Venous Gas Emboli and Ambulation at 4.3 psia

Johnny Conkin; Neal W. Pollock; Michael J. Natoli; Stefanie D. Martina; James H. Wessel III; Michael L. Gernhardt

INTRODUCTION: Ambulation during extravehicular activity on Mars may increase the risk of decompression sickness through enhanced bubble formation in the lower body. Hypotheses: walking effort (ambulation) before an exercise-enhanced denitrogenation (prebreathe) protocol at 14.7 psia does not increase the incidence of venous gas emboli (VGE) at 4.3 psia, but does increase incidence if performed after tissues become supersaturated with nitrogen at 4.3 psia.

METHODS: VGE results from 45 control subjects who performed exercise prebreathe without ambulation before or during a 4-h exposure to 4.3 psia were compared to 21 subjects who performed the same prebreathe but ambulated before and during the hypobaric exposure (Group I) and to 41 subjects who only ambulated before the hypobaric exposure (Group II). Monitoring for VGE in the pulmonary artery was for 4 min at about 12-min intervals using precordial Doppler ultrasound (2.5 mHz). Detected VGE were assigned a categorical grade from I to IV. The detection of Grade III or IV was classified as "high VGE grade."

RESULTS: The incidence of high VGE grade for Group I (57%) was greater than the control (17%) and Group II (15%). The incidence of pain-only decompression sickness was greater for Group I (20%) than the control (0%) and Group II (5%).

CONCLUSIONS: High-grade VGE are increased by mild ambulation conducted under a supersaturated state (Group I vs. II); however, no increase was observed with mild ambulation during the saturated state alone (control vs. Group II).

KEYWORDS: venous bubbles, hypobaric decompression sickness, Doppler ultrasound, bubble grade, micronuclei.

Conkin J, Pollock NW, Natoli MJ, Martina SD, Wessell JH III, Gernhardt ML. *Venous gas emboli and ambulation at 4.3 psia*. Aerosp Med Hum Perform. 2017; 88(4):370–376.

Evolution imbued *Homo sapiens* with bipedal locomotion. We walk everywhere on Earth and even on our Moon. We will soon explore other moons or planets by walking in a low-pressure spacesuit.[1,3] An otherwise adequate denitrogenation (prebreathe) protocol to protect from hypobaric decompression sickness (DCS) in microgravity may be inadequate if ambulation is part of the extravehicular activity (EVA) on a planetary surface.[17] So the effect of ambulation on DCS risk is an important consideration for exploration missions.[8,19] In this research, we quantify the contribution of mild ambulation before and during a simulated EVA to the risk of hypobaric DCS and venous gas emboli (VGE) in an otherwise conservative exercise prebreathe protocol that was used in the construction of the International Space Station (ISS). We provide several sources if the reader needs additional background on historical, current, or future prebreathe protocols that minimize the risk of DCS during EVA.[4,12,17] Hypotheses: walking effort (ambulation) before an exercise-enhanced prebreathe at 14.7 pounds per square inch absolute (psia) does not increase the incidence of VGE at 4.3 psia, but does increase incidence if done after tissues become supersaturated with nitrogen (N_2) at 4.3 psia.

METHODS

Subjects

During a 4-h exposure to 4.3 psia, 45 subjects performed exercise during prebreathe and did not ambulate before or during the exposure. These subjects served as the historical control group for 21 subjects in Group I who performed the identical exercise prebreathe but ambulated before and during exposure to 4.3 psia and 41 subjects in Group II who ambulated before but not during exposure to 4.3 psia. **Table I** shows the demographic information for the three groups of subjects stratified by gender.

Research to reduce prebreathe time while maintaining a low risk of DCS and VGE by means of exercise during prebreathe

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This manuscript was received for review in July 2016. It was accepted for publication in January 2017.

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DOI: https://doi.org/10.3357/AMHP.4733.2017

Table I. Subject Physical Characteristics.

GROUP	*N*	AGE ± SD (yr)	WT ± SD (kg)	HT ± SD (cm)	BMI ± SD ($kg \cdot m^{-2}$)	$\dot{V}O_{2PEAK}$ ± SD (mL O_2 (STPD) $\cdot kg^{-1} \cdot min^{-1}$)
control male	35	31.9 9.4	82.3 13.3	179 5.1	25.5 3.6	42.6 6.7
control female	10	31.3 7.8	61.2 10.7	165 6.2	22.4 4.6	34.5 5.1
control total	45	31.7 9.0	77.6 15.4	176 7.8	24.8 4.0	40.8 7.2
I male	16	35.4 6.5	81.0 8.8	180 6.9	24.9 1.8	45.8 7.6
I female	5	39.6 15.0	59.8 11.3	164 11.2	22.0 3.1	38.4 4.9
I total	21*	36.4 8.9	76.0 13.0	176 10.4	24.2 2.4	44.1 7.7
II male	32	36.7 10.4	76.3 9.8	178 7.7	24.1 2.8	45.9 7.3
II female	9	33.5 9.5	62.2 7.5	161 5.2	24.0 2.7	36.1 4.7
II total	41	36.0 10.2	73.2 11.0	174 10.1	24.1 2.8	43.8 7.9

* One asymptomatic subject removed at 115 min due to LVGE so does not count toward DCS incidence.

was conducted in hypobaric chambers at 4.3 psia by three laboratories from 1998 to 2008: the Center for Hyperbaric Medicine and Environmental Physiology at Duke University (Duke); the Memorial Hermann Center for Environmental, Aerospace, and Industrial Medicine at Hermann Hospital; and Defense Research and Development Canada - Toronto. Duke has conducted new research since 2013, described here, to quantify the consequences of ambulation before and during hypobaric exposure. An operationalized version of the control exercise prebreathe herein described, in conjunction with the *Quest* airlock, was one of four protocols available to U.S. astronauts to conduct safe EVAs from the ISS.[4,12]

All protocols were approved in advance by the NASA Institutional Review Board and the institutional review boards at the collaborating sites. Volunteer subjects provided written, informed consent before participating and were free to withdraw at any time. A modified NASA Class III Flight Physical, extensive medical history, and other selection criteria ensured a sample of subjects who approximated the health and fitness of astronauts who perform EVAs. Our current selection criteria include a 4:1 ratio of men to women; an age range from 25 to 60 yr with ≥25% greater than 34 yr; male body fat (estimated from seven-site skin folds) ≤30% and $\dot{V}O_{2peak}$ ≥35 mL O_2 (STPD) $\cdot kg^{-1} \cdot min^{-1}$, and female body fat ≤35% and $\dot{V}O_{2peak}$ ≥30 mL O_2 (STPD) $\cdot kg^{-1} \cdot min^{-1}$.

$\dot{V}O_2$ Peak

Peak oxygen consumption ($\dot{V}O_{2peak}$) normalized to bodyweight (mL O_2 (STPD) $\cdot kg^{-1} \cdot min^{-1}$) was measured at least 3 d before the hypobaric exposure to allow for a subsequent exercise prescription. Each subject was instrumented with ECG leads to obtain wave forms and heart rate (HR) (Model Omicare CMS 24, Hewlett Packard, Palo Alto, CA), and connected to a metabolic cart (Model TrueOne 2400, Consentius Technologies, Sandy, UT) to measure oxygen consumption and carbon dioxide production. Expired gas concentrations and volumes were measured in 30-s intervals. The subject was seated on a Monarch 818E (Monark Exercise AB, Sweden) leg ergometer with seat height adjusted to approximately 110% of leg length with the pedal in the lowest position. The subject then pedaled at a cadence of 75 rpm. Workloads, based on body mass, were manually increased in steps (see **Table A** online, https://doi.org/10.3357/AMHP.4733sd.2017) until volitional fatigue or upon reaching medical termination criteria.[14] $\dot{V}O_{2peak}$ was determined as the highest oxygen consumption averaged over two 30-s periods, which typically occurred in the last stage of the peak exercise test. Steady-state $\dot{V}O_2$ as a dependent variable was plotted against HR and workloads (W) at each exercise stage. Linear regressions of oxygen consumption vs. HR and workload were performed, then later used to create an exercise prescription.

Exercise Prebreathe Prescription

The targeted workloads for the four stages of prebreathe exercise for the day of the test were calculated as percentages of the measured $\dot{V}O_{2peak}$ for three 1-min incremental exercise stages on the duel-cycle ergometers at 75 rpm using 37.5%, 50.0%, 62.5%, and then 7 min at 75%. The required workload in watts was calculated so that 12% of total work was done by the arms and 88% by the legs. The proper wattage setting at 75 rpm was dialed into the Monarch 818E leg ergometer and 818 arm ergometer for each of the four exercise stages (see **Table B** online, https://doi.org/10.3357/AMHP.4733sd.2017).

Prebreathe and Depressurization

All subjects in the control group, Group I, and Group II performed the same prebreathe and depressurization steps. One to three subjects were tested at a time in a chamber and the accumulation of several chamber tests with new subjects over several months increased the sample size in our prospective, sequential trial design. Subjects were at 14.7 psia breathing 100% oxygen through a mask or head tent for 50 min; the first 10 min included dual cycle ergometer exercise at 75% of $\dot{V}O_{2peak}$. A check of HR (obtained from a Polar Heart watch) vs. prescribed workload in watts for each stage of exercise verified that subjects performed the appropriate work during exercise prebreathe. After 50 min, the chamber atmosphere was depressurized to 9.6 psia in 20 min and then repressurized to 10.2 psia in 10 min. The subjects began 40 min of intermittent light exercise from a semirecumbent position on an exercise cot 5 min into the depressurization phase (see **Table C** online, https://doi.org/10.3357/AMHP.4733sd.2017). Once at 10.2 psia the breathing gas was switched from 100% oxygen to 26.5% oxygen–balance nitrogen for 30 min. The subjects completed the remaining 15 min of light exercise at 10.2 psia breathing this mixture, then the mixture was switched back to 100% oxygen for the remaining 15 min at 10.2 psia. A 5-min repressurization to 14.7 psia was followed by 35 min of additional resting

prebreathe. Final depressurization to 4.3 psia required 30 min, followed by 4 h of EVA simulation activities from an exercise cot, including 4-min intervals of rest and VGE monitoring (see **Table D** online, https://doi.org/10.3357/AMHP.4733sd.2017). Exercise prebreathe and EVA work simulation performed from a semirecumbent position on the exercise cot approximated upper and lower body work associated with ISS assembly tasks.

Ambulation Status

The partial pressure of nitrogen in tissues relative to ambient pressure can be in one of three states: saturated before prebreathe, undersaturated during prebreathe, and supersaturated while at 4.3 psia. Exercise, particularly ambulation, may have different consequence to DCS and VGE outcomes depending on these saturation states. Nonambulation in the historical control meant no walking or standing 100 min before the start of exercise prebreathe and during all phases of the subsequent 7-h test. This was achieved by placing subjects in a semirecumbent position on the exercise cot before and during prebreathe and while at 4.3 psia in the altitude chamber. Subjects were helped to and from the leg ergometer to minimize walking and prevent a standing posture. Exercise at altitude included 20 4-min intervals of activity where the lower body experienced brief but sustained isometric contraction, but subjects did not stand or walk while at altitude. Subjects in Groups I and II were ambulatory during the 100-min period. Ambulation consisted of stepping in place at 80 steps/min for 4 min; this was conducted seven times with 8 min of rest between stepping intervals. Ambulation in Group I also included stepping in place while at 4.3 psia at 80 steps/min for 4 min seven times during the 4-h exposure. No ambulation was permitted at altitude in Group II; here subjects used the same semirecumbent exercise protocol at 4.3 psia as in the control group.

A variety of exercises simulated the efforts associated with preparation for EVA and for EVA tasks. The lower body exercise involves brief but intense isometric contraction to simulate engaging the shuttle portable foot restraint, our PS/AS2 activity. The remaining activities approximate upper body work in a spacesuit and work expended against the spacesuit to overcome its neutral, inflated shape. One stipulation was that overall oxygen consumption during exercise at 4.3 psia be similar for the three conditions. This was achieved by replacing some of the hand and leg tasks with ambulation in Group I. See Table D (online) for the exercise sequence and **Table E** (online, https://doi.org/10.3357/AMHP.4733sd.2017) for descriptions of the exercise.

VGE Collection

Interval VGE monitoring was performed for 4 min approximately every 12 min for 14 periods over the 4-h exposures to 4.3 psia (see Table D online). Monitoring was accomplished by a Doppler technician using a Techno Scientific (Concord, Ontario, Canada) Model DBM9005 precordial Doppler ultrasound bubble detector with a continuous wave 2.5 mHz circular flat array probe (TSI-DPA7) placed on the skin over the pulmonary artery. Venous blood from the right ventricle passes through the pulmonary artery on the way to the lungs for gas exchange, so the pulmonary artery is a common point to sample the entire venous return. Subjects were instructed to report any symptom at any time, but had no knowledge about their VGE status so as to minimize bias in symptom reporting.

While in a semirecumbent position during VGE monitoring periods, monitoring was first done with the subject at rest, then the subject was prompted to flex each limb in turn three times, moving all joints and contracting all muscles, to mobilize VGE in the vasculature to produce transient peak VGE scores. The Doppler technician evaluated the audio signal from the bubble detector in real time and assigned a 0 to IV categorical grade[16] for VGE during the initial resting period and then for peak scores after each of four limb flexion efforts. Each cycle of VGE monitoring was following by a two-dimensional echo imaging scan (SonoSite SonoHeart 180, Bothell, WA) of the left heart to look for the presence of left ventricular gas emboli (LVGE). We paraphrase the VGE categories:[16] Grade 0 is the complete lack of bubble signals in all cardiac cycles; Grade I is the occasional bubble signal detected in a cardiac cycle with the majority of cardiac cycles free of bubble signals; Grade II is when many, but less than half, of the cardiac cycles contain bubble signals; Grade III is when most of the cardiac cycles contain bubble signals, but not overriding the cardiac motion signals; and Grade IV is when bubble signals are detected continuously through the cardiac cycles such that the signal overrides the amplitude of the cardiac motion and blood flow signals. A complete record contains 70 measures of VGE grade over 4 h of altitude exposure. Not all subjects completed the 4-h exposure, primarily because of a symptom(s) diagnosed as DCS that terminated their participation. The presence of LVGE was also a test termination criterion.

The spatial and temporal information about VGE allows many options for analysis. The presence or absence of VGE during the exposure to compute incidences across the three conditions is evaluated. Also, the incidence of "high" VGE grade (Grade III or IV) is evaluated across the three conditions. Finally, how the incidence of VGE changes through time has been used to evaluate the efficacy of prebreathe protocols.[9,10] But in this case, the contribution of ambulation toward the incidence of VGE is evaluated in three otherwise identical prebreathe protocols. The highest VGE grade during each of the 14 monitoring periods was coded as 1 if the Grade was III or IV, else 0 if the Grade was 0, I, or II. This approach divided the subjects into those who produced "high" and "low" bubble grades and each 1 or 0 is associated with the elapsed time at which it was recorded.

VGE Response and Recovery

The incidence of VGE through time has three phases: lag, response, and recovery. We developed a trend model[7] that combined the lag and response phases (Hill equation) and the recovery phase (exponential) to describe the time course for the expected incidence of VGE in subjects undergoing a prebreathe validation trial. This model is suitable to describe a large

quantity of VGE data. Eq. 1 approximates the pattern of high VGE grade incidence as a function of time at 4.3 psia.

$$\text{high VGE grade incidence} = t^{\alpha} / \left(t^{\alpha} + \beta^{\alpha}\right) \times exp\left(-\gamma \times t\right). \quad \text{Eq. 1}$$

The three parameters (α, β, and γ) were estimated using nonlinear least squares regression for the three conditions.

Statistics

No subject from the control group participated in either Group I or II; however, five men in Group I did participate again in Group II. None had DCS on either exposure, four had no VGE on either exposure, and one had VGE on both exposures. We do not consider this a significant violation of the assumption of independence in the application of our test statistics because there were 102 distinct subjects who participated in 107 exposures across the 3 conditions. We used: 1) Fisher's exact one-tail directional test to compare VGE counts between the three conditions, with $P < 0.05$ taken as significant; 2) nonlinear regression to describe changes in high VGE grade incidence through time; 3) logistic regression (LR) to compare the incidence of high VGE grade during an exposure across the three conditions with the three categorical ambulatory states represented by indicator variables and evaluated the following explanatory variables: gender (1 = male, 0 = female), age (yr), height (cm), weight (kg), body mass index (BMI, as $kg \cdot m^{-2}$), and $\dot{V}O_{2peak}$ [mL O_2 (STPD) $\cdot kg^{-1} \cdot min^{-1}$]; 4) unpaired *t*-test for continuous data, for example, to compare mean ages among the three conditions, and finally 5) nonparametric Kaplan-Meier to compare survival curves of high VGE grade among the three conditions, all with SYSTAT® ver. 13.[21]

RESULTS

The primary DCS and VGE results for all three conditions are summarized by Pollock et al.[18] This paper reports on: 1) the incidence of VGE and high VGE grade, 2) the time-dependent change in high VGE grade, 3) a multivariable LR evaluating the contribution of explanatory variables in addition to ambulation status to high VGE grade over entire exposures, and 4) the distribution of the onset times of high VGE grade using survival analysis.

Incidence of VGE and High VGE Grade

Table II shows the incidence of VGE and high VGE grade for each group along with mean age and gender proportions. The incidence of VGE was greater in Group I (62%) compared to the controls (31%, $P = 0.01$) and Group II (27%, $P = 0.005$) using Fisher's exact directional test. The incidence of high VGE grade in Group I (57%) was also greater than the controls (17%, $P = 0.001$) and Group II (15%, $P = 0.0004$). However, the difference in mean ages between the controls (31.7 yr) and Groups I (36.4 yr, $P = 0.054$) and II (36.0 yr, $P = 0.042$) from the *t*-test were significant. This result and reference to age and DCS in the literature[20] were our motivations for using age as an explanatory variable in a LR analysis.

Time-Dependent High VGE Grade Incidence

Fig. 1 contrasts the fraction of high VGE grade through time at 4.3 psia between the nonambulatory control and the two ambulatory groups. The control group had 14 of 45 subjects with VGE but no DCS, so there was no VGE data dropout due to early termination for DCS symptoms. Group I had 13 of 21 subjects with VGE with 4 cases of DCS and 1 case of LVGE that initiated early termination. Group II had 6 of 41 subjects with VGE with 2 cases of DCS that initiated early termination. The regression curve for the controls was based on 618 classifications of high and low VGE grade at the 14 VGE monitoring intervals, 257 classifications for Group I, and 557 classifications for Group II. The pre-exposure ambulation in Group II had no influence on the fraction of high VGE grade through time compared to nonambulatory control. There is about a threefold difference in magnitude of response between Group I and control.

Logistic Regression with High VGE Grade

Table III shows results of a univariate and multivariate LR of high VGE grade across the three conditions. The ambulation status is a three-category variable transformed into two-indicator variables with the controls as reference. Ambulation status is the primary explanatory variable and a univariate analysis returned an odds ratio of 6.16 (1.9 to 19.5 95% CI) for Group I relative to the controls, but only 0.79 (0.25 to 2.5 95% CI) for Group II. Group II was not significantly different from the controls.

A multivariable analysis was then performed using: age (19-57 yr), height (155–195 cm), weight (44–115 kg), BMI (17.5–35.5 $kg \times m^{-2}$), $\dot{V}O_{2peak}$ (26.1–64.9 ml O_2 (STPD) $\cdot kg^{-1} \cdot min^{-1}$), and gender (83 men and 24 women), along with ambulation status as predictors. Besides ambulation status, age was the only other significant predictor for P(Grade III or IV) VGE. In this model, the odds ratio decreased from 6.16 to 5.25 (1.6 to 17.2 95% CI) for Group I relative to the controls and from 0.79 to 0.59 (0.17 to 2.0 95% CI) for Group II, with odds ratio for age at 1.062 (1.0 to 1.12 95% CI). For every 5-yr increase in age for a given ambulation status, the risk of high VGE grade increases

Table II. VGE Results with Age and Gender.

GROUP	AMBULATION BEFORE/DURING	*N*	% MALE	MEAN AGE ± SD	% VGE	% GD III OR IV
Control	no/no	45	78	31.7 9.0	31.1 (14/45)	17.8 (8/45)
I	yes/yes	21*	76	36.4 8.9	61.9 (13/21)	57.1 (12/21)
II	yes/no	41**	78	36.0 10.2	26.8 (11/41)	14.6 (6/41)

* Four subjects with VGE were removed early (60, 62, 112, and 145 min) for DCS symptoms in the lower body and one was removed early for LVGE (92 min), but had no symptoms.
** Two subjects with VGE were removed early at 80 and 115 min for DCS symptoms in the lower body.

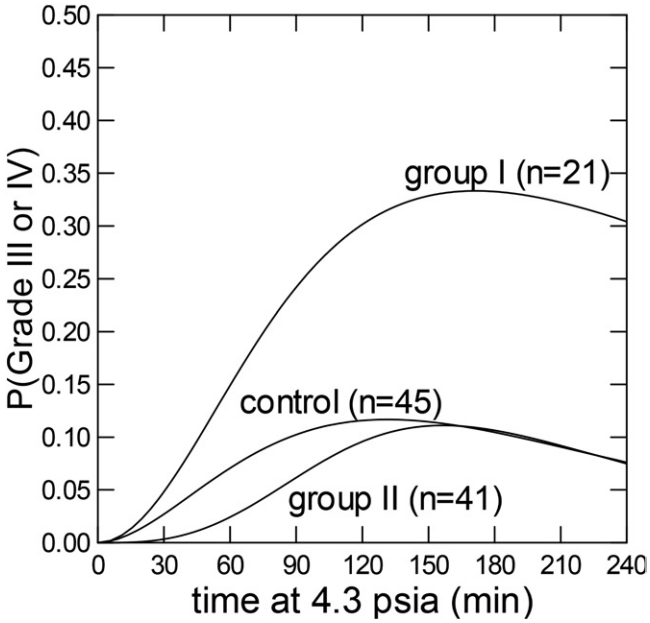

Fig. 1. The fraction of high VGE grade before and during exposure to 4.3 psia. The α, β, and γ coefficients to produce the curves using Eq. 1 are as follows: control (1.908, 169.5, 0.009); Group I (2.024, 123.1, 0.004); and Group II (3.341, 150.7, 0.010).

by 1.35 times [odds ratio = $exp(5 \times 0.060)$]. Eq. 2 is the LR model used to create the probability curves in **Fig. 2**.

$$P(\text{Grade III or IV}) = \frac{exp[-3.537 - 0.53\times(\text{AMBU\_0}) + 1.659\times(\text{AMBU\_1}) + 0.060\times(\text{AGE})]}{[1 + exp(-3.537 - 0.53\times(\text{AMBU\_0}) + 1.659\times(\text{AMBU\_1}) + 0.060\times(\text{AGE}))]}, \quad \text{Eq. 2}$$

where {0,0} for ambulation status denotes the controls, {0,1} denotes Group I, and {1,0} denotes Group II.

Onset Time of High VGE Grade

Fig. 3 shows nonparametric Kaplan-Meier survival curves for onset times of high VGE grade for the three conditions. The nonambulatory controls had lower incidence (8/45) of high VGE grade compared to Groups I (12/21) and II (6/41). The difference among the three survival curves is significant from three log-rank tests: Mantel ($P < 0.001$), Breslow-Gehan ($P < 0.001$), and Tarone-Ware ($P < 0.001$). Survival curves are not significantly different between the controls and Group II ($P > 0.64$) from log-rank tests. Mean onset times were similar for each condition: 105 ± 72 min standard deviation (SD, σ_{n-1}) for control, 104 ± 55 for Group I, and 102 ± 22 for Group II.

Table III. Logistic Regression Results for High VGE Grade.

	COEF.	SE*	*P*-VALUE	95% CI	ODDS RATIO	SE	95% CI
Univariate							
Constant	−1.531	0.39	0.00	−2.3 to -0.76			
AMBU_0	−0.232	0.59	0.69	−1.4 to 0.9	0.793	0.46	0.25 to 2.5
AMBU_1	1.819	0.59	0.00	0.66 to 3.0	6.16	3.63	1.94 to 19.5
Multivariate							
Constant	−3.537	1.00	0.00	−5.5 to −1.5			
AMBU_0	−0.53	0.62	0.39	−1.7 to 0.70	0.59	0.36	0.17 to 2.0
AMBU_1	1.658	0.60	0.00	0.5 to 2.8	5.25	3.18	1.6 to 17.2
Age	0.060	0.03	0.023	0.01 to 0.1	1.062	0.03	1.0 to 1.12

* SE: standard error of the coefficient.

DISCUSSION

We confirmed here that even mild ambulation at 4.3 psia with tissue nitrogen in a supersaturated state influences a conservative prebreathe protocol. We also confirmed that mild ambulation before the start of prebreathe when tissue nitrogen is in a saturated state did not influence a conservative prebreathe protocol. The exact mechanism(s) by which ambulation increases the risk of hypobaric DCS and VGE is unknown. There is likely a complex interaction between tribonucleation and micronuclei stabilized in hydrophobic crevices,[22] which we now discuss. Our previous communication[8] discussed the indirect evidence for activity-induced quasi-stable micronuclei formation and the related topic of tribonucleation.[13] A case was made that homogeneous nucleation, the de novo formation of a gas phase in bulk solution, required supersaturation in excess of what is possible in diving and aviation,[23] and yet divers and aviators produce VGE and succumb to DCS. So mechanisms that generate and stabilize micronuclei must be considered to permit heterogeneous nucleation, which reduces the required supersaturation to grow bubbles from micronuclei.

Ambulation imparts substantial compressive and decompressive forces in tendons, ligaments, and cartilage in the knees and ankles. Contraction and relaxation of muscles that move the body provide a large surface area for structures to slide against. Blood vessels of all diameters compress and expand due to muscle contraction and relaxation, potentially inducing bubble formation on the endothelium through viscous adhesion. These mechanical forces likely create a local distribution of quasi-stable micronuclei that in concert with inert gas supersaturation will grow bubbles that displace tissue and also travel with venous blood to the lungs. While the distribution of micronuclei is quasi-stable, the same mechanical perturbation to the lower body should logically result in different outcomes if the time to hypobaric exposure is delayed. Kumar et al.[15] showed that 16 h of rest before exposure to 6.5 psia following 3 consecutive days of treadmill exercise did not change the incidence of DCS and VGE as compared to the same subjects doing no treadmill exercise. In contrast, Dervay et al.[11] showed that vigorous lower body activity (leg squats) before ascent to 6.2 psia did result in a greater incidence of VGE from the lower body than if a 1- or 2-h recovery from the exercise preceded the ascent. There were 10 subjects who had VGE during all 3 exposures related to pre-exposure

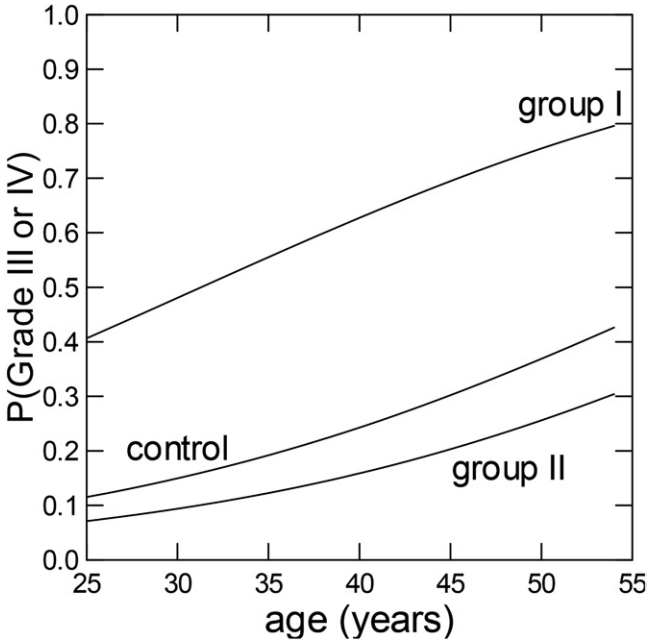

Fig. 2. Logistic regression result with age as an additional explanatory variable. Accounting for age with the LR model allows focus on the contribution of the ambulation variable to the P(Grade III or IV) VGE. The 95% upper and lower confidence limits are not shown, but for comparison consider a 40-yr-old from Group I and the controls. The best estimate of P(Grade III and IV) VGE is 0.63 (0.40-0.81) and 0.24 (0.12-0.42), respectively.

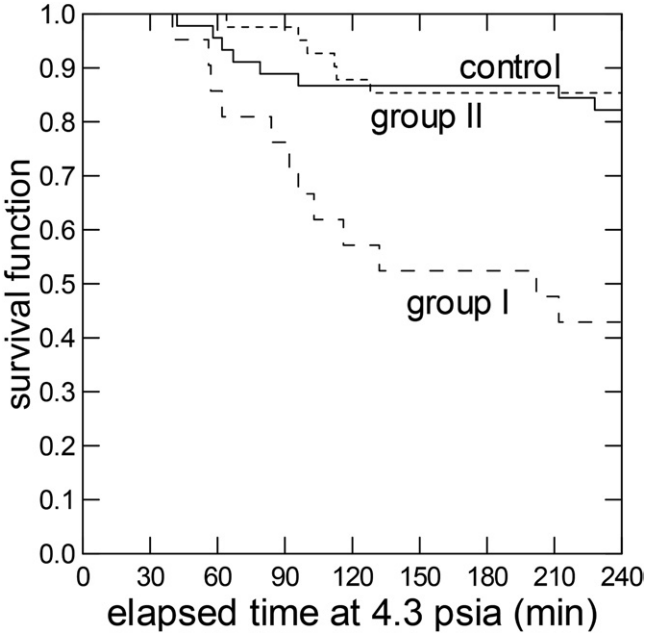

Fig. 3. Nonparametric Kaplan-Meier survival curves show the distribution of onset times of high VGE grade in nonambulatory controls and the ambulatory groups.

lower body exercise while 10 others had no VGE on any exposure. So other subject-specific factors are important to consider.[5,6,25,26] The effect of ambulation on DCS and VGE depends on the conditions of the exposure, including subject-specific factors. Others have found no influence of ambulation on the DCS outcome under their specific conditions.[2,24] We did not observe an effect of mild ambulation before prebreathe and subsequent nonambulatory hypobaric exposure (Group II vs. controls) on DCS[18] or VGE outcome. The ambulation was during a period before nitrogen supersaturation existed. But we did observe more DCS and VGE when the ambulation was at 4.3 psia (Group I vs. control and Group II), a period where any induced micronuclei could grow into detectable bubbles.

Our observation that mean onset times for high VGE grade were independent of ambulation status merits discussion. Mean onset times for the control, Group I, and Group II were about 104 min, but SDs were different. Mild ambulation before our prebreathe (Group II) had no subsequent effect on high VGE grade so we combined onset times for the controls and Group II to advance this discussion. Mean onset time for the 14 combined cases in 86 tests was 104.0 $\pm$ 54.9 min SD and 104.1 $\pm$ 54.7 min SD for the 12 cases in 21 tests from Group I. We observed that mild ambulation at 4.3 psia increased the proportion of subjects with high VGE grade and the proportion with Type I symptoms; however, mean onset time for high VGE grade was 104 min with or without mild ambulation at 4.3 psia. It is unclear what accounts for this observation, other than coincidence, since a logical assumption is that ambulation would also shorten onset times for high VGE grade. One hypothesis is that subjects had an anatomical site, possibly fat depots such as the infra-patellar fat pad, that contributed VGE based on the magnitude of nitrogen supersaturation and muscle contractions near the joint. Ambulation increases the proportion of subjects that make available evolved gas to the venous return from the site, but the distribution of onset times is otherwise unchanged. In addition, ambulation in some subjects induces evolved gas through tribonucleation that goes on to elicit a symptom in a knee or ankle. VGE detected in the pulmonary artery are not highly correlated with a subsequent symptom; they may be a necessary, but not sufficient condition for subsequent symptoms from a joint.[9] The positive predictive value for DCS of Grade IV VGE has been reported to be about 50%, whereas the negative predictive value of Grade 0 VGE is about 98%.[4,9] Similar mean onset times for high VGE grade irrespective of ambulation status at 4.3 psia could indicate a site(s) for bubble formation other than the site of perceived mechanical distortion within the joint. We infer a different anatomical site(s) for the origin of bubbles detected in the pulmonary artery than those induced to form and elicit symptoms from the knees or ankles due to ambulation. It is possible, given our conservative prebreathe, that much of the gas expanding through time in the cartilage, ligaments, tendons, and synovial space of a joint remains stationary, not contributing to what is detected through time in the pulmonary artery until the volume of gas in these structures exceeds some critical threshold. If this were not the case, then one might expect onset times for high VGE grade to be on average sooner in subjects who ambulate.

Finally, as ambulation during EVA is a significant part of future planetary exploration, then additional denitrogenation needs to compensate for the additional risk of DCS and VGE imposed by the ambulation. Our work for the future is to quantify the prebreathe compensation.

ACKNOWLEDGMENTS

We thank Alan H. Feiveson for editing and reviewing our statistical analyses. This work was made possible through contracts NNJ06HE93A and NNX-12AG22A between NASA and Duke University and the Human Health and Performance Contract (NNJ15HK11B) between NASA and KBRwyle. Funding for this research was provided by the NASA Human Research Program. Conclusions are those of the authors and are not necessarily endorsed by the National Aeronautics and Space Administration.

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