Hemoglobin Oxygen Saturation with Mild Hypoxia and Microgravity

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INTRODUCTION: Microgravity (μG) exposure and even early recovery from μG in combination with mild hypoxia may increase the alveolar-arterial oxygen (O_2) partial pressure gradient.

METHODS: Four male astronauts on STS-69 (1995) and four on STS-72 (1996) were exposed on Earth to an acute sequential hypoxic challenge by breathing for 4 min 18.0%, 14.9%, 13.5%, 12.9%, and 12.2% oxygen–balance nitrogen. The 18.0% O_2 mixture at sea level resulted in an inspired O_2 partial pressure (P_IO_2) of 127 mmHg. The equivalent P_IO_2 was also achieved by breathing 26.5% O_2 at 527 mmHg that occurred for several days in μG on the Space Shuttle. A Novametrix CO_2SMO Model 7100 recorded hemoglobin (Hb) oxygen saturation through finger pulse oximetry (S_pO_2, %). There were 12 in-flight measurements collected. Measurements were also taken the day of (R+0) and 2 d after (R+2) return to Earth. Linear mixed effects models assessed changes in S_pO_2 during and after exposure to μG.

RESULTS: Astronaut S_pO_2 levels at baseline, R+0, and R+2 were not significantly different from in flight, about 97% given a P_IO_2 of 127 mmHg. There was also no difference in astronaut S_pO_2 levels between baseline and R+0 or R+2 over the hypoxic challenge.

CONCLUSIONS: The multitude of physiological changes associated with μG and during recovery from μG did not affect astronaut S_pO_2 under hypoxic challenge.

KEYWORDS: oxygen dissociation curve, pulmonary edema, gas exchange, spaceflight.

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Early in human space exploration there were concerns that exposure to microgravity (μG) would lead to hypoxemia since cardiopulmonary physiology was disrupted. Any potential disruptions did not impact performance or operational success in short-duration flights, so detailed investigation into pulmonary gas exchange was delayed until experiments were devised and longer duration flights were possible. For example, there was no indication at the time that pulmonary gas exchange in μG was impeded in the normoxic Skylab atmosphere of 70% oxygen (O_2) at 5.0 psia, an inspired O_2 partial pressure (P_IO_2) of 148 mmHg. But there were concerns about gas exchange efficiency, given the mildly hypoxic atmosphere of the shuttle staged denitrogenation protocol. The implementation of the shuttle staged denitrogenation protocol in 1984 allowed for safe and effective extravehicular activity (EVA).[3] The challenge was to reduce the risk of decompression sickness (DCS) to an acceptable level on going from the 14.7 psia air atmosphere to the 4.3 psia 100% O_2 atmosphere in the spacesuit without lengthy in-suit denitrogenation (prebreathe) time. The solution was to stage the depressurization at 10.2 psia while breathing 26.5% O_2 for about 2 d before the first EVA. Tissue nitrogen (N_2) tension was assumed to approximate inspired N_2 partial pressure at 10.2 psia, about 353 mmHg, after about 2 d. Several competing flammability, materials, and medical constraints culminated in an acceptable mildly hypoxic atmosphere at 10.2 psia with a P_IO_2 of 127 mmHg; the staged condition was equivalent to breathing air at 1220 m (4000 ft) altitude.

Waligora et al.[21] in 1982 evaluated the combination of hypoxia and simulated μG exposure in two small groups of

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subjects with an 8-h exposure to 2440 m (8000 ft) in an altitude chamber with ($N = 4$) and without ($N = 3$) 28 h of 6° head-down bed rest. The expected hemoconcentration was observed in the bed rest group, with hematocrit increasing from 43 to 47%, but there was no indication that the simulated μG reduced arterial oxygenation below the decrease expected while breathing a P_IO_2 of 108 mmHg at 2440 m altitude. With bed rest, mean arterial blood oxygen (P_aO_2) and carbon dioxide (P_aCO_2) partial pressures were 61 and 35 mmHg, respectively, and without bed rest were 59 and 36 mmHg, respectively. Arterial blood hemoglobin (Hb) oxygen saturation (S_aO_2) was about 90% for each group (estimated from figures). One source shows alveolar oxygen (P_AO_2) at approximately 69 mmHg and alveolar CO_2 (P_ACO_2) at 36 mmHg for an acute exposure to 2440 m altitude.[5] The difference in alveolar-arterial oxygen partial pressure ($A\text{-}aDO_2$) with and without bed rest was about 9 mmHg, normal for resting young men at sea level.[5] Loeppky et al.[12,13] in 1993 concluded that 5° head-down bed rest and hypoxic exposure for 8 d accentuated the loss of fluids and electrolytes, thus reducing plasma volume, but there were no negative impacts from these fluid changes on pulmonary mechanics or gas exchange, suggesting no evidence of pulmonary interstitial edema. We concede that bed rest and head-down bed rest are imperfect analogs to μG exposure. Uncertainty about a widening $A\text{-}aDO_2$ in μG persisted, particularly following a 1990 report of a large decrease in plasma oxygen partial pressure (PO_2) from blood taken from the fingers of three cosmonauts after 171 d on the Mir space station and the slow recovery of PO_2 postflight.[7] Without corroboration of these PO_2 values with true arterial blood samples or even indirect pulse oximetry, these data remain questionable.

In response, and before results from Spacelab experiments on pulmonary function in μG were readily available from Prisk et al.,[15] investigators in the Environmental Physiology Laboratory (EPL) at the Johnson Space Center (JSC) developed a Detailed Supplemental Objective (DSO), designated 494, to collect basic cardiopulmonary data in μG. We report here on an experiment titled, "Pulmonary Oxygen Exchange in Microgravity" conceived in early 1990, implemented on two shuttle missions from 1995 to 1996, but not described in the scientific literature until now. Our conclusions have particular relevance to NASA. The next generation of exploration missions will likely use a mildly hypoxic atmosphere at 8.2 psia with 34% O_2 and a P_IO_2 of 128 mmHg to facilitate safe and effective EVAs by reducing prebreathe time while maintaining an acceptably low risk of DCS.[14]

Efficient gas exchange across the lung dictates P_aO_2. Hypoxemia results when there is a decrease in P_IO_2, hypoventilation, diffusion limitation, shunt, or ventilation-perfusion ($\dot{V}_A/\dot{Q}$) heterogeneity. Changes in the last three variables increase $A\text{-}aDO_2$. One could posit that exposure to μG might lead to hypoxemia through any number of mechanisms, but this communication is not a review of that vast literature; see Prisk et al.[16,17,18] for current findings about pulmonary gas exchange in μG. We focused on mild hypoxia during exposure to μG on the Space Shuttle and recovery from μG combined with an acute sequential hypoxic challenge. Our measure of Hb oxygen saturation was through indirect finger pulse oximetry, designated S_pO_2. Our first null hypothesis was that exposure to μG did not change S_pO_2 compared to what was measured when breathing a P_IO_2 of 128 mmHg in 1 G. Our second null hypothesis was that recovery from μG did not change S_pO_2 during an acute sequential hypoxic challenge with P_IO_2s of 128, 106, 96, 92, and 87 mmHg as compared to what was measured preflight.

METHODS

Subjects

Eight male astronauts volunteered and provided written informed consent to participate after the protocol was approved by the JSC Institutional Review Board. The eight astronauts, four on each flight of the Shuttle *Endeavor* (STS-69 in 1995 and STS-72 in 1996), had height, weight, and ages of 182.6 cm ± 7.8, 78.0 kg ± 5.3, and 40.9 yr ± 3.6 (mean ± SD), respectively.

Measurements

Between 5 and 45 d before launch, astronauts reported to the EPL at JSC, in Houston, TX. They submitted to a 20-min seated acute sequential hypoxic challenge by breathing for 4 min 18.0%, 14.9%, 13.5%, 12.9%, and 12.2% O_2 at sea level, all within ± 0.1% of specified concentration with ± 0.02% analysis. Longer intervals of hypoxic breathing were evaluated during the design phase, but were found to elicit unwanted symptoms in some subjects, including tingling sensations, light headedness, and lethargy. Nominal P_IO_2 for the bottle concentrations at 760 mmHg was 128, 106, 96, 92, and 87 mmHg, respectively, with ± 1.4 mmHg for the extreme of the specified concentration. P_IO_2 is computed from $[(P_B - 47) \times F_IO_2]$, where P_B is ambient pressure (mmHg), 47 is vapor pressure (mmHg) of water at 37° centigrade, and F_IO_2 is the dry-gas decimal fraction of oxygen in the breathing gas. Barometric pressure on the day of testing was not measured and was assumed to be 760 mmHg at JSC and at the Kennedy Space Center (KSC).

A Novametrix CO_2SMO $ETCO_2/S_pO_2$ Model 7100 was used to measure and display Hb-O_2 saturation through finger pulse oximetry (S_pO_2, %), heart rate (HR, bpm), end-tidal CO_2 partial pressure ($P_{ET}CO_2$, mmHg), and respiration rate (RR, breaths · min^{-1}). S_pO_2 and HR were measured using red and infrared wavelength light emitting diodes beamed into a finger. Heart rate was calculated by taking the inverse of the time interval between the peaks of the infrared light waveform. The capnograph measured CO_2 concentration and RR with a solid-state sensor, designated Capnostat II. The Capnostat II sensor was placed onto an airway adapter. The astronauts breathed through the adapter (bidirectional flow). Infrared light generated in one leg of the "U" shaped sensor was beamed through the window of the airway adapter to a detector in the other leg of the sensor. Some of this light is absorbed by the CO_2 as a result of respiration. Respiration rate was calculated by taking the inverse of the time interval between peaks of the CO_2 waveform.

While seated, the astronauts were fitted with a nose clip and then breathed through an on-demand scuba regulator (U.S.

Divers Octopus, Vista, CA) each of five hypoxic mixtures, starting with 18% O_2. They briefly held their breath until the new mixture was made available. Between the mouth piece and the scuba regulator was the Capnostat II sensor mounted onto the airway adapter. S_pO_2, $P_{ET}CO_2$, RR, and HR were digitally displayed with whole number resolution on the Novametrix. These data were transcribed each minute for 4 min per gas mixture by lab personnel to a data collection sheet. The same process was followed postflight. On R+0 and R+2, data from the sequential hypoxic challenge were collected by lab personnel with astronauts resting and seated at the KSC in Titusville, FL.

Following launch, between 17 to 95 h, the astronauts also breathed for 10 min the shuttle atmosphere at 10.2 psia with 26.5% O_2, which was an equivalent P_IO_2 of 128 mmHg as the 18.0% O_2 mixture at sea level. The Novametrix device was removed from an equipment locker on the middeck and prepared by an astronaut for use by the test astronaut. Then the device was returned to locker by the test astronaut until needed for another measurement. In total, 12 measurements were collected from 8 astronauts at various times during their exposure to a P_IO_2 of 128 mmHg. One astronaut on STS-72 had 4 measurements, another had 2, and the remaining 2 on STS-72 and 4 on STS-69 had a single measurement. No on-demand regulator was required in flight, just bidirectional breathing through the Capnostat II sensor mounted onto the airway adapter. The in-flight data were transcribed each minute for 10 min to a data collection sheet by the astronaut during quiet breathing. Lab personnel computed means for the 4-min pre/post data and the 10-min in-flight data and then transferred the means to an electronic datasheet for later statistical analysis.

Shuttle Environment

The STS-69 mission lasted 10.8 d and 8.9 d for STS-72. The atmospheric conditions at 14.7 psia for both missions combined were PO_2 of 3.16 ± 0.10 psia with PCO_2 of 3.03 ± 0.72 mmHg. The atmospheric conditions at 10.2 psia for the combined missions were PO_2 of 2.72 ± 0.07 psia (P_IO_2 of 128 mmHg) with PCO_2 of 3.29 ± 0.60 mmHg (P_ICO_2 of 3.0 mmHg). Inspiring even a small concentration of CO_2 caused a large instrument error in the measurement of $P_{ET}CO_2$, so in-flight $P_{ET}CO_2$ was not available for analysis. Both shuttle flights included EVAs and required the use of the staged 10.2 psia denitrogenation protocol with astronauts living at 10.2 psia while breathing 26.5% O_2 before EVA. This condition persisted for 170 h in STS-69 and 51 h in STS-72 before the first EVA. The mean time at 10.2 psia on STS-69 before data collection was 20.4 h (ranged from 19 to 22 h) and 41.4 h (ranged from 17 to 95 h) for STS-72. The partial tissue denitrogenation at 10.2 psia allowed for a subsequent short 40 to 70-min 100% O_2 prebreathe period in the suit to further reduce the risk of DCS during about a 6-h EVA at 4.3 psia. The P_IO_2 during the EVA was slightly hyperoxic at 175 mmHg.

Statistics

We fitted linear mixed effects regression models[19] to account for correlation between the unbalanced, repeated measures data collected on each astronaut to test our two research hypotheses. First, a main effects only model was estimated using maximum likelihood and compared to a model that additionally included potential interaction terms using a likelihood ratio test (LRT). Any influential interaction terms were then included in a final model that was fit using restricted maximum likelihood (REML), as maximum likelihood underestimates variance components by ignoring uncertainty attributable to fixed effects' estimation. We incorporated a random intercept term in each model, which accommodated random heterogeneity in astronauts' S_pO_2 levels that persisted throughout the study. We treated F_IO_2, RR, HR, and $P_{ET}CO_2$ as continuous covariates, and condition (baseline, in-flight, R+0, R+2) as a categorical covariate. Condition entered each model using indicator variables for each level. Interaction terms were generated by taking the product of each continuous covariate and condition level.

We first tested the hypothesis that S_pO_2 was not different between baseline, in-flight, R+0, and R+2 measures while breathing a P_IO_2 of 128 mmHg using the main effects only, mixed effects model in Eq. 1:

$$\begin{aligned} S_pO_{2\,ij} &= \beta_0 + \beta_1 \times (Baseline)_{ij} + \beta_2 \times (R+0)_{ij} \\ &\quad + \beta_3 \times (R+2)_{ij} + \beta_4 \times HR_{ij} \\ &\quad + \beta_5 \times RR_{ij} + b_{0\,j} + \varepsilon_{ij}, \end{aligned} \qquad \text{Eq. 1}$$

where

$$b_{0\,j} \sim N(0, {\sigma_{Astro}}^2) \text{ and } \varepsilon_{ij} \sim N(0, \sigma^2).$$

Eq. 1 models the response $S_pO_{2\,ij}$ at the i^{th} measurement for the j^{th} astronaut, where β_0 is the overall population intercept, $\beta_1,\ldots,\beta_5$ are the fixed effects for each covariate, and ε_{ij} is an independent error term. The random intercept takes the form of b_{0j} in this model's formulation, which allows for deviation from the population intercept for astronaut *j*. Here, we set the reference category for condition to in-flight to compare from μG. Additionally, we controlled for RR and HR in the event they influenced S_pO_2. Note that $P_{ET}CO_2$ was not available for in-flight measurements. In a secondary model, we compared the model in Eq. 1 to one that incorporated interaction terms between RR (HR) and condition level.

To answer our second research question, we again fitted a linear mixed effects model to S_pO_2 data to investigate if Hb-O_2 saturation during an acute sequential hypoxic challenge with F_IO_2s of 18.0, 14.9, 13.5, 12.9, and 12.2% in 1 G differed following spaceflight. Specifically, Eq. 2 is the main effects only, mixed effects model:

$$\begin{aligned} S_pO_{2\,ij} &= \beta_0 + \beta_1 \times (R+0)_{ij} + \beta_2 \\ &\quad \times (R+2)_{ij} + \beta_3 \times HR_{ij} + \beta_4 \times RR_{ij} \\ &\quad + \beta_5 \times P_{ET}CO_{2ij} + \beta_6 \times F_IO_{2ij} \\ &\quad + \beta_7 \times {F_IO_2}^2{}_{ij} + b_{0\,j} + \varepsilon_{ij} \end{aligned} \qquad \text{Eq. 2}$$

This model takes a similar form to that presented in Eq. 1. Eq. 2 includes a curvilinear relationship between F_IO_2 as percentage

and measured S_pO_2 by incorporating a squared term for F_IO_2, an acceptable approximation for the upper portion of the Hb-O_2 desaturation curve. As there was no sequential hypoxic challenge in spaceflight, we compared baseline (reference category) measures to those collected at R+0 and R+2. We were able to control for $P_{ET}CO_2$ in this model, in addition to RR and HR. Similar to our first approach, we compared Eq. 2 to a model including interactions between RR (HR, $P_{ET}CO_2$) and condition level.

RESULTS

Assessment of Change in S_pO_2 After Breathing P_IO_2 of 128 mmHg

Table I shows the means (M) and standard deviations (SD) for measurements taken at each time point. **Fig. 1** is a box and whisker plot of S_pO_2 for astronauts in each condition. Table I and Fig. 1 show an S_pO_2 of about 97%, regardless of the measurement time. **Fig. 2** is a scatter plot of S_pO_2 for astronauts in each condition, providing a visual assessment of within- and between-subject variability in S_pO_2 at a P_IO_2 of 128 mmHg. With the exception of the astronaut indicated with a ° symbol at R+0, we found that S_pO_2 levels remained relatively constant for each astronaut, regardless of measurement time.

Comparing the model presented in Eq. 1 to a model that additionally incorporated interaction terms using a LRT, we failed to reject the null hypothesis (interaction term's regression coefficients = 0) at the 0.05 α-level with a *P*-value = 0.24. Thus, we found no evidence to suggest a need to include interactions between condition and HR or RR in the final model. Results from the final model for our first research question are shown in **Table II**.

We conclude from Table II that there is not a statistically significant difference (0.05 α-level) in S_pO_2 at baseline, R+0, and R+2 conditions compared to in flight for the typical astronaut ($b_{0j} = 0$), holding all else constant (*P*-values = 0.582, 0.099, and 0.100, respectively). By typical astronaut we mean the random intercept term is zero so there is no astronaut-specific modification of estimated S_pO_2. Thus, there is not enough evidence in the data to claim S_pO_2 changed across baseline, in-flight, R+0, and R+2 measures after breathing P_IO_2 of 128 mmHg for the typical astronaut.

Assessment of Acute Sequential Hypoxic Challenge

Table III summarizes the data collected during the hypoxic challenge at baseline, R+0, and at R+2. Note that as mean S_pO_2 decreases as F_IO_2 decreases the SD roughly increases fivefold. There is a slight increase in mean HR as F_IO_2 decreases, little change in RR, and slight increase in $P_{ET}CO_2$ on R+2 (see **Table IV** for statistical significance).

Comparing the model presented in Eq. 2 to a model that additionally incorporated interaction terms using a LRT, we failed to reject the null hypothesis (interaction term's regression coefficients = 0) at the 0.05 α-level with a *P*-value = 0.25. Thus, we found no evidence to suggest a need to include the interactions effect between condition and HR, RR, or $P_{ET}CO_2$ in the final model. Results from the final model for our second research question are shown in Table IV.

We conclude from Table IV that there is no statistically significant difference (0.05 α-level) in S_pO_2 from baseline to R+0 or R+2 for the typical astronaut ($b_{0j} = 0$), holding all else constant (*P*-values = 0.873 and 0.052, respectively). Thus, there is not enough evidence in the data to claim that recovery from μG changes S_pO_2 saturation compared to what is measured during an acute sequential hypoxic challenge with P_IO_2s of 128, 106, 96, 92, and 87 mmHg in 1 G.

DISCUSSION

Our contribution appears after much has been learned about gas exchange physiology in μG.[18] Pulmonary diffusion capacity (D_{LCO}) from single-breath carbon monoxide breathing and membrane diffusing capacity (D_m) both increase to parallel the increase in pulmonary capillary blood volume (V_c) in μG. The persistent increase in D_{LCO} and D_m is evidence that pulmonary edema does not occur in μG. In addition, gravity imposes a degree of matching between ventilation and perfusion. Prisk et al.[18] concluded that, "… *the increases* (D_{LCO}, D_m, and V_c) *rapidly revert to preflight levels on return to 1g. This in-flight increase was attributed to a transition of the pulmonary circulation from a 1g configuration (ie, zones 1, 2, 3) to a situation in which the lung vasculature is entirely zone 2 or 3. This would result in more uniform filling of the pulmonary capillary bed and an attendant increase in the surface area available for gas exchange*". So an otherwise normal lung with no change in the apparent range of $\dot{V}_A/\dot{Q}$ in μG[15] is expected to have no impediment to gas transfer. Hypoxemia is just attributable to breathing a mildly hypoxic P_IO_2, assuming normal red blood cell (RBC) function. This conclusion is supported here and by earlier efforts[12,13,21] to address the issue by combining bed rest with hypoxic altitude exposure.

Table I. Results After Breathing P_IO_2 of 128 mmHg.

	MEASUREMENT TIME			
	BASELINE (*N* = 8)	IN-FLIGHT (*N* = 12)	R+0 (*N* = 8)	R+2 (*N* = 8)
MEASUREMENT	M (SD)	M (SD)	M (SD)	M (SD)
S_pO_2 (%)	96.8 (0.7)	97.0 (0.5)	96.8 (1.0)	97.1 (0.9)
$P_{ET}CO_2$ (mmHg)	39.5 (4.6)	not available	39.1 (4.5)	39.3 (6.0)
RR (breaths · min^{-1})	8.6 (2.5)	15.2 (4.4)	9.6 (4.4)	8.5 (2.8)
HR (bpm)	68.5 (10.0)	65.7 (4.9)	75.5 (8.0)	68.7 (7.5)

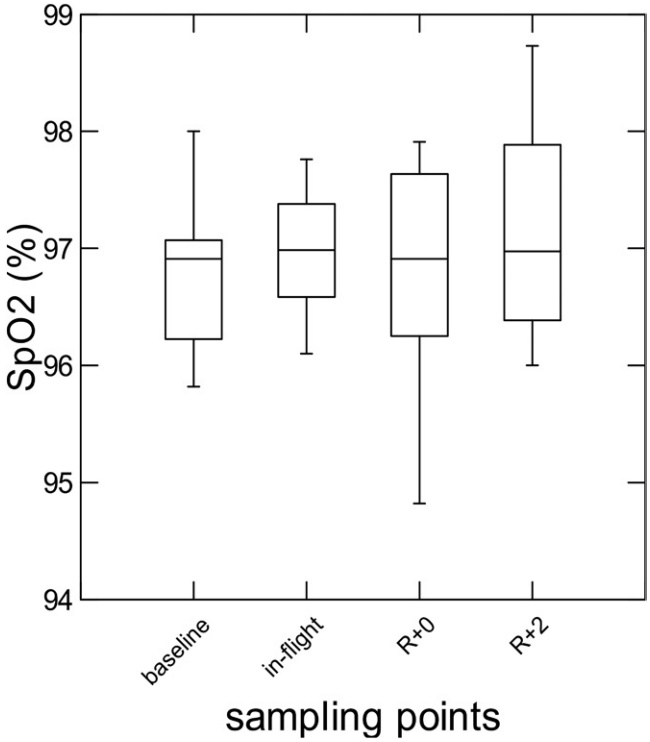

Fig. 1. Box and whisker plots of S_pO_2 at P_IO_2 of 128 mmHg from baseline, in-flight, R+0, and R+2.

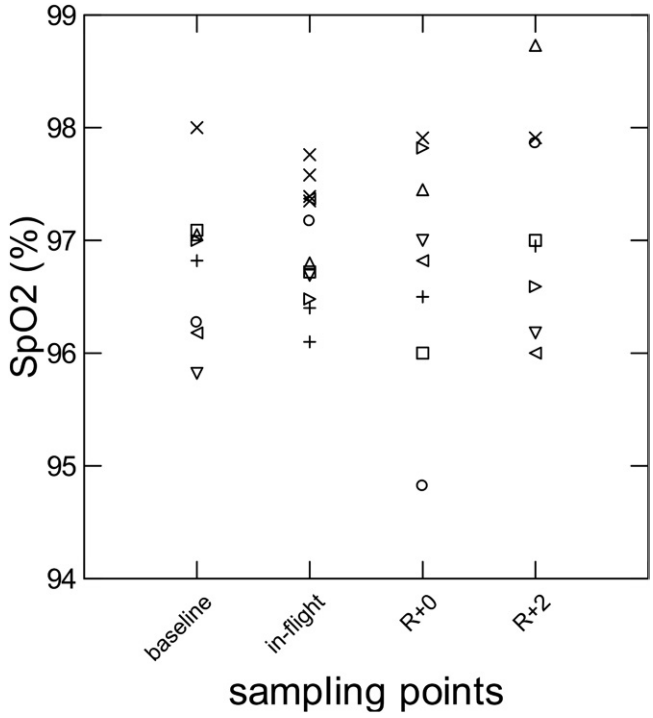

Fig. 2. Scatter plot of S_pO_2 at P_IO_2 of 128 mmHg from baseline, in-flight, R+0, and R+2. Each different symbol represents each of the eight astronauts. Note that during the in-flight measurements one astronaut (x) provided four measurements and a second (+) provided two, all others provided one. The plot is useful to visualize variations within and between astronauts across time points.

No two humans are exactly the same, so the same response to hypoxic challenge is not expected. S_pO_2 is the final integrated result of O_2 transport from the environment to RBCs. Multiple coupled events through time dictate how O_2 from the environment finally binds to Hb, so it is not surprising that we measured large subject-specific variations in S_pO_2 during the acute, sequential hypoxic challenge across conditions. A similar conclusion about subject-specific factors was reached after an extensive review of the Hypoxic Ventilatory Response in mammals.[20] When O_2 supply is limited, certain subject-specific factors influence S_pO_2 whereas when O_2 supply is not limited those factors have lesser effect. Subject variations in hypoxia-induced arteriovenous shunting,[9] ventilatory response,[20] or modifications of $\dot{V}_A/\dot{Q}$ in response to hypoxia[6] are a few such considerations.

We did observe that no two astronauts responded in the same way to the repeated hypoxic challenges, except all showed some decrease in S_pO_2 while breathing reduced F_IO_2s. For example, Table III records a sevenfold increase in SD for S_pO_2 while breathing 12.2% O_2 compared to breathing 18.0% O_2 for the baseline condition. Horiuchi et al.[8] shows that even when the hypoxic intervals are extended to 60 min in 11 males there was an eightfold increase in SD for S_pO_2 while breathing 12% O_2 compared to breathing 18% O_2 (see their Table 1). In contrast, Laurie et al.[9] show in 12 subjects a 3.5-fold increase in SD for S_pO_2 while breathing 10% O_2 for 30 min compared to breathing 16% O_2 (see their Table 3). Replacing P_IO_2 with P_AO_2 would likely reduce the within- and between-subject variability in S_pO_2 to a progressive hypoxic challenge as defined by P_AO_2. P_AO_2 reflects the integrated ventilatory response to an increasing hypoxic dose while P_IO_2 does not change in response to ventilatory drive.

Transition to μG initiates many adaptive changes in physiology that then revert on return to 1 G. There are adjustments to RBCs and the plasma volume they occupy due to μG exposure,[1,2] not even considering mild hypoxia at 10.2 psia that could induce a small increase in 2,3-Diphosphoglycerate (2,3-DPG) within the RBCs[4] or the chronic inspiration of about 3 mmHg CO_2. And then there

Table II. Mixed Effects REML Regression for P_IO_2 of 128 mmHg Across Four Conditions.

FIXED EFFECT	COEFFICIENT (95% CI)	STANDARD ERROR	z-SCORE	*P*-VALUE
Intercept, β_0	100.61 (98.49, 102.73)	1.082	92.97	<0.001
Condition				
Baseline, β_1	0.195 (-0.500, 0.891)	0.355	0.55	0.582
R + 0, β_2	0.617 (-0.117, 1.351)	0.374	1.65	0.099
R + 2, β_3	0.587 (-0.112, 1.286)	0.357	1.64	0.100
HR, β_4	-0.062 (-0.093, -0.032)	0.016	-4.01	<0.001
RR, β_5	0.029 (-0.032, 0.091)	0.031	0.94	0.349
RANDOM EFFECT	**ESTIMATE (95% CI)**	**STANDARD ERROR**		
σAstro	0.156 (0.011, 2.151)	0.209		
σ	0.619 (0.468, 0.819)	0.089		

Table III. Physiological Responses to Three Acute Sequential Hypoxic Challenges.

	S_pO_2 (%)	$P_{ET}CO_2$ (mmHg)	RR (breaths · min^{-1})	HR (bpm)
F_IO_2 (%)	M (SD)	M (SD)	M (SD)	M (SD)
Baseline				
18.0	96.7 (0.7)	39.5 (4.6)	8.6 (2.5)	68.5 (10.0)
14.9	94.8 (1.6)	38.0 (5.1)	7.5 (2.4)	67.6 (8.9)
13.5	93.1 (2.5)	36.4 (5.5)	7.6 (2.4)	68.9 (9.2)
12.9	90.3 (3.7)	36.2 (4.8)	7.3 (2.4)	70.5 (9.7)
12.2	87.5 (5.0)	35.2 (6.1)	7.4 (3.0)	71.7 (10.8)
R+0				
18.0	96.8 (1.0)	39.1 (4.5)	9.6 (4.4)	75.5 (8.0)
14.9	94.2 (2.3)	38.6 (5.5)	9.9 (4.8)	78.3 (8.2)
13.5	91.3 (3.2)	38.6 (5.3)	10.0 (4.5)	80.1 (10.2)
12.9	88.8 (4.2)	37.6 (4.9)	9.9 (4.4)	80.4 (10.2)
12.2	88.0* (4.3)	35.6* (4.5)	9.7* (5.0)	82.5* (10.1)
R+2				
18.0	97.1 (0.9)	39.3 (5.9)	8.5 (2.8)	68.7 (7.5)
14.9	94.9 (1.9)	39.2 (5.4)	8.2 (2.8)	71.2 (7.1)
13.5	92.6 (2.1)	39.4 (5.8)	8.0 (3.0)	72.2 (7.2)
12.9	91.7* (1.6)	38.8* (5.6)	7.7* (3.6)	73.9* (9.2)
12.2	87.6! (2.3)	38.6! (4.7)	6.8! (3.4)	72.8† (6.7)

* $N = 7$, † $N = 6$.

are physiological readjustments on return to 1 G. We entertained the possibility that changes in RBCs and the plasma environment of the RBCs during or after μG exposure could have modified Hb affinity for O_2, and yet astronaut S_pO_2 was no different in μG with a P_IO_2 of 128 mmHg compared to baseline, R+0, or R+2. Also, there was no difference in astronaut S_pO_2 following μG exposure between baseline, R+0, or R+2 during the acute sequential hypoxic challenge.

Future studies about S_pO_2 and hypoxic challenge before, during, and after μG exposure would benefit by measuring variables that influence S_pO_2, like P_AO_2, Hb, and 2,3-DPG concentrations in RBCs from venous blood. These additional explanatory variables in combination with the mixed effects regression model would reduce the large within- and between-subject variability in S_pO_2 at increasing hypoxic dose, as defined by P_AO_2. Our sample size was not dictated by an a priori power analysis; this was a pilot, exploratory study. The absence of additional explanatory variables limited our ability to account for the variability in S_pO_2 and so limited our ability to detect all but the greatest impediment to O_2 transfer onto Hb. A future study design is enabled by the few data collected in this pilot study.

Table IV. Mixed Effects REML Regression for Hypoxic Challenge Across Three Conditions.

FIXED EFFECT	COEFFICIENT (95% CI)	STANDARD ERROR	z-SCORE	*P*-VALUE
Intercept, β_0	2.384 (-24.267, 29.035)	13.598	0.18	0.861
CONDITION				
R + 0, β_1	-0.087 (-1.151, 0.977)	0.543	-0.16	0.873
R + 2, β_2	0.918 (-0.009, 1.846)	0.473	1.94	0.052
HR, β_3	-0.065 (-0.127, -0.003)	0.032	-2.05	0.041
RR, β_4	0.111 (-0.059, 0.282)	0.087	1.28	0.201
$P_{ET}CO_2$, β_5	-0.252 (-0.389, -0.135)	0.060	-4.22	<0.001
F_IO_2, β_6	12.464 (8.953, 15.975)	1.791	6.96	<0.001
$F_IO_2^2$, β_7	-0.360 (-0.475, -0.246)	0.058	-6.17	<0.001
RANDOM EFFECT	**ESTIMATE (95% CI)**	**STANDARD ERROR**		
σAstro	0.627 (0.227, 1.727)	0.324		
σ	2.000 (1.742, 2.292)	0.140		

We showed that exposure to and recovery from μG did not significantly alter the gas exchange process that dictates S_pO_2 response to mild hypoxia in 8 astronauts. We now discuss the association between HR and exposure to μG. Changes in HR are potentially attributable to recovery from μG but are superimposed on the hypoxic challenge. For example, postflight tachycardia is a common response to decreased plasma volume associated with orthostasis.[2,17] However, this study added a stimulus to potentially increase HR as part of the postflight hypoxic challenge. We reanalyzed the data from the hypoxic challenge to investigate changes in HR across condition levels. In this supplemental analysis, we fitted a linear mixed effects model, similar to Eq. 2, but only controlled for a linear effect of F_IO_2. We also evaluated a potential interaction between F_IO_2 and condition level to see if F_IO_2 moderated the association between μG recovery and the HR response. We found a significant difference in HR during the hypoxic challenge on R+0 ($\beta_1 = 10.0$, $P < 0.001$) and R+2 ($\beta_2 = 2.95$, $P = 0.024$) compared to baseline. Recall that the value of the β coefficient is the magnitude of the difference of the outcome variable for the condition R+0 or R+2 referenced to baseline. This difference was not unexpected since HR is increased following exposure to μG[17] but recovers to normal in the ensuing days. The interaction between condition level and F_IO_2 was not significant ($P = 0.49$); therefore, we conclude the change in HR ($\Delta HR / \Delta F_IO_2$) at R+0 and R+2 compared to baseline was not moderated by changes in F_IO_2 during the hypoxic challenge. We posit that the offset increases in HR on R+0 and R+2 compared to baseline are attributable to recovery from μG associated with orthostasis and not increased sensitivity of the HR response to the sequential hypoxic challenge. We do not have the appropriate data to support a full discussion about the ventilatory response to hypoxia during and after exposure to μG, see Prisk et al.[16]

The middeck of the *Endeavor* was less than an ideal controlled laboratory setting. There are more stressors in spaceflight (confinement, disrupted sleep, anxiety, elevated PCO_2) than just μG and mild hypoxia while breathing 26.5% O_2 at 10.2 psia, which apparently did not dominate our results. There were few in-flight data samples (12

samples with 8 astronauts), and missing data on R+0 and R+2 with our lowest O_2 concentrations. Unfortunately, pre/post measurements while breathing air at sea level (an F_IO_2 of 20.9% with a P_IO_2 of 149 mmHg) were not collected. A reasonable value for S_pO_2 at sea level is 98%, which compares closely to two in-flight S_pO_2 measurements of 97.8% and 97.4% taken under normoxic conditions. These values dropped by 1 to 96.7% and 96.5% while exposed to a P_IO_2 of about 127 mmHg in the shuttle. Table I shows pre/post results compared to in-flight results where P_IO_2 was about 127 mmHg. But the pre/post results are after breathing 18.0% O_2 at sea level for 4 min whereas the in-flight results are after breathing 26.5% O_2 at 10.2 psia for 19 to 95 h. We contend that our comparisons are valid but would have preferred more time breathing 18% O_2. The original experiment design was to include an in-flight hypoxic challenge. However, the cost and potential hazard to launch the five pressurized hypoxic mixtures was prohibitive, leaving only the results from breathing 18% O_2 for 4 min to compare with in-flight results.

Our assumption of a "nominal" in-flight environmental condition of 10.2 psia with 26.5% O_2 appears valid. The atmospheric conditions at 10.2 psia were maintained at a P_{O_2} of 2.72 $\pm$ 0.07 psia, which at 10.2 psia provides a P_IO_2 of 128 mmHg when F_IO_2 is 26.6%. But the mean P_{CO_2} of 3.29 $\pm$ 0.60 mmHg combined with the mild hypoxia may have contributed to the in-flight increase in RR. There is a long history about the challenge to control ambient P_{CO_2} in spaceflight and the health and performance consequences if not maintained below about 8 mmHg.[10,11] However, Prisk et al.[15] attributes an increase in in-flight RR to factors other than just increased P_{CO_2}. Also, the absence of the on-demand regulator in-flight, which was used to deliver the hypoxic breathing gases pre- and postflight, makes comparison of RRs problematic. It was decided not to build and launch a pressurized breathing system with 26.5% O_2 with the same on-demand regulator to provide a P_IO_2 of 128 mmHg while at 10.2 psia since the ambient conditions in the shuttle at 10.2 psia with 26.5% O_2 provided the equivalent hypoxia, even if comparisons of RR would be problematic.

In summary:

1) There was no difference in astronaut S_pO_2 (about 97%) after breathing 18% O_2 for 4 min at 760 mmHg before and after spaceflight and breathing 26.5% O_2 at 527 mmHg for 10 min after days in μG, both at a P_IO_2 of about 128 mmHg.
2) There was no difference in astronaut S_pO_2 between baseline, R+0, or R+2 during the acute, sequential hypoxic challenge.
3) We conclude that there was no acclimatization to mild hypoxia during spaceflight that alters S_pO_2 levels upon return to 1 G.

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