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Analysis of Human Physiology in Simulated Lunar and Martian Hypogravity With and Without Virtual Reality

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Abstract

Hypogravity environments expected in future Lunar and Martian missions require a deeper understanding of their effects on human physiology, and virtual reality (VR) has emerged as a complementary tool in experimental simulations, providing enhanced immersion and sensorimotor integration. To investigate how exposure to simulated hypogravity, with and without VR, affects cardiovascular responses during walking under partial gravity, thirty healthy volunteers completed 10-minute walking trials in six randomized scenarios—Earth ($\mathbf{1}\,\boldsymbol{G}$), Moon ($\mathbf{0.20}\,\boldsymbol{G}$), and Mars ($\mathbf{0.38}\,\boldsymbol{G}$), each with and without VR—using an AlterG treadmill for body-weight reduction via lower-body positive pressure. During each condition, systolic and diastolic blood pressure, heart rate, and peripheral oxygen saturation were monitored, and the data were analyzed using repeated-measures ANOVA and comparison of marginal means. The findings revealed that while ANOVA did not identify significant effects of scenario or VR on systolic, diastolic, or mean arterial pressure ($\boldsymbol{p} > \mathbf{0.05}$), significant interaction effects were observed for VR $\times$ Time ($\boldsymbol{p} = \mathbf{0.03}$) and Scenario $\times$ Time ($\boldsymbol{p} = \mathbf{0.01}$). Marginal means comparisons showed that heart rate was higher in the Earth condition at all evaluated time points compared with the Moon and Mars conditions ($\boldsymbol{p} < \mathbf{0.001}$), and in the VR $\times$ Time interaction, heart rate increased more rapidly in the absence of VR, whereas VR delayed this physiological response; additionally, peripheral $\boldsymbol{O_2}$ saturation was slightly lower with VR ($\boldsymbol{p} = \mathbf{0.01}$). In conclusion, simulated hypogravity reduces cardiovascular demand during walking, and VR modulates the dynamics of the physiological response by delaying the rise in heart rate, suggesting that the AlterG + VR combination is safe, reproducible, and effective for physiological preparation in crewed missions of the Artemis program and future missions to Mars.

Keywords: hypogravity; virtual reality; human physiology; AlterG; Artemis

1 Introduction

Space exploration, initially driven by geopolitical competition, has evolved into a field of scientific and technological collaboration. In the twentieth century, advances in rocketry enabled missions originally designed for military purposes to be progressively redirected toward space research. During the Cold War, the space race between the United States and the Soviet Union led to pioneering achievements such as the Luna 2 mission (1959), the first human-made object to reach the lunar surface [1], and the flights of Yuri Gagarin and Alan Shepard (1961), which marked the beginning of human presence in space [2]. This progression culminated in the Apollo Program (1968–1972), responsible for the first lunar walk in 1969 [3], and later in the Space Shuttle Program (1981–2011), which enabled long-duration microgravity experiments and the construction of the International Space Station (ISS), a major milestone in international collaboration among space agencies [3, 4].

The current "Second Space Age" is marked by the growing involvement of private industry, expanding access to space and accelerating the pace of innovation. Companies such as SpaceX, Blue Origin, and Axiom are now leading launch activity and crewed

missions [3, 5], including Inspiration4—the first fully private orbital mission—which contributed to the development of the Space Omics and Medical Atlas (SOMA) [4, 6]. Between 2024 and 2025, record numbers of orbital launches were documented, with more than 50% conducted by SpaceX, effectively democratizing civilian access to space [7]. Programs such as Artemis and proposals for crewed missions to Mars reinforce the notion that sustained human presence beyond low Earth orbit is increasingly within reach [3, 8].

The prospect of sustained human presence in extraterrestrial environments makes it essential to understand how the human body responds to intermediate gravity levels. Although the effects of microgravity are well documented, particularly from studies conducted in orbit and on the ISS, data on hypogravity remain scarce [9, 10], including partial-gravity levels such as those on the Moon (≈1/6 g) and Mars (≈1/3 g). Preliminary evidence indicates that these environments may affect venous return, cardiovascular control, balance, and the sensory mechanisms responsible for spatial orientation, thereby increasing the risk of motion sickness [11–14].

The use of lower-body positive pressure (LBPP) systems, such as the AlterG treadmill, enables simulation of reduced body load during walking and represents one of the most promising approaches for hypogravity simulation. In parallel, virtual reality (VR) has emerged as a relevant complementary technology by providing immersive visual, vestibular, and proprioceptive stimuli, thereby enhancing the validity of experimental models of reduced gravity [15].

Although the effects of microgravity have been extensively investigated, human physiological responses to hypogravity under controlled conditions remain poorly understood, particularly when combined with immersive sensory stimulation provided by virtual reality. Prior to this experiment, our group conducted a systematic review across PubMed, Scopus, Web of Science, the Cochrane Library, and IEEE Xplore, identifying 1,412 records and including 23 studies after applying eligibility and internal validity criteria [16]. The review showed that virtual reality–based hypogravity simulators can influence physiological responses; however, the literature remains fragmented, with heterogeneous protocols and limited studies examining active walking in partial hypogravity. Based on this gap, the present study investigated how exposure to simulated hypogravity (Moon and Mars) affects cardiovascular variables and whether virtual reality modifies these responses.

2 Methods

The study was conducted in accordance with the principles of the Declaration of Helsinki and national regulations for research involving human participants. It was approved by the Ethics Committee of the Lisbon Academic Medical Center (CAML), which comprises the Faculty of Medicine of the University of Lisbon (FMUL), the João Lobo Antunes Institute of Molecular Medicine (iMM), and Santa Maria Hospital (CHULN), under registration No. 219/24, on 25 November 2024. All participants were fully informed about the procedures and risks of the experiment and signed the informed consent form, with the guarantee of voluntary withdrawal at any time without penalty or justification.

2.1 Study design

This was a randomized, repeated-measures experimental study involving the simulation of partial hypogravity (Moon and Mars) on an anti-gravity treadmill, with and without virtual reality. Each participant completed six 10-minute walking trials under different gravity and sensory-immersion conditions, with serial physiological monitoring throughout.

Data collection took place between 5 and 12 May 2025 at the Center for Aerospace Medicine Studies (CEMA), School of Medicine, University of Lisbon, Portugal, in a laboratory composed of two rooms:

- Room 1: anthropometric assessment (height, abdominal circumference, and lower-limb length);
- Room 2: body mass measurement, selection of the neoprene shorts compatible with the AlterG treadmill, and computerized randomization of the virtual scenarios.

2.2 Participants

Healthy volunteers (30) were recruited through digital outreach on social media and individual contact for clarification of questions. Inclusion criteria were: age between 18 and 40 years, non-smokers, no continuous medication use (except hormonal contraceptives), and height between 154 and 190 cm, required for AlterG treadmill calibration.

Before data collection, participants were instructed to: abstain from stimulants (coffee, energy drinks, tea) for 24 hours beforehand; wear sports clothing.

2.3 Equipment and materials

The following equipment was used: BIC Bem Estar YK 80B pulse oximeter; Riester Big Ben pedestal aneroid sphygmomanometer; 3M Littmann Classic II SE stethoscope; two Cescorf measuring tapes; Mondial BL-03 digital scale; VR SHINECON virtual reality headset; Samsung Galaxy A35 5G and iPhone 7 smartphones (iOS 15.8.4); and an AlterG VIA 500/500X Anti-Gravity Treadmill® (LBPP).

2.4 Experimental procedures

Upon arrival at the Center for Aerospace Medicine Studies (CEMA), each participant was welcomed by the research team, informed about the study objectives, and invited to sign the informed consent form. Next, while seated, baseline physiological measurements were collected: systolic and diastolic blood pressure, heart rate, and peripheral oxygen saturation (SpO_2), using an aneroid sphygmomanometer, stethoscope, and digital oximeter.

Next, participants were taken to Room 1 of the CEMA, where anthropometric measurements were obtained: height (using a wall-mounted measuring tape), abdominal circumference, and lower-limb length (from the greater trochanter to the floor, without shoes). Subsequently, in Room 2, body mass was measured and the neoprene

shorts compatible with the AlterG treadmill were selected to ensure proper fit and adequate sealing of the lower-body positive pressure (LBPP) system.

The order of the six experimental scenarios was individually randomized for each participant using a spreadsheet (RAND function in Microsoft Excel). The Optimal Walking Speed (OWS) was calculated individually for each participant and for each gravitational condition (Earth, Moon, and Mars). The calculation was based on the principle of dynamic similarity, which relates locomotion speed to gravity and leg length through the Froude number ($Fr = V^2/gL$) [17]. Under this principle, geometrically similar bodies exhibit comparable gait dynamics at the same Froude number. Therefore, walking speed scales with gravitational acceleration and characteristic leg length, allowing the estimation of dynamically equivalent walking speeds across different gravity levels. This approach ensured comparable locomotion conditions across the experimental scenarios.

Before the tests began, the virtual reality (VR) headset was adjusted to each participant's head circumference to ensure comfort and stability during walking. Physiological sensors were positioned in a standardized manner: the blood pressure cuff on the left arm and the pulse oximeter on the right index finger. After entering the AlterG VIA 500/500X Anti-Gravity Treadmill®, the chamber was sealed, the equipment calibrated, and the internal pressure equalized before initiating the protocol. These initial steps are shown in Figure 1.

A 10-minute walking period was chosen for each scenario, considering that cardiovascular and respiratory steady state is typically reached between 3 and 6 minutes under terrestrial gravity, whereas in hypogravity and microgravity, exercise durations of 5 to 10 minutes are recommended to capture slower hemodynamic and autonomic adaptations [10, 18, 19]. Because the 3- to 10-minute interval is widely validated for obtaining reliable physiological data in humans [10, 18, 20], the upper limit of this interval was adopted to ensure stabilization of the physiological responses.

Each participant completed six 10-minute walking trials across the following scenarios: Earth (1G), Moon (0.20 G), and Mars (0.38 G), each with and without virtual reality. During all walks, systolic and diastolic blood pressure, heart rate, and peripheral oxygen saturation (SpO_2) were recorded at 0, 5, and 10 minutes. After completing the six scenarios, participants remained under medical observation for 10 minutes in the rest area for monitoring and recovery, as shown in Figure 2.

The virtual reality device was disinfected with an antiseptic solution between sessions to ensure biosafety and comfort for subsequent participants.

2.5 Selection of Virtual Reality Applications

The selection of virtual reality applications used in the immersive scenarios was based on predefined criteria organized into three dimensions: the immersion and realism of the environment, suitability for hypogravity simulation during walking, and usability.

For the "immersion and realism" dimension, the following aspects were evaluated: visual fidelity to the terrestrial, Lunar, or Martian environment, including landscapes, colors, topography, and lighting conditions, as well as auditory elements, when present, such as background music and sound effects capable of enhancing the sense of presence in the simulated environment. The degree of engagement (the feeling of "being there,"

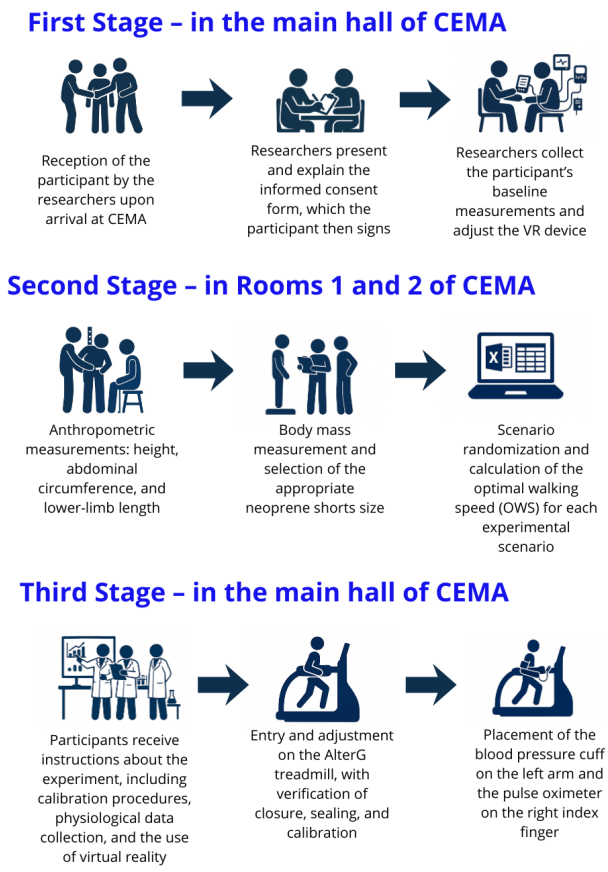


Figure 1 – Initial stages of the experiment prior to walking.

with reduced awareness of the real surroundings) and the possibility of interacting with the scenario, such as walking, exploring, or performing simple actions in the virtual environment, were also considered.

For the "suitability for hypogravity simulation" dimension, applications were assessed for compatibility with use during walking on the AlterG treadmill, functioning alongside physical exertion without requiring constant manipulation. Priority was given to applications that offered smooth visual motion during walking, with visual feedback coherent with the participant's displacement and kinetic stimuli capable of enhancing the sensation of walking under different gravitational fields (such as on the Moon or Mars).

Criteria for the "usability" dimension included ease of operation for both researchers and participants, simple and fast setup, absence of crashes during continuous use, and compatibility with the available devices, including smartphones and VR

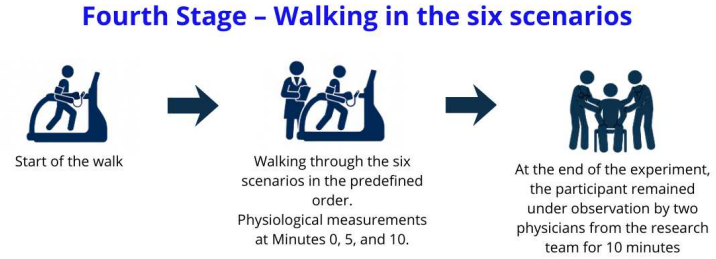


Figure 2 – Fourth stage of the experiment: walking across the six scenarios.

headsets. Language, audio, and brightness adjustments were also considered, as well as the availability of free or educational-license versions.

Each application was individually tested in 15 to 30 minute sessions conducted between March and April 2025, during which impressions regarding performance, strengths, limitations, and subjective immersion were recorded. As limitations of the selection process, it is acknowledged that the evaluation was performed by a single assessor, a biomedical informatics undergraduate student from UFCSPA, which may limit perceptual variability, and that the small number of mobile devices tested may have influenced performance differences across applications.

After screening, two main applications were selected: VR Moon Walk (for the Lunar scenario) and Mars Walk (for the Martian scenario). For the Earth scenario, VR-format videos available on YouTube were chosen due to their higher graphic quality and 360° visualization, which allowed participants to explore the virtual environment with a greater sense of real displacement. Additionally, the videos demonstrated better technical performance on the devices used, required less smartphone processing, and combined visual and auditory stimuli, resulting in greater stability and enhanced perceptual immersion compared with the tested applications.

2.6 Collected Variables and Outcomes

During the experiment, anthropometric, physiological, and subjective variables were collected to assess participants' responses to walking under simulated hypogravity, with and without the use of virtual reality.

I. **Anthropometric variables:**
   (a) Height, abdominal circumference, and lower-limb length (right and left). These measurements were obtained using a measuring tape, with a wall reference for height. Lower-limb length was measured from the greater trochanter to the floor, without footwear. In cases of discrepancy between limbs, the higher value was used.
   (b) Body mass measured using a digital scale, followed by the selection of the appropriate neoprene shorts for use on the AlterG treadmill.

II. **Physiological variables:**

(a) Systolic and diastolic blood pressure, heart rate, and peripheral oxygen saturation (SpO_2). Measurements were taken with the participant seated before the walk, using a sphygmomanometer, stethoscope, and pulse oximeter.
(b) In each scenario, physiological responses were monitored, with heart rate progression recorded at 0, 5, and 10 minutes, allowing comparisons among Earth, Moon, and Mars conditions.

III. **Primary outcome:**
(a) To evaluate the impact of virtual reality combined with hypogravity on physiological responses

IV. **Secondary outcomes:**
(a) To compare differences in physiological responses across Earth, Moon, and Mars gravity levels.

The data collected during the experiment were recorded in a standardized manner using digital forms developed in Google Forms, which were completed in real time by the research team during the execution of the experimental scenarios. The forms were structured with mandatory fields to prevent information loss and to standardize the collection process. After each session, responses were automatically exported to Google Sheets spreadsheets, ensuring traceability and integrity of the records. The resulting spreadsheets were then organized and submitted to the statistical analysis procedures described in the following subsection.

2.7 Statistical analysis and reproducibility

Initially, a descriptive analysis of the data was performed using tables, boxplots, and profile plots to examine the distribution of the mean values of the physiological variables over time, grouped by Virtual Reality (with/without), Scenario (Earth, Moon, and Mars), and the interaction between these factors. The physiological variables analyzed included systolic, diastolic, and mean arterial blood pressure, heart rate, and peripheral oxygen saturation (SpO_2).

For the inferential analysis, a Repeated-Measures ANOVA model was applied, considering the factors Scenario, Virtual Reality, and Time (0, 5, and 10 minutes), as well as their interactions. In all analyses, a significance level of $\alpha = 0.05$ was adopted. The choice of this model aimed primarily to understand the behavior of physiological variables as a function of the experimental factors over time, evaluating how the effects of Scenario and Virtual Reality manifest and evolve across different time points, rather than performing only pointwise comparisons between factors.

Model assumptions were assessed prior to the analysis, also adopting a significance level of $\alpha = 0.05$. Normality was evaluated using the Shapiro–Wilk test, and sphericity was assessed using Mauchly's test. No additional corrections or transformations were applied to data that did not meet these assumptions, and analyses were conducted using the original data.

When the Repeated-Measures ANOVA showed statistical significance ($p \leq 0.05$), results were further explored using estimated marginal means, considering $\alpha = 0.05$, in order to identify which combinations of factors presented statistically significant

differences. All statistical analyses were performed using R software, version 4.4.3 (2025-02-28 ucrt).

3 Results

The data for this study were collected according to the protocol described in Section 2, at the CEMA, between 5 and 12 May 2025. The research team organized the CEMA facilities one day prior to the start of the experiment in order to optimize data collection.

3.1 Sample Characterization

The sample consisted of 30 participants, 16 male and 14 female. The total sample presented a mean height of 170.78 cm (SD = 8.82; median = 171.5; IQR = 164.88–177.38). When segmented by sex, the mean height was 176.1 cm among men (SD = 6.5; median = 176.0) and 164.7 cm among women (SD = 7.1; median = 165.3). The observed ranges were 160.8 to 188.0 cm for men and 153.0 to 180.0 cm for women, consistent with the mean difference and without relevant overlap in medians. Greater variability was noted among the women compared with the men.

The initial descriptive analysis of the sample characteristics showed that men presented higher mean values for height, abdominal circumference, body mass, lower-limb length, and blood pressure, whereas women exhibited lower dispersion in these variables. For heart rate, wide variability was observed in both sexes, and peripheral oxygen saturation remained high for most of the sample (overall mean = 96.43%). In both groups, the distribution was strongly concentrated at the upper limit (Q3 = 99 and maximum = 99), with greater variability in the female group due to lower minimum values.

The description of the variables is presented in Table 1, and selected box-plot graphs for these variables are shown in Figure 3.

Men displayed higher mean values for height, abdominal circumference, body mass, lower-limb length, and blood pressure, with generally larger standard deviations and wider ranges, particularly for abdominal circumference and blood pressure.

Women presented lower means for these variables and lower relative dispersion in body mass and systolic and diastolic pressures. Regarding heart rate, means and medians were similar between sexes, although broad variability was present in both groups. Oxygen saturation remained high for most participants, with a distribution highly concentrated near the upper limit and a slight difference in mean values favoring the male group.

Overall, the sample demonstrates low variability and no markedly discrepant values.

Table 1 Values obtained during the experiment for sample description.

Variable	Group	N	Mean	Std Dev	Minimum	Maximum
Height (cm)	Men	30	170.78	8.82	153.00	188.00

Variable	Group	N	Mean	Std Dev	Minimum	Maximum
	Total	14	164.71	7.07	153.00	180.00
	Women	16	176.08	6.54	160.80	188.00
	Men	30	68.25	13.99	44.60	106.30
Body Mass (kg)	Total	14	59.35	7.11	44.60	69.70
	Women	16	76.04	14.01	56.90	106.30
	Men	30	112.27	11.28	86.00	140.00
Systolic BP (mmHg)	Total	14	106.00	8.98	86.00	118.00
	Women	16	117.75	10.37	100.00	140.00
	Men	30	71.37	8.70	55.00	93.00
Diastolic BP (mmHg)	Total	14	67.29	4.39	60.00	72.00
	Women	16	74.94	10.03	55.00	93.00
	Men	30	75.63	14.49	46.00	99.00
Heart Rate (bpm)	Total	14	77.14	15.80	55.00	99.00
	Women	16	74.31	13.63	46.00	97.00
	Men	30	96.43	6.34	76.00	99.00
Oxygen saturation (%)	Total	14	95.50	8.07	76.00	99.00
	Women	16	97.25	4.43	81.00	99.00
	Men	30	88.05	4.62	78.50	99.00
Selected Lower Limb Length (cm)	Total	14	86.54	4.78	78.50	92.50
	Women	16	89.38	4.19	81.50	99.00
	Men	30	24.43	3.78	18.00	39.00
Age (years)	Total	14	22.79	2.75	18.00	27.00
	Women	16	25.88	4.03	22.00	39.00
	Men	30	73.82	10.75	58.00	99.00
Abdominal Circumference (cm)	Total	14	65.69	5.65	58.00	76.50
	Women	16	80.94	8.94	69.50	99.00
	Men	30	23.32	3.91	17.43	33.18
BMI	Total	14	21.95	3.07	17.43	29.78
	Women	16	24.53	4.28	17.56	33.18
	Men	30	85.00	8.25	68.67	105.33
Mean Arterial Pressure (mmHg)	Total	14	80.19	5.28	68.67	87.33
	Women	16	89.21	8.17	73.33	105.33

3.2 Analysis of VR and Scenario Over Time – Blood Pressure

Mean values of systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) showed only small variations across the experimental scenarios (Earth, Moon, and Mars) and between the conditions with and without virtual reality. However, the standard-error intervals of the means overlapped extensively,

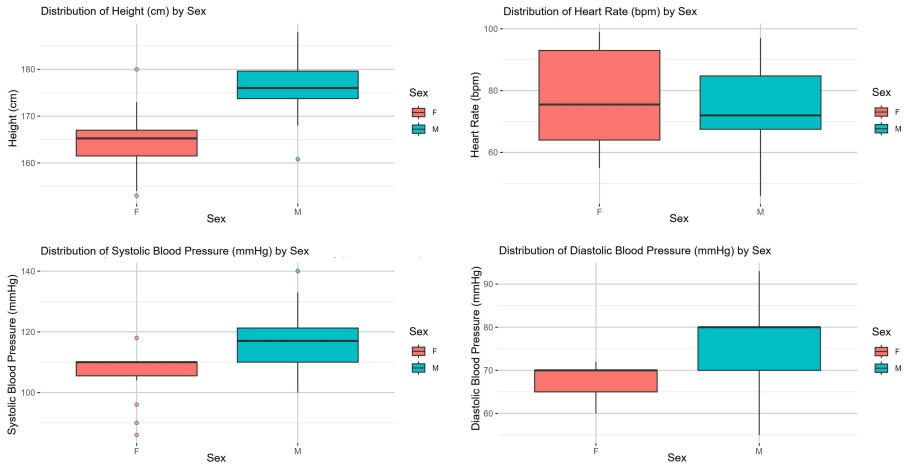


Figure 3 – Box-plot charts describing the experimental sample.

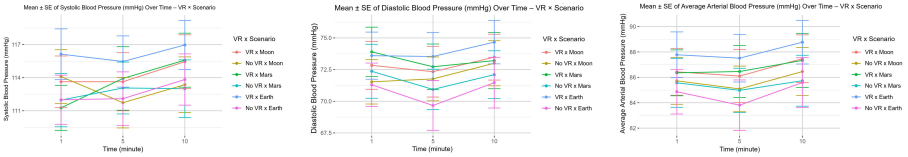


Figure 4 – Profile plots showing blood pressure measures over time.

indicating similarity across groups and no consistent trend of increase or decrease associated with hypogravity or VR use.

The repeated-measures ANOVA (RM-ANOVA) showed that for all three blood pressure variables—SBP, DBP, and MAP—none of the experimental factors (simulated gravity: Earth, Moon, Mars; VR use: with/without; or measurement time: 0, 5, and 10 minutes), nor their interactions, reached statistical significance (all $p > 0.05$). Thus, at the 5% significance level, there was no evidence that simulated hypogravity or virtual reality influenced mean blood pressure values during walking on the anti-gravity treadmill. Figure 4 presents profile plots illustrating these relationships over time.

3.3 Analysis of VR and Scenario Over Time – Heart Rate

A descriptive analysis of the data was performed using profile plots to examine the distribution of mean heart rate values over time, grouped by virtual reality, scenario, and their combined conditions, as shown in Figure 5.

Mean heart rate values varied over time and across the different experimental scenarios. The Earth (1G) condition consistently showed higher mean heart rate values

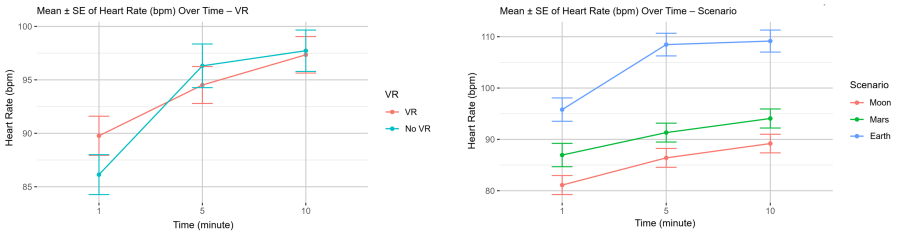


Figure 5 – Profile plots of heart rate over time.

Table 2 Differences in marginal means of heart rate for the Scenario × Time interaction (lowercase letters within the same row indicate equal mean values; uppercase letters within the same column indicate equal mean values).

TIME / SCENARIO	MOON*	MARS*	EARTH*
0*	81.08 ± 1.89 aA	87.00 ± 2.31 bA	95.80 ± 2.27 cA
5*	86.42 ± 1.87 aAB	91.32 ± 1.84 aAB	108.47 ± 2.21 bB
10*	89.19 ± 1.81 aB	94.07 ± 1.86 aB	109.15 ± 2.15 bB

compared with the hypogravity scenarios (Moon and Mars), indicating greater physiological demand during walking under terrestrial gravity. Although closer to each other, the Moon and Mars conditions also displayed a distinct difference, as shown in Figure 5, with Mars presenting higher values than the Moon at all time points.

To statistically investigate these effects, a repeated-measures ANOVA (RM-ANOVA) model was fitted, considering the factors Time (0, 5, and 10 minutes), Scenario (Earth, Moon, and Mars), and Virtual Reality (with/without), as well as their interactions. The results indicated statistical significance for the main factors Time ($p < 0.001$) and Scenario ($p < 0.001$), as well as for the interactions Time × Virtual Reality ($p = 0.033$) and Time × Scenario ($p = 0.014$). These findings demonstrate that heart rate progression depends not only on walking time but also on the interactions between simulated gravity level and virtual reality, highlighting physiological modulation induced by the experimental environment.

Given the significant results identified by the RM-ANOVA, marginal means comparisons were conducted to determine which factor levels presented statistically significant differences. The results of these comparisons are summarized in Table 2.

When examining the differences among scenarios at each measurement time point (Table 2), it was observed that at Time 0, the mean heart rate values were 81.08 bpm in the Moon scenario, 87.00 bpm in the Mars scenario, and 95.80 bpm in the Earth scenario. Group comparisons indicated that the Earth scenario presented significantly higher values than both Mars and Moon, and that Mars was statistically higher than Moon at Time 0.

At Time 5, the Moon and Mars scenarios remained statistically similar ($p = 0.09$). However, the Earth scenario (108.47 ± 2.21 bpm) exhibited a significantly higher heart rate than both Moon (p ≈ 0.00) and Mars (p ≈ 0.00).

Table 3 Differences in marginal means for VR × Time (lowercase letters within the same row indicate equal mean values; uppercase letters within the same column indicate equal mean values).

TIME / VR	WITH*	WITHOUT*
0	89.92 ± 1.87 A	86.13 ± 1.87 A
5	94.62 ± 1.74 AB	96.31 ± 2.04 B
10	97.34 ± 1.71 B	97.72 ± 1.93 B

At Time 10, the Moon and Mars scenarios again did not differ significantly from each other ($p = 0.09$). The Earth scenario (109.15 ± 2.15 bpm), however, continued to show a significantly higher heart rate compared with both ($p \approx 0.00$ for Moon and $p \approx 0.00$ for Mars).

These findings indicate that the Earth scenario was primarily responsible for the higher heart rate values, showing consistently elevated measurements compared with both Moon and Mars at all time points analyzed.

The comparison across time points within each scenario (Table 2) showed that, in the Moon scenario, a significant increase in heart rate occurred only between Time 0 and Time 10 ($p = 0.01$), whereas Time 5 did not differ significantly from either Time 0 ($p = 0.06$) or Time 10 ($p = 0.34$). In the Mars scenario, a similar pattern was observed, with a significant difference only between Time 0 and Time 10 ($p = 0.01$).

In contrast, in the Earth scenario, there was a significant increase between Time 0 and Time 5 ($p \approx 0.00$) and between Time 0 and Time 10 ($p \approx 0.00$), with no difference between Time 5 and Time 10 ($p = 0.81$), indicating stabilization of the response from the midpoint of the walk onward.

These results demonstrate that:

1. Earth produces a faster and more intense cardiovascular response, reaching stability as early as Time 5.
2. Moon and Mars show a more gradual increase, with differences appearing only between the beginning and the end of the test.

In summary, Table 2 shows that heart rate varies significantly as a function of both scenario and time, and that the temporal pattern of HR increase differs according to the simulated gravity level, reinforcing the modulatory effect of the experimental environment.

The interaction between the marginal means of virtual reality within each time point did not show statistically significant differences. However, as illustrated in the profile plot (Figure 5), a statistically significant difference was observed when comparing the marginal means of time between the virtual reality categories, as shown in Table 3.

Table 3, which presents the differences between the marginal means of the virtual reality × time interaction, shows that the effect of time on heart rate progression differs according to the presence or absence of virtual reality. In the VR condition, heart rate showed a significant difference only between Time 0 and Time 10, indicating a more

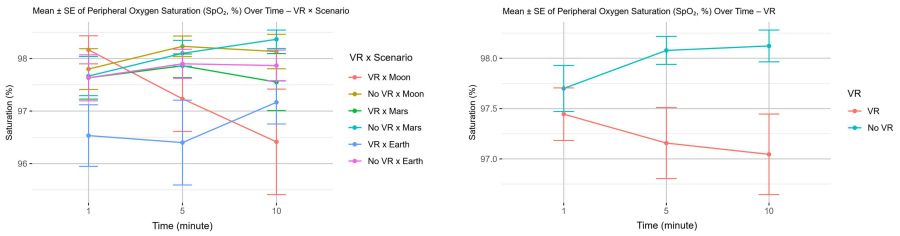


Figure 6 – Profile plots of oxygen saturation over time.

gradual increase throughout the walk. No significant difference was observed at Time 5, suggesting an intermediate transition without an abrupt change in HR behavior.

In contrast, in the condition without VR, Time 0 differs significantly from the other measurement points, indicating a faster rise in heart rate at the beginning of the walk, followed by subsequent stabilization.

These findings reinforce the notion that virtual reality acts as a modulator of the temporal pattern of cardiovascular response, attenuating the rise in heart rate throughout the experiment.

The temporal modulation of heart rate observed in the virtual reality condition suggests that the immersive stimulus may act as a perceptual regulator of exertion, promoting a slower increase in heart rate between minute 0 and minute 10.

3.4 Analysis of VR and Scenario Over Time – O_2 Saturation

Oxygen saturation showed small oscillations over time across the different experimental scenarios. Despite the discrete variation between measurement time points, a distinct pattern emerged when the data were grouped according to the presence or absence of virtual reality (Figure 6). Visually, it can be observed that the presence of virtual reality tends to slightly reduce SpO_2 over time, whereas the absence of virtual reality tends to increase saturation.

The repeated-measures ANOVA (RM-ANOVA) indicated that the virtual reality factor had a significant effect on oxygen saturation ($p = 0.01$), demonstrating that the presence or absence of immersive stimulation alters SpO_2 behavior. No significant effects were identified for the other factors (scenario and time) or for their interactions at the 5% significance level.

Given the relevance of the virtual reality factor, marginal means comparisons were performed between its categories (Table 3). The results showed a statistically significant difference between saturation with VR (97.2%) and without VR (97.97%), indicating a slight reduction in SpO_2 in the presence of virtual reality (Table 4). Although statistically significant, the effect is of low clinical magnitude.

In summary, although all oxygen saturation means remained within normal physiological ranges, virtual reality produced a modest reduction in O_2 saturation during walking under simulated gravity.

Table 4 Differences in marginal means for oxygen saturation between VR conditions (lowercase letters indicate significant differences between columns).

VARIABLE / VR	WITH VR	WITHOUT VR
Oxygen Saturation (%)	97.20 ± 0.20 a	97.97 ± 0.10 b

4 Discussion

This study investigated the effects of simulated Lunar (0.20 G) and Martian (0.38 G) hypogravity on human physiology during walking, with and without virtual reality (VR), through the monitoring of heart rate, blood pressure and peripheral oxygen saturation. The results showed that partial-gravity simulation using the AlterG treadmill, combined with VR, is feasible, safe, and reproducible, allowing simultaneous assessment of physiological and perceptual variables in a controlled environment.

Heart rate (HR) showed distinct behavior across scenarios. The Earth condition presented higher mean HR values throughout the walk, whereas the Moon and Mars conditions exhibited lower values, with slower and less pronounced increases. This difference may be related to the action of Lower Body Positive Pressure (LBPP), which increases venous return and reduces cardiac demand during exercise under simulated gravity—an effect previously described in studies involving LBPP and microgravity [23, 24]. HR was lower in the Moon and Mars scenarios than in the Earth scenario, as reported in the literature [23, 24]. A significant increase from Time 0 to Time 10 was observed in both the Moon and Mars scenarios. The heart rate of the astronauts who performed the first Lunar landing (Apollo 11) reached marked peaks during Lunar extravehicular activity (non-simulated hypogravity) [25]. This HR change was also interpreted as indicative of significant neuroemotional and physical stress, required to overcome inertia and perform tasks under partial gravity in a context that was unprecedented at the time. For participants in the present study, the novelty of walking in simulated Lunar and Martian hypogravity may have been a major motivator for engaging in the experiment, potentially influencing the HR changes observed.

In our study, HR in the Earth scenario showed consistently higher values than those observed in the Moon and Mars scenarios at all time points during the walk. Although Moon and Mars presented closer values, with statistically significant differences between the scenarios, only at minute 0 did they exhibit a distinct difference, as shown in Figure 5, where Mars displayed higher values than the Moon at every time point. Considering the time points 0, 5, and 10 minutes, in the Moon scenario a statistically significant increase in HR occurred only between Time 0 and Time 10 ($p = 0.01$). A similar pattern was observed in the Mars scenario, where the significant difference in HR also occurred only between Time 0 and Time 10 ($p = 0.01$). Thus, the hypogravity scenarios showed a similar pattern to each other and a distinct pattern compared with the 1G Earth condition. In contrast, in the Earth scenario there was a significant increase in HR between Time 0 and Time 5 ($p \approx 0.00$) and between Time 0 and Time 10 ($p \approx 0.00$), but no significant difference between Time 5 and Time 10 ($p = 0.81$). This indicates that HR increased more rapidly and intensely in the Earth

condition compared with the Moon and Mars scenarios. Taken together, these findings suggest that hypogravity reduces cardiovascular stress at the onset of activity, but as walking progresses, the metabolic demand becomes comparable across conditions. Such a profile may help guide adjustments to the duration of simulated-hypogravity training sessions for astronauts or for clinical rehabilitation populations.

SpO_2 showed an unexpected pattern. Descriptive interpretation of the data revealed a slight reduction in oxygen saturation during walking with virtual reality, whereas in the no-VR condition saturation increased progressively. Although the spaceflight literature describes SpO_2 as relatively stable in microgravity [26–28], the effect observed in this study may indicate that sensory immersion increases autonomic activation or reduces breathing depth—phenomena reported in immersive VR studies that include ventilation monitoring [29]. There is no evidence that visual stimuli directly interfere with peripheral oximetry measurements, which suggests that the effect observed in this study results from a physiological modification induced by the immersive experience rather than from technical interference in the oximeter reading. One hypothesis is that visually directed attention toward the virtual environment reduces respiratory awareness, leading to shallower ventilation. Future studies may include direct respiratory measurements to test this hypothesis.

The findings have relevance for the planning of physiological adaptation training for Lunar and Martian missions:

- Walking duration in hypogravity may be extended in future studies to capture cardiovascular behavior after the stabilization point observed in this study.
- Including respiratory measures and heart rate variability may broaden the understanding of autonomic modulation induced by VR.

Taken together, the results of this study demonstrate that simulated hypogravity combined with virtual reality modulates physiological and perceptual responses during walking, influencing in particular the dynamics of heart rate and peripheral oxygen saturation. Hypogravity reduced the initial cardiovascular demand, whereas virtual reality subtly altered peripheral oxygenation, suggesting that the immersive environment may modulate the autonomic response to exertion. These findings reinforce the idea that the interaction among vestibular, proprioceptive, and visual stimuli is a key determinant of human responses in gravitational environments. Future investigations with larger samples, respiratory monitoring, and extended exposure protocols may deepen the understanding of these effects and contribute to safer and more effective physiological preparation strategies for astronauts undertaking long-duration space missions.

During the study, noise levels in the experimental environment were measured using the Decibel X app (iOS) to characterize the acoustic conditions of the setting. In the room without data collection, with the AlterG turned off, the average ambient noise level at CEMA was 42.3 dBA, equivalent to the sound level of a quiet office. During data collection, with the AlterG operating, noise levels increased to approximately 75.3 dBA, corresponding to the acoustic profile of an urban traffic environment. This increase in noise during data collection may have acted as a facilitator of immersion in the virtual reality (VR) conditions, as the additional auditory stimulus may have contributed to a more realistic and engaging sensory context. The medical literature on

virtual reality shows that background noise can enhance participant immersion in VR experiments, especially when integrated multimodally with other sensory stimuli [30]. Studies show that the presence of auditory stimuli—including ambient or background sounds—increases the sense of presence and engagement in the virtual environment, making user behavior more similar to that observed in real settings. The inclusion of background sounds also brings user behavior in virtual environments closer to that observed in real environments, suggesting that omitting such stimuli may compromise the ecological validity of experiments. Thus, the noise generated by the operation of the AlterG, although not a controlled experimental variable, likely played a secondary, positive role in enhancing participant immersion during the locomotor task under virtual reality.

Data Availability

The datasets generated and analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Author Contributions

RPG: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. JSH: Supervision, Methodology, Writing -review & editing. JCC: Methodology, Formal analysis, Investigation. JFG: Investigation. AAFR: Investigation. GAR: Investigation. DC: Investigation. TR: Methodology, Formal analysis, Validation. AAOJ: Supervision, Validation.

Competing Interests

The authors declare no competing interests.

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