Autonomic Cardiovascular Responses to Orthostatic Stress After a Short Artificial Gravity Exposure

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BACKGROUND: Intermittent artificial gravity (AG) training over days and weeks has been shown to improve the human orthostatic tolerance limit (OTL) and improve cardiovascular regulation in response to orthostatic stress. Effects of a single AG exposure are currently unknown.

METHODS: We tested cardiovascular responses to orthostatic stress in 16 hypovolemic subjects (9 men and 7 women), once following a single, short (~90 min) bout of AG and once following a similar period of head-down bed rest (HDBR). Hypovolemia was produced by intravenous furosemide infusion (20 mg) and orthostatic stress was produced by combined 70° head-up tilt (HUT) and progressively increasing lower body negative pressure until symptoms of presyncope developed. To assess reflex-induced changes in cardiovascular regulation, heart rate and blood pressure variability were analyzed by spectral analysis and baroreflex activity was evaluated by transfer function analysis.

RESULTS: Compared to HDBR, a short AG exposure increased men's low frequency (0.04–0.15 Hz) power of systolic blood pressure (SBP_{LF}), but did not change women's SBP_{LF} responses to orthostatic stress. In response to 70° HUT, compared to supine, low frequency phase delay ($Phase_{LF}$) between systolic blood pressure and RR intervals increased by ~20% following HDBR, but did not change following AG, reflecting improved baroreflex activity at a milder level of orthostatic stress after AG.

CONCLUSIONS: These results indicate that a short bout of AG increased both sympathetic and baroreflex responsiveness to orthostatic stress in hypovolemia-induced, cardiovascular-deconditioned men and women, which may contribute to the AG-induced improvement of OTL shown in our previous reports.

KEYWORDS: artificial gravity, cardiovascular deconditioning, orthostatic stress, gender difference.

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Microgravity and its ground-based simulations, such as head-down bed rest (HDBR),[24] result in the development of multiple cardiovascular dysfunctions that become apparent on returning to gravitational environments,[27] one of which is the development of orthostatic intolerance (OI). Depending on the length of the spaceflight, applied countermeasures, and the characteristics of postflight testing, approximately 28–65% of astronauts experience symptoms of OI during postflight stand/tilt tests,[7] posing a great risk to the safety and performance of astronauts. Therefore, development of effective countermeasures to protect against OI is an important area of research.

Among countermeasures[27] proposed to prevent OI, artificial gravity (AG) provided by a short-arm centrifuge has been suggested as a gravity-based countermeasure to combat the deconditioning of spaceflight.[3] Although no data are currently available concerning the effects of AG on the cardiovascular system during spaceflight, results from ground-based studies using intermittent artificial gravity (IAG) provided by a short-arm centrifuge are promising. Our previous studies have shown that 3 wk of IAG exposure improves the orthostatic tolerance limit (OTL) of ambulatory men[9,30] and prevents OI in cardiovascular-deconditioned men.[29] However, these aforementioned IAG protocols required substantial time to complete; therefore,

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the development of an effective countermeasure that could be applied over a relatively short time period would be of particular importance. Using a single, sustained exposure to 30-min +3-G_z AG, Schlegel et al.[26] found that OTL and baroreflex responsiveness were improved in ambulatory men. Nevertheless, as plasma volume loss is one factor contributing to postflight OI,[17,24] cardiovascular control of hypovolemic subjects is more relevant to returning astronauts.[13] Therefore, the effects of a single AG exposure on cardiovascular responses during hypovolemic-induced deconditioning, which are currently unknown, need to be studied. In addition, women have been reported to be more predisposed to OI than men.[11] However, most AG studies,[3,9,29] and in particular studies using a single AG exposure,[26] have involved only male subjects. Convertino et al.[5] indicated that cardiovascular adaptations to IAG were different in men and women. Stenger et al.[30] reported passive IAG exposure did not significantly improve women's OTL. Thus, whether a single bout of AG exposure is effective to improve women's OTL needs to be investigated.

To determine whether OTL would be improved by a single bout of AG exposure during spaceflight, we tested cardiovascular responses to orthostatic stress, once following ~90 min AG exposure and once following ~90 min HDBR exposure, in a pharmacologically induced hypovolemic condition. Our previous report[8] indicated that a short bout of AG improved the OTL of hypovolemic men by 30% and women by 22%, while men and women showed different blood pressure responses to orthostatic stress following AG exposure. To further identify potential underlying mechanisms for improved OTL, we sought to determine effects of this short-duration AG exposure on autonomic cardiovascular function and baroreflex function during orthostatic stress. In the present study, we reanalyzed the same dataset used in the previous report.[8] We have carefully avoided replication of data between reports, except where common variables were necessary to describe subjects' physiological status at the time points of interest [e.g., heart rate (HR) and blood pressure (BP)].

METHODS

Subjects

Nine men (38 ± 4 yr in age, 175 ± 3 cm in height, and 81 ± 5 kg in weight) and seven women (30 ± 2 yr in age, 168 ± 2 cm in height, and 71 ± 4 kg in weight), who were nonsmokers and normotensive, were recruited. None was a trained athlete. Each subject gave informed written consent to the experimental protocol, approved by the NASA Ames Research Center and University of Kentucky Institutional Review Boards for the Protection of Human Subjects. Selection of subjects was based on a screening evaluation that consisted of a medical history questionnaire, a 12-lead electrocardiogram, and BP measurement.

Experimental Design and Protocol

Each subject attended two experimental sessions separated by 21 d which occurred at the same time of day as the previous session. Both experimental sessions included each of the following: 1) dehydration, 2) ~90 min HDBR exposure or AG exposure, and 3) an OTL test. The order of treatment assignment (HDBR vs. AG) was randomized and counterbalanced. By the combination of these sessions, we can model the cardiovascular responses during spaceflight with (dehydration and AG) or without (dehydration and HDBR) employing countermeasures and after reentry to gravitational environments (OTL test).

Dehydration. To model spaceflight-induced plasma volume loss, subjects were given guidelines for sodium intake for 48 h preceding each experimental session. On the day of each session, after a check of the subject's potassium level, 20 mg furosemide was infused intravenously to reduce plasma volume. Urine output and BP were monitored for up to 2 h after the injection. Tests started after urine output and BP had stabilized.

Head-down bed rest. To model the cardiovascular response to spaceflight without employing countermeasures, hypovolemic subjects were placed in the −6° HDBR position for ~90 min before their OTL test.

Artificial gravity exposure. To model cardiovascular response to spaceflight with employing countermeasures, the Human Performance Centrifuge at the NASA Ames Research Center was used to provide AG. Details of the protocol were reported elsewhere.[8] Briefly, the hypovolemic subject lay on the centrifuge with his/her head toward the center in a fully extended body position and underwent an individualized "step up" AG protocol. To determine the tolerance limit to AG, the acceleration was increased +0.1 G_z every 3 min until the subject experienced presyncopal sympotoms (systolic blood pressure < 90 mmHg, HR drop > 20 bpm, or the subject experienced nausea, dizziness, or lightheadedness). After a short rest, each subject underwent a 45-min AG training protocol consisting of several "step up" AG ramps that stopped +0.2 G_z below that subject's tolerance limit.

Orthostatic tolerance limits test. To model cardiovascular responses to reentry to gravitational environments, OTL was tested using combined head-up tilt (HUT) and lower body negative pressure. Following AG or HDBR treatment, those hypovolemic subjects lay supine for at least 15 min for instrumentation and equilibrium. Supine control data were taken for 10 min before HUT. The tilt table was then brought to 70° for 10 min, after which pressure inside the chamber was reduced 20 mmHg below atmospheric pressure for 3 min; subsequent 10-mmHg reductions in pressure were made at 3-min intervals until the onset of presyncopal symptoms. At the onset of presyncope, the subject was placed in the Tredelenburg position (−6°) until BP and HR stabilized.

Instrumentation and Data Acquisition

During the OTL test, a standard lead II electrocardiogram (Model 90623A, SpaceLabs, Inc., Redmond, WA) was continuously

monitored and recorded. Continuous BP and HR were obtained at the middle finger of the left hand using photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands) with the hand positioned at heart level using a sling. The height correction feature of the Finometer was used to correct for hydrostatic differences between heart and finger sensor. Brachial artery BP was also measured periodically using a manometer (UA-767, A&D Medical, San Jose, CA) placed around the upper arm for the calibration of continuous BP. Changes in blood volumes of body segments were estimated with the use of a tetrapolar high-resolution impedance monitor four-channel digital impedance plethysmograph (UFI Model 2994D, Morro Bay, CA). Impedance was obtained for four anatomic segments, i.e., thorax, abdomen, upper leg, and lower leg.[6] Respiration was estimated using respiration-induced changes of thoracic impedance. All data were collected by computer acquisition software (WinDAQ, DATAQ Instruments, Akron, OH) at 1000 Hz with subsequent analysis using MATLAB (R2012b, Mathworks, Natick, MA).

Data Analysis

Data were summarized as 2-min averages. Segments including 2 min before HUT, 2 min after tilt to 70°, 2 min before presyncope, and 2 min following tilt back were chosen to determine cardiovascular regulation at supine control, the initial response to tilt (early tilt, ET), preceding presyncope (late tilt, LT), and recovery for OTL tests following AG compared to HDBR exposures. Due to the intersubject differences of OTL, the late tilt period includes some levels of lower-body negative pressure for some subjects.

Preprocess. Locations of the R wave peak were identified in the ECG and R-R interval (RRI) time series were constructed. Local maxima and minima of BP within each heartbeat were identified and used to construct systolic (SBP) and diastolic blood pressure (DBP) time series, respectively. Respiratory rate was obtained by identifying local minima of the respiratory waveform (i.e., the start of expiration). Thoracic (Z_{THX}) and abdominal (Z_{ABD}) impedance were normalized to the distance between electrodes. All artifacts were removed by visual inspection. Mean values of each 2-min data section were used to provide hemodynamic parameters. Each time series was then linearly interpolated, resampled at 4 Hz, and linearly detrended for spectral and transfer function analyses.

Spectral power. Spectral power of RRI was calculated based on Welch's averaged periodogram method. Power spectral density estimates were made from 256-point (64-s) windows with 32-point (8-s) increments. This process resulted in eight segments of data for each recording. Mean values of spectral power in the low- (LF, 0.04–0.15 Hz) and high- (HF, 0.15–0.4 Hz) frequency ranges were calculated.[21] The same methodology with SBP yielded SBP_{LF} and SBP_{HF}. The ratio of LF and HF spectral power of RRI ($RR_{LF/HF}$) and normalized HF spectral power of RRI (RR_{HFnu}, by the summation of LF and HF power) were also calculated to reflect sympathetic and vagal control of HR.[21]

Transfer function analysis. Coherence and transfer function gain and phase between spontaneous oscillations in SBP and RRI were determined using cross-spectral analysis in the LF range as this range is thought to be predominantly determined by the baroreflex.[25] To ensure robust gain and phase estimates within the LF band, we averaged only those gain and phase values where the corresponding coherence was greater than 0.5 (all subjects in the present study were above this threshold). Transfer function gain (magnitude of transfer) was used to quantify the amplitude of signal transmission from arterial pressure to RR intervals. Phase was used to estimate the temporal relationship between these two variables.

Statistical analysis. A three-way mixed model analysis of variance was used to determine the effects of gender, treatment (AG vs. HDBR), and time (supine, ET, LT, and recovery) with two repeated factors (treatment and time). Least mean square method post hoc was used to assess pairwise comparisons. Logarithmic transformation was performed for parameters not normally distributed. Analysis was completed using SAS 9.3 (SAS Institute Inc., Cary, NC). Significance was accepted at $P < 0.05$. Values are shown in mean ± SEM.

RESULTS

Hemodynamics

Table I shows group averages of hemodynamic parameters in response to orthostatic stress following AG compared with HDBR. Compared with HDBR, AG changes SBP responses differently in men and women (Gender × Treatment interaction, $P = 0.0442$). Compared with HDBR, AG reduced men's SBP ($P = 0.0192$), but did not change women's SBP. Compared to supine, SBP (Time, $P < 0.0001$) decreased during the mild level (i.e., ET) and severe level (i.e., LT) of orthostatic stress, and was not restored during recovery, while DBP (Time, $P < 0.0001$) increased at ET and decreased at recovery. Compared with ET, lower SBP ($P < 0.0001$) and DBP ($P < 0.0001$) were observed at LT. Orthostatic stress increased HR (Treatment × Time interaction, $P = 0.0166$). During recovery, HR following HDBR was lower than supine ($P = 0.0008$) and lower compared with HR following AG ($P = 0.0472$). Overall, women had higher HR responses (Gender × Time interaction, $P = 0.0005$), due primarily to ET response ($P = 0.0109$) after both AG and HDBR. Respiratory rate was not different for AG compared with HDBR and was not altered by orthostatic stress. Compared to supine, Z_{THX} increased (Time, $P < 0.0001$), while Z_{ABD} (Gender × Time interaction, $P = 0.0102$) decreased during tilt in both men and women. At recovery, Z_{THX} were restored to supine values in both men and women, while Z_{ABD} was restored in men, but not in women ($P = 0.0006$). Compared with ET, Z_{ABD} decreased in both men ($P = 0.0002$) and women ($P = 0.0019$) during LT.

Spectral Power

Heart rate and blood pressure variability parameters are shown in **Table II**. With respect to supine, $RR_{LF/HF}$ (Time, $P < 0.0001$)

Table I. Hemodynamic Response to Orthostatic Stress After AG vs. HDBR in Hypovolemic Men and Women.

	FOLLOWING AG				FOLLOWING HDBR			
	SUPINE	ET	LT	RECOVERY	SUPINE	ET	LT	RECOVERY
				MEN (N = 9)				
HR	68.9 ± 4.9	84.9 ± 4.1*	104.8 ± 7.0*§	70.3 ± 4.5†	67.4 ± 3.4	80.8 ± 4.0*	94.4 ± 5.1*§	64.0 ± 3.6*
SBP	119.7 ± 4.4†	122.3 ± 5.1†	100.1 ± 4.8*†§	107.8 ± 3.3*†	131.5 ± 3.3	130.0 ± 4.0	112.4 ± 4.5*§	113.7 ± 3.8*
DBP	69.3 ± 3.0	76.9 ± 2.4*	68.6 ± 3.1§	65.8 ± 2.5*	75.8 ± 2.2	79.6 ± 2.5*	73.7 ± 2.7§	67.8 ± 2.5*
fR	17.0 ± 1.0	16.3 ± 1.2	15.6 ± 1.2	17.9 ± 1.6	17.6 ± 1.5	15.1 ± 1.1	16.1 ± 1.4	16.2 ± 1.3
Z_{THX}	0.43 ± 0.03	0.46 ± 0.04*	0.47 ± 0.04*	0.43 ± 0.03	0.46 ± 0.03	0.50 ± 0.03*	0.50 ± 0.03*	0.46 ± 0.03
Z_{ABD}	0.85 ± 0.03	0.80 ± 0.03*	0.79 ± 0.03*§	0.84 ± 0.03	0.88 ± 0.04	0.83 ± 0.04*	0.82 ± 0.04*§	0.88 ± 0.04
				WOMEN (N = 7)				
HR	70.7 ± 1.7	90.9 ± 2.2*‡	101.3 ± 4.3*§	69.6 ± 2.4†	71.2 ± 2.1	94.4 ± 2.5*‡	106.6 ± 4.7*§	64.4 ± 2.4*
SBP	126.8 ± 2.1	127.0 ± 3.3	113.4 ± 4.0*§	109.6 ± 3.0*	124.5 ± 3.5	125.2 ± 2.9	111.0 ± 3.9*§	107.0 ± 5.7*
DBP	70.8 ± 2.0	75.5 ± 2.9*	71.3 ± 3.0§	66.1 ± 2.1*	71.2 ± 1.7	77.4 ± 2.5*	72.6 ± 2.5§	64.2 ± 3.4*
fR	17.6 ± 1.6	17.1 ± 2.3	17.2 ± 2.0	18.7 ± 2.1	17.0 ± 2.5	17.6 ± 2.3	17.6 ± 1.9	18.4 ± 1.9
Z_{THX}	0.59 ± 0.01‡	0.63 ± 0.01*‡	0.64 ± 0.01*‡	0.59 ± 0.01‡	0.56 ± 0.04‡	0.60 ± 0.04*‡	0.60 ± 0.04*‡	0.56 ± 0.04‡
Z_{ABD}	1.00 ± 0.02‡	0.93 ± 0.03*‡	0.91 ± 0.03*§‡	0.99 ± 0.02*‡	1.04 ± 0.06‡	0.97 ± 0.06*‡	0.96 ± 0.06*§‡	1.03 ± 0.07*‡

Values are mean ± SEM. AG, artificial gravity; HDBR, head-down bed rest; ET, early tilt; LT, late tilt; HR, heart rate, bpm; SBP, systolic blood pressure, mmHg; DBP, diastolic blood pressure, mmHg; fR, respiratory rate, breaths/min; Z_{THX}, normalized (by distance between electrodes) thoracic impedance, Ohm/cm; Z_{ABD}, normalized (by distance between electrodes) abdominal impedance, Ohm/cm. *Significantly different from supine, $P < 0.05$; †significantly different from HDBR, $P < 0.05$; ‡significantly different from male subjects, $P < 0.05$; §significant difference between ET and LT, $P < 0.05$.

increased and RR_{HFnu} (Time, $P < 0.0001$) decreased during tilt. Lower $RR_{LF/HF}$ ($P = 0.0022$) and higher RR_{HFnu} ($P = 0.0015$) were observed at recovery compared with supine. Compared to HDBR, AG increased SBP_{LF} in women ($P = 0.0441$), but tended to decrease SBP_{LF} in men ($P = 0.0524$) at supine (**Fig. 1**). During orthostatic stress, men and women showed different SBP_{LF} responses after different treatments (Gender × Treatment × Time interaction, $P = 0.0235$). In female subjects, compared to supine, SBP_{LF} increased at ET ($P = 0.0003$) following AG, while increasing at ET ($P < 0.0001$) and LT ($P = 0.0077$) following HDBR. In male subjects, compared to supine, SBP_{LF} increased at ET ($P = 0.0023$) and LT ($P = 0.0035$) following AG, but this pattern was not as pronounced during orthostatic stress following HDBR. Compared to male subjects, women had greater SBP_{LF} at supine ($P = 0.0222$), ET ($P = 0.0219$), and recovery ($P = 0.0278$) following AG, and greater SBP_{LF} at ET ($P = 0.0302$) following HDBR. Compared to supine, SBP_{LF} decreased at recovery in men ($P = 0.0017$ and 0.0103) and women ($P = 0.0224$ and 0.0195) following AG and HDBR, respectively. With respect to supine, SBP_{HF} increased during tilt (Time, $P < 0.0001$) and was restored during recovery. Compared with ET, SBP_{HF} increased during LT ($P = 0.0422$).

Transfer Function Analysis

Table III shows baroreflex parameters calculated using transfer function analysis. A significant three-way interaction was detected in coherence in the LF range (COH_{LF}; Gender × Treatment × Time interaction, $P = 0.0010$). Compared to results following HDBR, women had lower COH_{LF} at LT ($P = 0.0025$) and men had similar COH_{LF} during the OTL test following AG. Compared to men, women had higher COH_{LF} at ET ($P = 0.0190$) and LT ($P = 0.0035$) following HDBR and at supine ($P = 0.0053$) and ET ($P = 0.0042$) following AG. Compared with supine, women's COH_{LF} did not change during the OTL test following HDBR, but decreased at LT ($P = 0.0028$) and recovery ($P = 0.0068$) following AG. However, men's COH_{LF} was reduced at LT ($P = 0.0180$) following HDBR and did not change during the OTL test following AG. Compared with ET, women's COH_{LF} decreased at LT ($P < 0.0001$) following AG while men's COH_{LF} did not change following

Table II. Heart Rate Variability and Blood Pressure Variability Responses to Orthostatic Stress Following AG vs. HDBR in Hypovolemic Men and Women.

	FOLLOWING AG				FOLLOWING HDBR			
	SUPINE	ET	LT	RECOVERY	SUPINE	ET	LT	RECOVERY
				MEN (N = 9)				
$RR_{LF/HF}$	7.0 ± 2.2	11.5 ± 2.3*	19.2 ± 3.2*	4.1 ± 1.4*	6.7 ± 1.5	14.3 ± 4.3*	18.4 ± 4.5*	4.4 ± 1.6*
RR_{HFnu}	0.24 ± 0.07	0.11 ± 0.03*	0.07 ± 0.02*	0.30 ± 0.05*	0.18 ± 0.03	0.10 ± 0.02*	0.10 ± 0.04*	0.29 ± 0.06*
SBP_{LF}	7.2 ± 1.2	22.4 ± 9.0*	22.6 ± 5.8*	3.4 ± 1.0*†	10.3 ± 1.7	17.3 ± 3.9	18.9 ± 6.3	4.9 ± 0.9*
SBP_{HF}	1.3 ± 0.5	3.6 ± 0.7*	6.0 ± 1.2*§	1.0 ± 0.1	1.1 ± 0.2	2.5 ± 0.6*	6.2 ± 1.6*§	1.1 ± 0.2
				WOMEN (N = 7)				
$RR_{LF/HF}$	5.8 ± 1.6	16.1 ± 4.8*	12.4 ± 3.8*	3.0 ± 0.6*	4.2 ± 1.2	13.3 ± 3.3*	14.4 ± 3.6*	2.2 ± 0.5*
RR_{HFnu}	0.19 ± 0.04	0.09 ± 0.02*	0.13 ± 0.04*	0.30 ± 0.05*	0.23 ± 0.03	0.10 ± 0.03*	0.10 ± 0.03*	0.35 ± 0.05*
SBP_{LF}	12.5 ± 1.5†‡	36.0 ± 6.3*‡	26.9 ± 7.3	5.9 ± 0.7*‡	9.4 ± 2.4	34.5 ± 8.3*‡	25.8 ± 5.9*	4.2 ± 1.4*
SBP_{HF}	1.2 ± 0.4	5.4 ± 2.1*	5.8 ± 1.7*§	1.7 ± 0.7	1.2 ± 0.4	5.9 ± 1.2*	7.4 ± 2.3*§	0.8 ± 0.4

Values are mean ± SEM. AG, artificial gravity; HDBR, head-down bed rest; ET, early tilt; LT, late tilt; $RR_{LF/HF}$, ratio of low frequency (0.04–0.15 Hz) power and high frequency (0.15–0.4 Hz) power of RR intervals, normalized unit; RR_{HFnu}, normalized high frequency power of RR intervals, normalized unit; SBP_{LF}, low frequency power of systolic blood pressure, $mmHg^2$; SBP_{HF}, high frequency power of systolic blood pressure, $mmHg^2$. *Significantly different from supine, $P < 0.05$; †significantly different from HDBR, $P < 0.05$; ‡significantly different from male subjects, $P < 0.05$; §significant difference between ET and LT, $P < 0.05$.

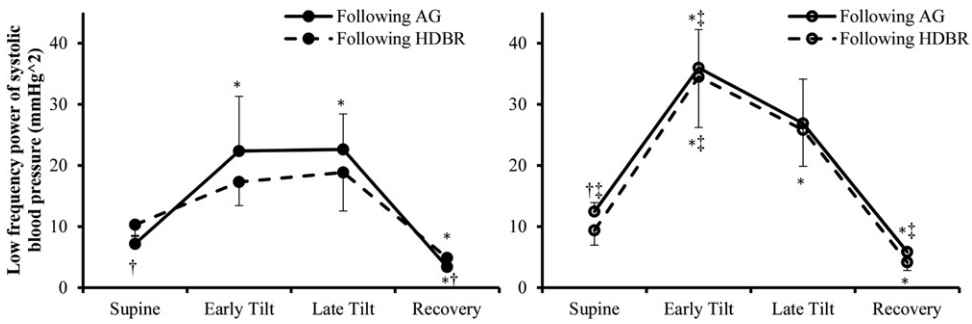

Fig. 1. Low frequency (0.04–0.15 Hz) power of systolic blood pressure response to orthostatic stress following artificial gravity (AG, solid line) and head-down bed rest (HDBR, dashed line) exposure in both male (left) and female (right) subjects. *Significantly different from supine, $P < 0.05$; †significantly different from HDBR, $P < 0.05$; ‡significantly different from male subjects, $P < 0.05$.

either AG or HDBR. Orthostatic stress reduced $Gain_{LF}$ (Time, $P < 0.0001$) at ET ($P < 0.0001$) and LT ($P < 0.0001$) and increased $Gain_{LF}$ ($P < 0.0001$) at recovery, compared to supine (**Fig. 2**). Compared with ET, $Gain_{LF}$ decreased at LT ($P < 0.0001$). Compared to supine, $Phase_{LF}$ (Treatment × Time interaction, $P = 0.0271$) decreased at ET ($P = 0.0260$) following HDBR, but did not change in response to orthostatic stress following AG.

DISCUSSION

Cardiovascular responses to orthostatic stress induced by combined HUT and progressive lower body negative pressure were tested in hypovolemic men and women following short exposures to AG and HDBR, respectively. Primary findings of this study are: 1) a short AG exposure increased women's, but tended to decrease men's, SBP_{LF} at supine, relative to those following HDBR exposure; 2) compared to supine, men's, but not women's, SBP_{LF} was still elevated at the severe level of orthostatic stress (i.e., LT) following AG exposure; and 3) in response to the mild level of orthostatic stress (i.e., ET), the $Phase_{LF}$ between SBP and RRI was unchanged following AG, but became more negative following HDBR exposure in both men and women compared with the $Phase_{LF}$ at supine.

In male subjects, the tendency of reduced supine SBP_{LF} following AG may indicate a beneficial effect of AG, since higher tolerance for upright posture has been observed in subjects with lower supine muscle sympathetic nerve activity.[1] Fu et al.[12] indicated that each subject may have a limited reserve for sympathetically medicated vasoconstriction; therefore, if resting sympathetic outflow is higher, then the sympathetic discharge upon upright posture would increase less. Waters et al.[31] reported that nonpresyncopal male astronauts have greater standing-induced norepinephrine increases than presyncopal men on landing day. In the present study, when exposed to orthostatic stress, male subjects had a significant elevation of SBP_{LF} following AG, but not HDBR, reflecting an increased sympathetic responsiveness to orthostatic stress in men after AG[9,29,30] compared to HDBR.

In addition to changes in autonomic control, increased baroreflex sensitivity[4,5,23] and increased operating point[26] have been reported following AG exposure. In the present study, we did not find elevated baroreflex sensitivity (as indicated by $Gain_{LF}$) following AG, compared to that observed following HDBR. Consistent with our previous IAG training study,[28] these results indicate that the sensitivity of baroreceptors was not enhanced by a short AG exposure. However, not only the gain response but also the time delay response determine the efficiency of baroceptors. Gulli et al.[15] have reported that subjects with different orthostatic tolerance have no differences in baroreflex sensitivity, while subjects with poor orthostatic tolerance have significantly longer phase delay between SBP and RRI using cross-spectral analysis in the LF range. In a study investigating patients with history of vasovagal syncope and healthy controls using spontaneous sequence analysis, Gulli et al.[14] indicated that most of the baroreflex responses occurred within 1 s in controls while it took more than 2 s in patients. These results emphasize that in a closed-loop feedback system, a delayed response in the output signal (e.g., RRI) may lead to system instability.[20] Therefore, the increased LF phase delay in response to 70° HUT following HDBR indicates a delayed response of

Table III. Transfer Function Gain, Phase, and Coherence Between Systolic Blood Pressure and RR Intervals in Response to Orthostatic Stress After AG vs. HDBR in Hypovolemic Men and Women.

	FOLLOWING AG				FOLLOWING HDBR			
	SUPINE	ET	LT	RECOVERY	SUPINE	ET	LT	RECOVERY
				MEN ($N = 9$)				
COH_{LF}	0.62 ± 0.03	0.65 ± 0.04	0.63 ± 0.05	0.57 ± 0.04	0.69 ± 0.02	0.64 ± 0.04	0.56 ± 0.04*	0.63 ± 0.06
$Gain_{LF}$	11.0 ± 2.7	5.0 ± 0.6*	2.2 ± 0.7*§	16.9 ± 1.7*	8.5 ± 1.5	5.2 ± 0.7*	2.8 ± 0.5*§	19.9 ± 4.0*
$Phase_{LF}$	−1.4 ± 0.1	−1.3 ± 0.2	−1.0 ± 0.3	−1.2 ± 0.2	−1.2 ± 0.2	−1.5 ± 0.2*	−1.5 ± 0.1	−1.5 ± 0.2
				WOMEN ($N = 7$)				
COH_{LF}	0.78 ± 0.06‡	0.82 ± 0.03‡	0.58 ± 0.04*†§	0.59 ± 0.02*	0.72 ± 0.03	0.77 ± 0.04‡	0.75 ± 0.03‡	0.63 ± 0.09
$Gain_{LF}$	9.8 ± 1.6	4.7 ± 0.5*	3.5 ± 0.9*§	13.7 ± 2.5*	11.1 ± 1.6	5.5 ± 0.9*	3.2 ± 0.8*§	22.8 ± 5.2*
$Phase_{LF}$	−1.3 ± 0.1	−1.2 ± 0.2	−1.4 ± 0.2	−1.3 ± 0.4	−1.2 ± 0.2	−1.3 ± 0.1*	−1.6 ± 0.2	−1.4 ± 0.3

Values are mean ± SEM. AG, artificial gravity; HDBR, head-down bed rest; ET, early tilt; LT, late tilt; COH_{LF}, coherence in the low frequency range, a.u.; $Gain_{LF}$, transfer function gain in the low frequency range, ms/mmHg; $Phase_{LF}$, transfer function phase in the low frequency range, radians. *Significantly different from supine, $P < 0.05$; †significantly different from HDBR, $P < 0.05$; ‡significantly different from male subjects, $P < 0.05$; §significantly difference between ET and LT, $P < 0.05$.

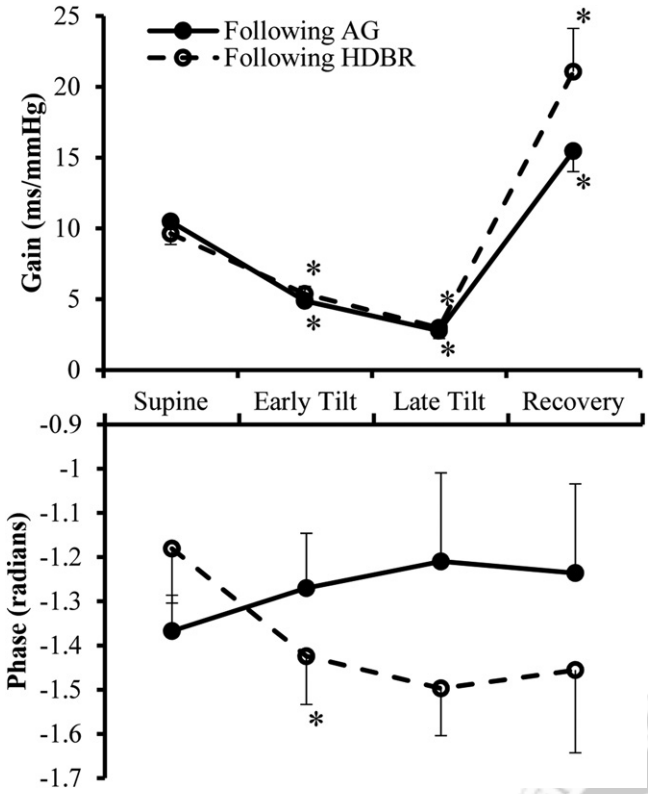

Fig. 2. Low frequency (0.04–0.15 Hz) transfer function gain and phase responses to orthostatic stress following artificial gravity (AG, solid line) and head-down bed rest (HDBR, dashed line) exposures. *Significantly different from supine, $P < 0.05$.

HR to dampen BP oscillations, and the delayed effector response could generate an unstable state of regulation,[18] which may lead to early onset of presyncope. Compared to the more negative phase following HDBR in response to 70° HUT, the sustained $Phase_{LF}$ between SBP and RRI following AG reflects enhanced baroreflex responsiveness to orthostatic stress. This indicates that even a short AG exposure can enhance baroreflex responsiveness, although the pattern of this improvement is different from previous AG studies.[9,23,26] Furthermore, it has been determined that the delay between SBP and RRI oscillations increased when vagal tone was low.[18] Westerhof et al.[32] found that subjects who presented presyncopal symptoms during 70° and 90° HUT had extended phase delay during the first 2 min of 70° and 90° HUT compared with those who did not, indicating sympathetic excitation. Gulli et al.[16] found a less negative phase 2–3 min before and during presyncope in fainters compared with nonfainters, indicating a disengaged sympathetic activity. These results reflect that fainters seem to engage,[32] and disengage,[16] sympathetic activity earlier. Therefore, in the present study, compared to supine, the increased $Phase_{LF}$ at ET indicated early sympathetic activation following HDBR exposure, while the well maintained $Phase_{LF}$ following AG may preserve the sympathetic adjustment and contribute to greater OTL than that observed following HDBR.

In the present study, female subjects were less tolerant of orthostatic stress, as evidenced by ~30% lower OTL following both AG and HDBR exposure.[8] Several mechanisms, such as differences in hemodynamic responses,[11] autonomic cardiovascular regulation,[10] sympathetic neural responses,[13] and baroreflex responses[19] to orthostatic stress, may contribute to poorer orthostatic tolerance in women. We previously reported that female subjects had smaller stroke volume (SV) during the orthostatic tolerance limit test.[8] In the present study, the greater HR in women at ET indicated the presence of an important compensatory mechanism for relatively smaller SV;[13] however, HR responses were similar when approaching syncope even though SV was still lower in women. The loss of HR compensation may induce less cardiac filling and contribute to the lower OTL. The significantly greater SBP_{LF} at ET in women indicated that female subjects achieved greater sympathetic excitation at milder levels of orthostatic stress than men, but might not have enough vasoconstrictor reserve to compensate for further central hypovolemia.[12] Indeed, we observed slightly reduced SBP_{LF} (~10%) in women during LT with respect to ET, reflecting reduced sympathetic outflow to the vasculature. In contrast to women, men maintained their SBP_{LF} levels during LT, compared with those during ET. In this study, although OTL improved significantly for both men and women, the OTL improvement of the women was not statistically significant as a separate group. This difference is not likely to be attributed to different dehydration levels, since our previous report[8] has shown comparable plasma volume changes at the beginning of the OTL test for both men (8.98 ± 1.79% on the AG day vs. 6.80 ± 1.59% on the HDBR day) and women (7.69 ± 1.84% on the AG day vs. 8.63 ± 1.74% on the HDBR day). This difference is also not likely to be attributed to the relatively lower level of AG exposure since our previous studies[28,30] indicated that 3 wk of passive IAG exposure did not significantly improve women's OTL. However, IAG exposure with exercise training did increase women's OTL.[28,30] Convertino et al.[5] reported that cardiovascular adaptations to hypergravity training was dependent of gender and indicated that women had an inherently limited capacity to improve their orthostatic performance. Therefore, efficient AG protocols or a combination of AG and other countermeasures need to be further investigated to support optimal performance of both men and women during subsequent orthostatic stress.

We acknowledge two limitations of this study. First, we were unable to conduct AG and HDBR protocols during the same stage of the women's menstrual cycle, which may affect the results due to hormone variations.[22] However, orthostatic tolerance and cardiovascular control have been shown not to be affected by the phase of the menstrual cycle.[2] Second, although this study was designed to study a passive AG countermeasure, subjects did a small amount of exercise during and following the AG exposure. During AG exposure, subjects were asked to bend their toes upward when development of presyncope was expected. After AG, to maintain gravitational exposure, subjects walked to the OTL station (~20 m). After HDBR, to maintain the simulation of spaceflight, subjects were transported to the OTL station via gurney. These differences in activity may have had some influences on the results.

We conclude that a short-duration exposure to artificial gravity increased some aspects of baroreflex activity and sympathetic responsiveness to orthostatic stress, compared with exposure to 90 min of head-down bed rest, in a pharmacologically induced hypovolemic condition in both men and women. Cardiovascular adaptions to artificial gravity may contribute to improved orthostatic tolerance when reentering a gravitational environment.

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