase in HF power. These results are in line with our research, as it has been demonstrated that pNN50 is closely correlated with parasympathetic nervous system activity [41] and HF [29]. However, they are not entirely comparable, as Vigo's study recorded long-term ECG measures using a digital Holter, whereas our measurements were short-term. Additionally, the Mars500 crew was composed exclusively of men.

HRV allows for a comprehensive assessment of astronauts' physical and emotional state (a key indicator of fatigue, stress, and well-being) and provides valuable information for adjusting the intensity of training sessions according to each individual's heart's autonomous regulatory capacity. Psychological stressors, such as prolonged isolation and confinement, can exacerbate physiological issues, leading to increased strain on cardiovascular health. Incorporating nonlinear HRV indices emerges as a helpful tool in the diagnostic realm and for evaluating the effects of individual treatments [24]. Their ability to provide more consistent and reliable measurements over time makes them a standout option for precise and detailed monitoring.

The main limitation encountered during the analog mission was the limited number of subjects evaluated, as crew sizes in space missions are similarly small [30]. Additional challenges included adhering to a strict schedule, variations in workload between missions, sleep quality issues, fluid intake, and exposure to extreme temperatures. Furthermore, the lack of specialized medical equipment, refrigeration units for sample preservation, and sufficient electrical power severely restricted the ability to conduct biomolecular studies and blood sampling. There were also no designated spaces for ECG recording, further complicating data collection.

The results of our study should be interpreted with caution due to its exploratory nature and the small sample size. We conducted the research with limited resources, aiming to replicate the conditions astronauts often face, which typically lack access to sophisticated equipment. These constraints further highlight the necessity for non-invasive tools like HRV to assess physiological adaptation in extreme environments, where blood sampling may not always be a viable option. HRV has been shown to effectively analyze changes in various biochemical or hematological variables during stressful situations, such as cortisol and histamine levels. For example, Pulopulos [42] demonstrated that a greater decrease in HRV during the anticipation of a stress task is associated with a higher cortisol increase induced by the task, though not with cortisol recovery. Anticipatory HRV, reflecting differences in stress regulation and prefrontal activity before encountering the stressor, is crucial for understanding the stress-induced cortisol response. Similarly, *Murakami* [43] identified alterations in the histamine H1 receptor through HRV analysis in genetically modified mice, further validating the usefulness of HRV as a non-invasive tool to assess physiological changes in extreme environments.

Future research should focus on comparing the HRV of astronaut crews who have traveled to space or are in training with analog astronauts; it should also explore new nonlinear parameters and standardize experimental procedures among different analog space missions to obtain a comparative analysis with long-term records and a larger sample size.

Understanding these variations is essential for enhancing physical and mental health monitoring and developing adaptive support systems for astronauts in future space missions, considering the circadian influences on their physiological states.

Recognizing that the limited duration of our analog mission imposes certain constraints on the generalizability and fidelity of our findings compared to longer space missions, we plan to address these limitations by conducting follow-up missions of extended duration in various analog environments. By increasing both the duration and diversity of these environments—specifically in regions of Mexico that present extreme environmental conditions, such as the Pinacate Desert in Sonora and the Cueva de Villa Luz in Tabasco—we aim to validate and expand upon our preliminary results. These sites will enable the implementation of more advanced physiological monitoring protocols and the integration of specialized medical technologies, further enhancing our understanding of human adaptation in hostile environments.

5. Conclusions

This study demonstrates that analog space environments are valuable for investigating human adaptation and social bonding under isolation and confinement. Exposing a crew to Mars analog conditions led to increased vagal activity during certain adaptation periods, which may be crucial for cardiovascular adaptation and survival. Changes in autonomic function, as shown by HRV indices, were observed, highlighting the physiological impact of the simulated environment. Psychological stressors, such as isolation, likely exacerbated these physiological changes. The Borg scale effectively assessed perceived exertion, underscoring its utility in managing workloads in analog environments. However, results should be interpreted cautiously due to this study's exploratory nature and small sample size.

Future research should focus on comparing HRV in space-trained astronauts and analog astronauts, exploring new nonlinear parameters, and standardizing experimental procedures across analog missions. This will enhance health monitoring and develop adaptive support systems for astronauts, considering circadian influences on physiological states.

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