Eur J Appl Physiol (2015) 115:2631–2640
DOI 10.1007/s00421-015-3261-2

ORIGINAL ARTICLE

Hypovolemic men and women regulate blood pressure differently following exposure to artificial gravity

Joyce M. Evans[1] · L. Christine Ribeiro[2] · Fritz B. Moore[3] · Siqi Wang[1] · Qingguang Zhang[1] · Vladimir Kostas[1] · Connor R. Ferguson[1] · Jorge Serrador[4] · Michael Falvo[4] · Michael B. Stenger[2] · Nandu Goswami[5] · Jon C. Rask[3] · Jeffrey D. Smith[3] · Charles F. Knapp[1]

Received: 12 May 2015 / Accepted: 6 September 2015 / Published online: 5 October 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract
Purpose In addition to serious bone, vestibular, and muscle deterioration, space flight leads to cardiovascular dysfunction upon return to gravity. In seeking a countermeasure to space flight-induced orthostatic intolerance, we previously determined that exposure to artificial gravity (AG) training in a centrifuge improved orthostatic tolerance of ambulatory subjects. This protocol was more effective in men than women and more effective when subjects exercised.
Methods We now determine the orthostatic tolerance limit (OTL) of cardiovascularly deconditioned (furosemide) men and women on one day following 90 min of AG compared to a control day (90 min of head-down bed rest, HDBR).
Results There were three major findings: a short bout of artificial gravity improved orthostatic tolerance of hypovolemic men (30 %) and women (22 %). Men and women demonstrated different mechanisms of cardiovascular regulation on AG and HDBR days; women maintained systolic blood pressure the same after HDBR and AG exposure while men's systolic pressure dropped (11 ± 2.9 mmHg) after AG. Third, as presyncopal symptoms developed, men's and women's cardiac output and stroke volume dropped to the same level on both days, even though the OTL test lasted significantly longer on the AG day, indicating cardiac filling as a likely variable to trigger presyncope.
Conclusions (1) Even with gender differences, AG should be considered as a space flight countermeasure to be applied to astronauts before reentry into gravity, (2) men and women regulate blood pressure during an orthostatic stress differently following exposure to artificial gravity and (3) the trigger for presyncope may be cardiac filling.

Keywords Orthostatic tolerance · Cardiovascular deconditioning · Centrifugation · Cardiac output · Stroke volume · Heart rate

Abbreviations

AG	Artificial gravity
OTL	Orthostatic tolerance limit
HDBR	Head-down bed rest
DLR	Deutches Zentrum fur Luftwaft und Raumfahrt
gz	Gravity applied in the body's long (head/toe) axis
gx	Gravity applied in the body's anterior/posterior (front/back) axis
g	Earth gravity
HUT/LBNP	Head up tilt combined with lower body negative pressure
Hz	Cycles/second
mg	Milligrams
ml	Milliliters

Communicated by Keith Phillip George.

✉ Joyce M. Evans
jevans1@uky.edu

[1] Department of Biomedical Engineering, University of Kentucky, 514G, Robotics and Manufacturing Building, 143 Graham Avenue, Lexington, KY 40506, USA

[2] Wyle Science, Technology and Engineering Group, Houston, TX, USA

[3] NASA Ames Research Center, Moffett Field, CA, USA

[4] Veterans Affairs New Jersey Health Care System, East Orange, NJ, USA

[5] Medical University of Graz, Graz, Austria

Introduction

Decreased plasma volume (hypovolemia) and disruption of regulatory mechanisms contribute to space flight-induced cardiovascular deconditioning and may lead to post-flight orthostatic intolerance. A limited ability to counteract this loss when re-presented with gravitational stress is a significant problem in returning astronauts (Buckey et al. 1996). Therefore, since the duration of space missions will lengthen in the future, identifying countermeasures to space flight-induced cardiovascular deconditioning is increasing in priority. This study was developed to model cardiovascular effects of spaceflight with and without an AG countermeasure applied before return to a gravity environment.

Gravity as an effective countermeasure to bed rest-induced cardiovascular deconditioning was established many years ago in a study demonstrating that exposure to Earth's gravity (2 h of standing each day) improved orthostatic tolerance in bed-rested men (Vernikos et al. 1996). However, since gravity is absent during space missions, we previously conducted a series of studies using exposure to short radius AG (centrifugation) in a long-term "training" model to explore its efficacy as a countermeasure to deconditioning (Evans et al. 2004; Stenger et al. 2007; Stenger et al. 2012). Results from those studies (Evans et al. 2004; Stenger et al. 2007; Stenger et al. 2012) indicated that 3 weeks of daily exposure to AG tended to reduce the loss of plasma volume (PV) resulting from bed rest and significantly improved orthostatic tolerance through enhanced activation of cardiovascular reflexes. Specifically, we found that 3 weeks of AG training (45–60 min a day) on a short radius centrifuge increased orthostatic tolerance in ambulatory men and women and in bed-rested men: In ambulatory, normovolemic subjects (Evans et al. 2004; Stenger et al. 2007), AG training was more effective in men than women and more effective in subjects who exercised during AG exposure than in those who passively rode the centrifuge (Evans et al. 2004; Stenger et al. 2007). Subsequently, we determined that the orthostatic tolerance of bed-rested (21 days) men who underwent an hour of AG training per day was greater than that of their untrained male controls (Stenger et al. 2012). However, bed-rested women were not included in that study.

In a companion (to the present) study, we observed that 90 min of AG exposure to a matched protocol (individualized to provide a common stimulus to each person) improved orthostatic tolerance in normovolemic men and women, who were mildly deconditioned by 60 min of supine rest (Goswami et al. 2013; Goswami et al. 2015). We now seek to determine effects of this protocol on men and women who were deconditioned by furosemide infusion following a 24-h low salt diet, a combination shown to induce plasma volume loss similar to that observed in astronauts returning from space flight (Fu et al. 2005; Romero et al. 2011). On the control day, a 90-min exposure to −6° head-down bed rest (HDBR) followed the furosemide infusion. Specifically, on this day of study, we used low salt diet, furosemide, 90-min HDBR, and a test of subjects' orthostatic tolerance limit (OTL) to model the cardiovascular response to spaceflight followed by return to Earth without employing a countermeasure. On the other day of study, we followed the same protocol except that we substituted 90 min of AG for 90 min of HDBR to model spaceflight with a short exposure to the AG countermeasure before return to Earth. We hypothesized that the day that included the 90-min exposure to AG would increase orthostatic tolerance compared to the day without the AG countermeasure.

In light of our previous findings of gender differences in response to AG training, our second focus was to examine responses to acute AG and HDBR exposures for differences between these hypovolemic men and women, in regard to blood pressure, cardiac output, stroke volume, heart rate and peripheral vascular resistance regulation during orthostatic stress. We hypothesized that men would demonstrate greater orthostatic tolerance than women.

Methods

Subjects and protocol overview

Nine men (Mean ± SD: 37.7 ± 4.0 years, 174.8 ± 3.2 cm, 81.2 ± 4.4 kg) and eight women (29.6 ± 2.2 years, 168 ± 1.9 cm, 70.8 ± 3.5 kg) gave written informed consent to the experimental protocol, approved by the NASA Ames Research Center and University of Kentucky Human Research Institutional Review Boards. Subjects were familiarized with each aspect of the study approximately 20 days before their first of two study visits. Study visits consisted of the following, performed in sequential order: (1) furosemide infusion, (2) experimental condition, and (3) test of the subject's orthostatic tolerance limit, Fig. 1. The experimental conditions were 90 min of −6° HDBR and 90 min of an individualized AG protocol. The two study visits were separated by 21 days and half the subjects had their HDBR day first and the other half had their AG day first.

Voluntary salt restriction

The following advice on voluntary salt reduction was given orally and in writing: "The day before both of the study days you will need to be very conscientious of restricting your salt intake (don't forget to keep an eye on the sodium

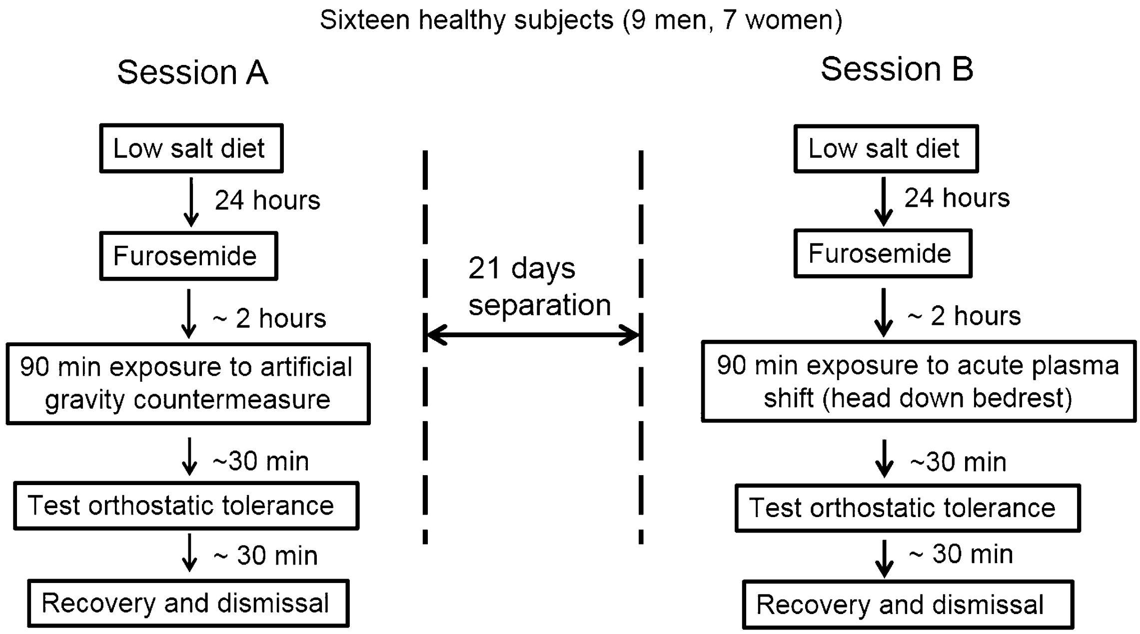

Fig. 1 Outline of study protocol. On both study day A and B, induction of plasma volume loss approximating that of space flight, preceded the study intervention. On study day A, subjects received 90 min of artificial gravity exposure to simulate application of an artificial gravity countermeasure during spaceflight before return to Earth gravity (simulated by the orthostatic tolerance test). On study day B, subjects received 90 min of head-down bed rest (HDBR) to enhance the effect of spaceflight by simulating the acute, headward plasma shift of spaceflight before testing their tolerance for return to gravity (orthostatic tolerance test). To randomize the protocol, eight subjects completed study day A first, then completed study day B 21 days later. The other eight subjects completed study day B first then completed study day A 21 days later

content in beverages).” A reference to sodium content of food was also given to each subject.

Pre-furosemide baseline

Prior to other experimental interventions, including furosemide infusion, subjects lay supine for a 15-min data acquisition session that included continuous blood pressure and heart rate (Portapres, Finapres Medical Systems BV, Amsterdam, The Netherlands). The only response to furosemide reported here will be the change in plasma volume.

Cardiovascular deconditioning (furosemide) protocol

To induce hypovolemia on each day of study, 20 mg furosemide was administered IV following 24 h of voluntary salt intake reduction to induce hypovolemic cardiovascular deconditioning (Fu et al. 2005; Romero et al. 2011).

Artificial gravity protocol

Studies were performed aboard the Human Performance Centrifuge at NASA Ames Research Center. Subjects rode supine and were oriented radially, with head towards the center of rotation. The individualized AG training protocol used in this study was similar to the one used for subjects at DLR, Cologne, Germany (Goswami et al. 2013; Goswami et al. 2015). However, due to the loss of plasma volume resulting from the furosemide infusion, ramping was started at lower g levels in the present study At the end of a 5-min supine control period, a blood sample was drawn and each subject underwent a “step up” AG protocol consisting of a determination of their presyncopal limit (~45 min) followed by a training period (~45 min) at g levels below their presyncopal limit: Hypovolemic men were taken to 0.6 gz (measured at the heart), as compared to 0.8 gz in the DLR study, at increments of 5 rpm/sec/sec and held there for 5 min, then incremented by 0.1 gz at 3-min intervals until the development of presyncopal conditions. Subjects were brought back to rest for 10 min. A training protocol was then initiated, similar to the one for determining the presyncopal limit, but the maximum g level was held 0.2 g below the step that produced presyncopal symptoms. The training protocol lasted an additional 45 min. Hypovolemic women tended to be less tolerant of gz stress than men. For that reason, we brought women to 0.4 gz at the heart (in the DLR study they were started at 0.6 gz), and followed the same protocol as men. Following training, the subject rested for 5 min and the second blood sample was drawn at the end of this recovery period. To maintain gravity stress in the time between the AG exposure and the OTL test, subjects walked to the rest room (~2 min) and then were placed supine and re-instrumented in the OTL station (total time ~30 min) to undergo their orthostatic tolerance test.

Blood pressure, heart rate (Portapres) and Gz (spinal axis, Crossbow) acceleration level were measured continuously during AG exposure. Data collected during the AG exposure are not given here.

Head-down bed rest protocol

On the test day, and following blood pressure and urine output stabilization from furosemide infusion (~2 h), subjects underwent 90 min of −6° HDBR in a semi-dark room with temperature between 70 and 75 °F. Blood samples were drawn at the end of the first 5 min and end of the 90 min of HDBR. During the 90-min HDBR, blood pressure (Portapres, FMS), ECG (Spacelabs) and heart rate (Portapres FMS) were measured continuously. At the end of the HDBR period, subjects were taken by gurney (to maintain the weightlessness simulation) to the bathroom and the OTL station. Data collected during the HDBR exposure are not given here.

OTL protocol (final procedure on each test day)

Following both the AG and HDBR procedures, subjects underwent the OTL protocol detailed below, using a head up tilt (HUT), lower body negative pressure (LBNP) device (a tilt table, fitted with a chamber sealed at the subject's waist, in which external pressure on the lower body could be controlled).

Control (supine) data were taken for 10 min before HUT. The tilt table was then brought to 70° HUT 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. When presyncopal symptoms developed, LBNP was released and the subject was brought back to supine or, in cases of more severe presyncope, to 6° head down until symptoms abated. Blood samples were drawn at the end of supine control and at 1-min post-OTL-induced presyncope to determine plasma volume shifts. Continuous measurements: blood pressure, heart rate, estimates of stroke volume, cardiac output and total peripheral resistance (Finometer with height correction). Manual brachial artery blood pressures (LifeSource UA-767) were acquired at each step of the protocol and used for calibration of Finometer measurements.

Blood samples were drawn at the end of control and beginning of recovery.

OTL data analysis

Data were acquired at 1000 Hz using Windaq (DATAQ, Akron, OH) and stored for later analysis. 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. All artifacts were removed by visual inspection. Each time series was then linearly interpolated and resampled at 4 Hz for further analyses. Because each subject had his/her own OTL time, downsampled (4 Hz) data for all subjects were aligned at the beginning and end of tilt. For this report, we present group mean ± SEM data in the following epochs: the last 2 min of supine control, the first 2 min of the OTL test (tilt only). The last 2 min of the OTL test (tilt plus LBNP in some subjects, tilt only in others) and the first 2 min of recovery. One woman was unable to participate in the AG study, therefore, her data were removed from all data sets prior to analysis. Data are presented for nine men and seven women.

Blood samples

A 2-ml blood sample was drawn from the antecubital vein prior to furosemide infusion, and at control and recovery from each protocol to assess plasma volume (Hematocrit/Hemoglobin) changes (Greenleaf et al. 1979) in response to furosemide and in response to 90 min of HDBR, 90 min of AG and the OTL test. Samples were sent to Quest Diagnostics for analysis.

Statistical analysis

All subjects received all treatments. Cardiovascular variables were examined with analysis of covariance in an unstructured pattern model, using four factors: treatment (AG training vs HDBR), gender (men vs. women), time point (control, first 2 min of the OTL test, last 2 min of the OTL test and recovery) and period (a test for first day effects). Tests of hypothesized differences in main effects and interactions were conducted using $p < 0.05$ as the cutoff for significance. Post hoc comparisons were assessed from differences of least squares means. A one-tailed, paired t test was used to determine the significance of the orthostatic tolerance limit following a 90-min AG exposure compared with that following a 90-min HDBR exposure. Analysis was completed using SAS 9.3 (SAS Institute Inc., Cary, NC). Values are shown as mean ± standard error of the mean (SEM).

Results

Plasma volume changes

Plasma volume changes in response to voluntary salt restriction and furosemide infusion were similar on the 2 days; an average of −7.14 ± 0.20 % ($p < 0.001$) for subjects on the day in which they were to undergo HDBR and −8.64 ± 0.24 % ($p < 0.001$) for subjects on the day they were to undergo AG. Subsequent plasma volume changes in response to 90 min of AG averaged −6.15 ± 0.38 % and plasma volume changes in response to 90-min HDBR averaged +1.36 ± 0.15 %; this difference was significant, $p < 0.001$. By the time of supine control for OTL, however, this difference had disappeared as evidenced by hematocrit and hemoglobin that were not different on the 2 days. During OTL testing, plasma volume decreased similarly on the 2 days: −8.47 ± 0.31 % ($p < 0.001$) for the HDBR day and −8.28 ± 0.24 % ($p < 0.001$) for the AG day. This small difference was not significant, even when normalized by the length of the OTL test. There was no difference in OTL-induced plasma volume changes between male and female subjects.

Individualized artificial gravity protocol

We determined that we could bring each person to a similar physiological point in their artificial gravity exposure with the application of the individualized protocol given here. As with the companion study of normovolemic men and women (Goswami et al. 2013; Goswami et al. 2015), each of our hypovolemic subjects completed the 90-min AG protocol and the subsequent OTL protocol.

Orthostatic tolerance response

We determined that a single, 90-min exposure to individualized artificial gravity profiles, significantly ($p < 0.033$) increased hypovolemic subjects orthostatic tolerance limit (the time required to produce a presyncopal response to the OTL protocol), compared to their OTL response following 90 min of HDBR. This increase of 30.1 % in men and 22 % in women is shown in Fig. 2.

Overlays of OTL Data

To contrast differences with and without AG, we show overlays of AG and HDBR day blood pressure, heart rate, cardiac output and total peripheral resistance waveforms aligned at the beginning and end of OTL for 9 men (left) and 7 women (right) in Fig. 3. These traces represent downsampled data, averaged (center line ± SEM) over each group on each day.

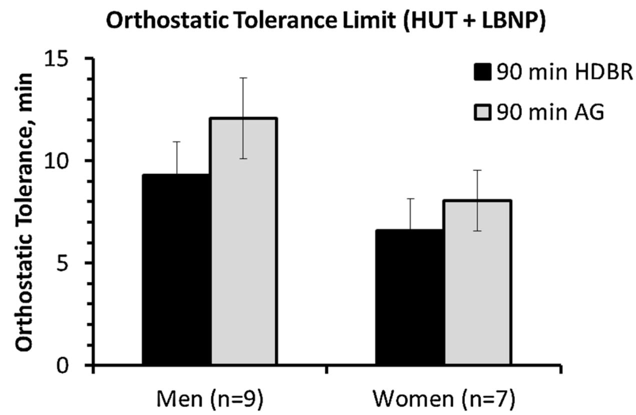

Fig. 2 Orthostatic tolerance limit of 9 men and 7 women on their day with artificial gravity (*AG*) exposure (*gray*) and their control day with head-down bed rest (*HDBR*) (*black*). Values are mean ± SEM. OTL was greater on the AG day, $p < 0.033$, differences between men and women were not significant

Across control, the OTL test and recovery, men's blood pressure and total peripheral resistance were lower, and cardiac output was higher, on their AG, (light band), compared to their HDBR (dark band), day. Also, throughout the OTL test, men's blood pressure and cardiac output declined, while heart rate rose and peripheral resistance was maintained fairly constant. By the end of OTL, cardiac output and peripheral resistance reached the same values on both days, even though the OTL test lasted 30 % longer on the AG day.

Overlays of HDBR and AG data under the same conditions are shown for the seven women of this study on the right side of Fig. 3. Unlike men, women's blood pressure was not different on the 2 days even though they demonstrated a trend to lower total peripheral resistance on the AG day. As with men, women's cardiac output and total peripheral resistance reached the same values at presyncope on both days, and, in women, the OTL test on the AG day lasted 22 % longer compared to the HDBR day. Also different from men, women's heart rate rise at the end of OTL was not as great on the HDBR, as on the AG, day. Significant effects of gender, HDBR vs AG, time point and interactions between these factors will be discussed by variable below.

Two-minute averaged values

Group means were tested on the 2 days for the data segments shown in Fig. 3 for: (1) the last 2 min of control, (2) the first 2 min of the OTL test, (3) the last 2 min of the OTL test and (4) the first 2 min of recovery. Summaries of results for each cardiovascular variable thus tested follow:

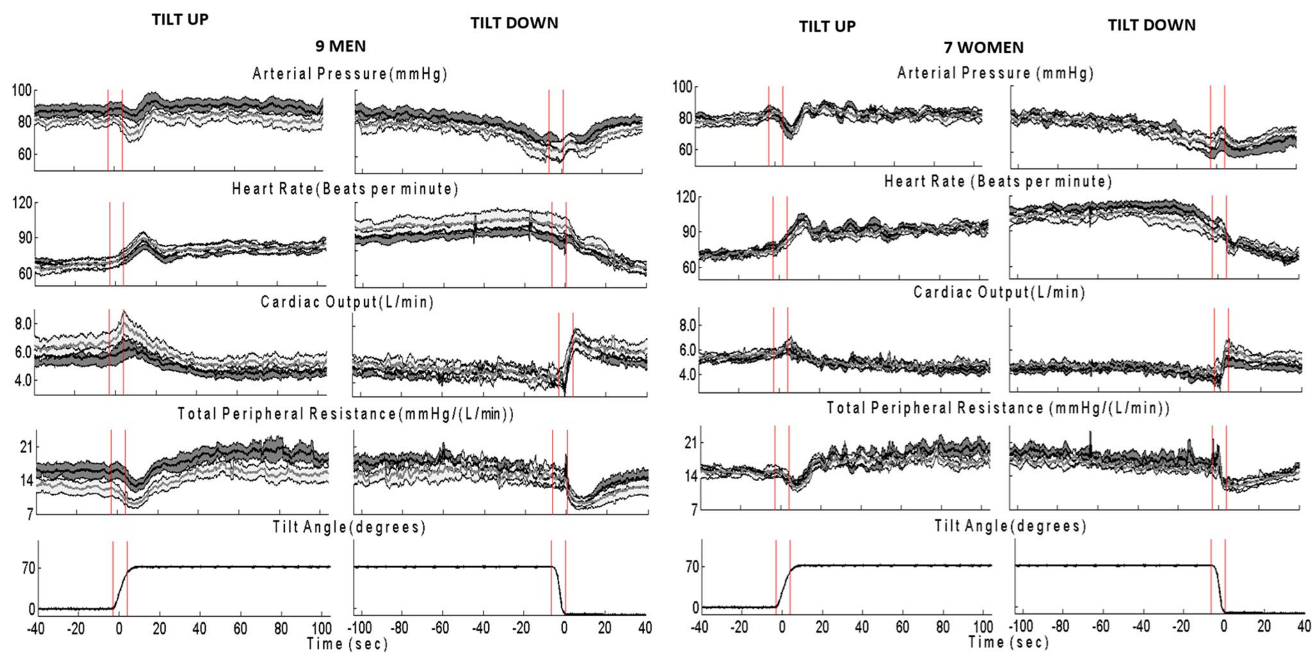

Fig. 3 Overlay of cardiovascular variables: arterial pressure, heart rate, cardiac output, peripheral resistance and tilt angle, at the end of supine control, start of OTL, end of OTL and start of recovery on HDBR (*black*) and AG (*gray*) days for 9 men (*left*) and 7 women (*right*). Values are mean ± SEM

Group (men and women) cardiac output was marginally greater on the AG day than on the HDBR day ($p < 0.056$). Cardiac output dropped significantly ($p < 0.001$) during the first 2 min of OTL and continued to decline ($p < 0.007$) between the first and last 2 min with a full recovery during the first 2-min post-OTL. Presyncopal cardiac output was not different on the AG and HDBR days in either men or women.

Men demonstrated greater stroke volume than women ($p < 0.042$) in all conditions. On both days, group stroke volume dropped significantly ($p < 0.0001$) during the first 2 min of OTL and continued to decline ($p < 0.0001$) between the first and last 2 min with a full recovery during the first 2-min post-OTL. In addition a significant ($p < 0.019$) treatment by time point interaction for the group indicated that the greater stroke volume seen during control and the first 2 min of OTL on the AG day was gone by the last 2 min of the OTL test.

Group heart rate rose ($p < 0.001$) at the beginning and end ($p < 0.001$) of the OTL test. Women's heart rate was greater than men's during the first 2 min of tilt ($p < 0.004$). Men's and women's heart rates during the last minute of OTL were not significantly different.

Group peripheral resistance was lower on the AG day than on the HDBR day (main effect of study day, $p < 0.028$). On both days, resistance rose significantly (main effect of time point, $p < 0.003$) during the first 2 min of the OTL test, declining slightly during the last 2 min and returning to control values within a minute of the end of tilt.

On both days, diastolic blood pressure rose in the first 2 min of the OTL test (time point, $p < 0.036$), dropped significantly during the last 2 min ($p < 0.02$) and remained lower in recovery ($p < 0.01$).

Similarly, mean blood pressure rose in the first 2 min of the OTL test on the HDBR, but not on the AG day (study day × time point interaction, $p < 0.035$). On both HDBR and AG days, MBP was lower during the last 2 min of OTL ($p < 0.008$) and remained lower in recovery ($p < 0.001$).

Mean values of systolic blood pressure are shown in Fig. 4: In addition to the significant drop in systolic pressure during the last 2 min of the OTL test and beginning of recovery (time point, $p < 0.0001$), men and women had different reactions to HDBR and AG (gender × study day, $p < 0.012$): men's overall systolic pressure on their HDBR day was higher compared to their AG day ($p < 0.005$) and higher than women's on their HDBR day ($p < 0.013$). The significant rise in diastolic and mean blood pressure during the first 2 min of tilt was not significant for systolic pressure.

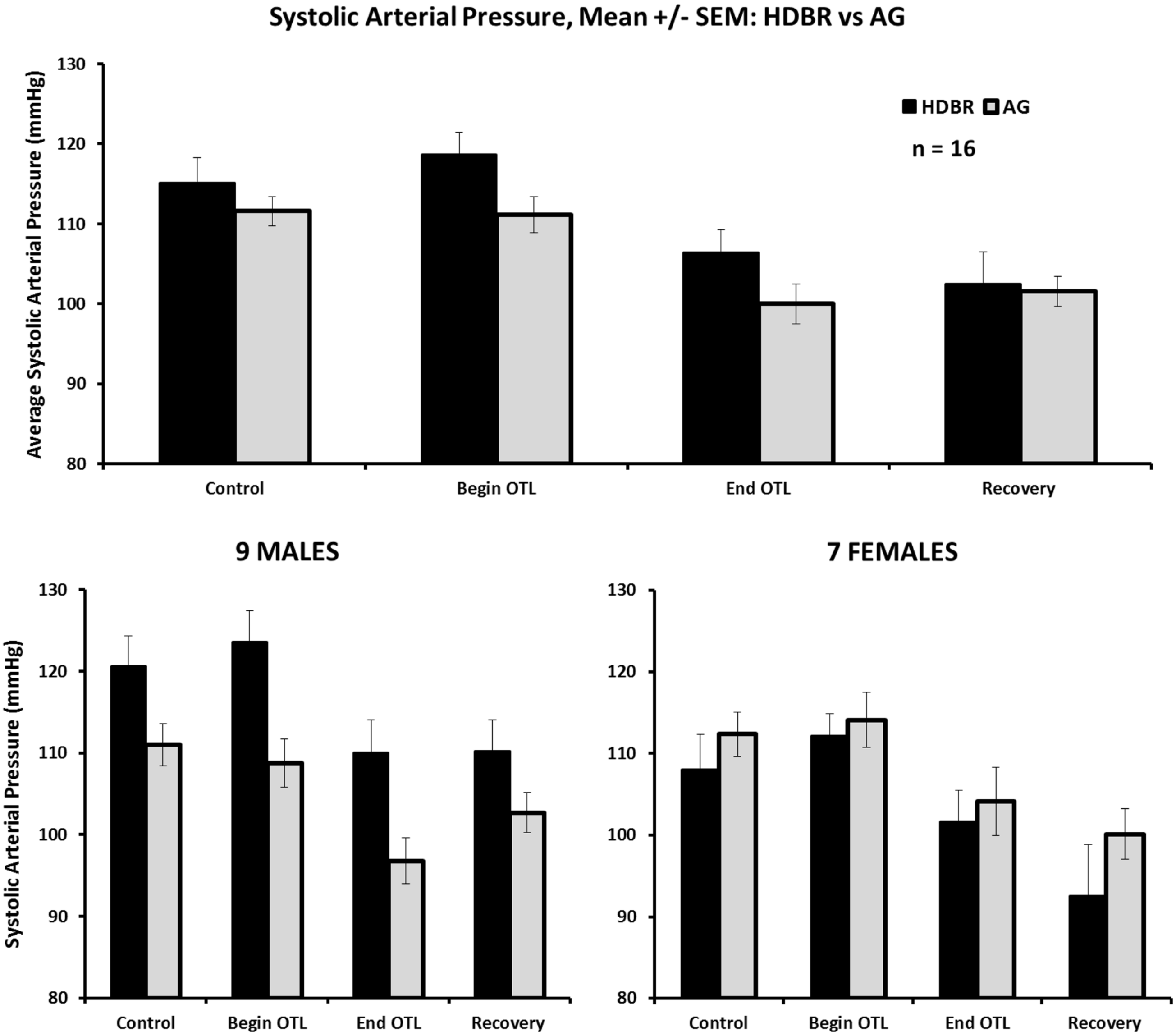

Fig. 4 Mean ± SEM systolic blood pressure for control, the first 2 min (*Begin OTL*), last 2 min (*End OTL*) and the first 2 min of recovery from presyncopal orthostatic stress for the group of 16 subjects (*top*) consisting of 9 men and 7 women (*below*). Values on the HDBR day are shown in *black* and values on the AG day are shown in *gray*. Significant ANOVA effects: time point (control > end OTL and recovery ($p < 0.001$) and begin OTL > end OTL $p < 0.001$). Day × gender: males on AG day < males on HDBR day ($p < 0.04$) and males > females on HDBR day ($p < 0.04$), time point × gender: males > females at recovery, $p < 0.03$

Discussion

This study had three major findings: A short bout of artificial gravity improved the orthostatic tolerance of hypovolemic men and women through decline of peripheral resistance and increase of cardiac output. Men and women demonstrated different mechanisms for regulating their cardiovascular responses to OTL after AG exposure as compared to the HDBR day; women appeared to regulate blood pressure while men appeared to regulate tissue perfusion. Finally, as presyncope developed, both men's and women's cardiac output (due to stroke volume) dropped to the same level on both days, independent of the length of the tolerance test or which protocol preceded the OTL test, indicating that cardiac filling was a likely variable to trigger the presyncopal response.

The fact that these deconditioned men's and women's orthostatic tolerance was greater on the day in which they were exposed to a short bout of artificial gravity confirmed our study hypothesis and is in agreement with results we obtained in a companion study that used a similar single bout of individualized AG training in ambulatory, normovolemic men and women (Goswami et al. 2013; Goswami et al. 2015). The hypothesis for both studies was based on results from previous studies we, and others, conducted in which it was established that artificial gravity training or standing in earth gravity improved orthostatic tolerances of ambulatory men and women (Evans et al. 2004; Stenger et al. 2007), and of deconditioned (bed rest) men (Stenger et al. 2012; Vernikos et al. 1996). A major difference between the present (and its companion) study and previous studies was the fact that the current studies demonstrate effects of a single bout of AG exposure on subsequent orthostatic tolerance. In addition, this is the first study to show how individualized AG training improved orthostatic tolerance in hypovolemic men and women. Finally, this study allows us to make the very broad inclusive statement that a single AG exposure, in addition to long-term AG training, improves orthostatic tolerance in normo-and hypovolemic men and women. Mechanisms associated with increased orthostatic tolerance following exposure to artificial gravity included a significant decline in peripheral vascular resistance (main effect of treatment, $p < 0.028$) and a tendency to increase cardiac output (main effect of treatment, $p < 0.056$) following AG, compared to HDBR exposure. Other principal effects were associated with gender × treatment interactions: systolic blood pressure ($p < 0.011$) and stroke volume ($p < 0.06$) declined in men but not in women following AG compared to HDBR exposure. Therefore, the decrease in systolic pressure in men following AG exposure came primarily from decreases in both resistance and stroke volume. Women maintained systolic pressure on their AG day through an increase in stroke volume that overcame their decrease in peripheral resistance.

Since the present study demonstrated that a single, acute bout of AG improved orthostatic tolerance, investigation into effects on bed-rested subjects is warranted. Given the positive results obtained in the previous study of AG training effects on bed-rested men (Stenger et al. 2012), the effectiveness of AG exposure on bed-rested women should be determined. Then, if a bed rest model of space flight-induced cardiovascular deconditioning indicates a positive effect of AG on orthostatic tolerance in both men and women, the feasibility of short exposures to AG, during longer spaceflights or prior to entry into a gravity (Earth, Mars or the Moon) environment, becomes a viable countermeasure that warrants consideration.

Of similar importance was the current study's demonstration of differences between men's and women's cardiovascular responses to this acute exposure to artificial gravity: men's blood pressure was lower following AG exposure while women's blood pressure was the same following both AG and HDBR exposures. Men's blood pressure response to AG exposure was quite similar to the post-exercise hypotensive response described previously (Cote et al. 2015; MacDonald 2002). Those studies determined that decreased blood pressure following exercise was a result of decreased peripheral vascular resistance, indicating that AG exposure may, like exercise, influence cardiac output to meet perfusion needs of the vasculature. These data are consistent with evidence for the dominance of peripheral vascular demand as a major controller of the response to numerous stresses, a topic recently highlighted by Joyner and Limberg (Joyner and Limberg 2014). We were surprised that women of our study did not exhibit post-AG hypotension. Differences in men's and women's strategies for regulating blood pressure may be related to gender differences in the rate of orthostatic cardiac output reduction (Convertino 1998), in beta adrenergic (Joyner and Limberg 2014; Convertino 1998) or alpha adrenergic (MacDonald 2002) responsiveness, in basal vagal tone (Evans et al. 2001; Convertino 1998), in carotid-cardiac responsiveness (Convertino 1998), and/or in sympathetic neural responsiveness to orthostatic stress (Yang et al. 2012). The latter study indicated that during orthostatic stress, men and women demonstrated different firing patterns of muscle sympathetic nerve activity, with men demonstrating a greater increase in coherence between blood pressure and muscle sympathetic nerve activity during orthostatic stress than did women (Yang et al. 2012). We also previously determined a dominance of sympathetic vascular regulation in men compared with a dominance of parasympathetic influence on heart rate regulation in women, consistent with results of the present study (Evans et al. 2001).

Our third major finding was that stroke volume and cardiac output appeared to predict impending presyncope. By the time of presyncope, both had reached the same low point on both days even though the OTL test lasted longer (30.1 % in men and 22 % in women) on the AG day. Since plasma volume started from the same values on both days and declined similarly, the significantly greater values of cardiac output (due to stroke volume) at rest and during early tilt on the AG exposure day appear to be functions of a resetting of cardiovascular regulatory control, rather than a function of plasma volume. Therefore, the decline in cardiac output and stroke volume to the same presyncopal level on both AG and HDBR days indicated that reduced cardiac filling was a likely source of the signal that evoked presyncope. This is an old concept, clearly shown in animal studies (Oberg and White 1970), and indirectly shown in humans (Thoren 1979), that supports the results of the present study.

Toward the end of OTL, the rise in men's heart rate on the AG day probably contributed to their increased orthostatic tolerance, even though men's heart rate averaged over the last 2 min was not significantly different on the

2 days. This was in contrast to the women's heart rate rise during their last 2 min of OTL which was not as robust on their AG, as on their HDBR, day, but a significant interaction between gender, day and time point would have been required to establish heart rate as a significant component of the difference in final OTL responses on the 2 days.

The hypovolemia model of space flight used in the present investigation is supported by a study of men in normo- and hypovolemic states whose decline in plasma volume of 10 % in response to 40 mg IV furosemide was similar, as were their declines in stroke volume and systolic blood pressure in response to head up tilt (Romero et al. 2011). That study did not provide data that could be used to compare results between men and women or responses to AG. Similar results were reported in which voluntary salt reduction plus the Lasix dosage (20 mg) used in the present study, followed by a 2-h stabilization period, induced a plasma volume loss similar to that occurring with space flight (Romero et al. 2011; Fu et al. 2005). Therefore, our subjects' 7.1–8.6 % reduction in plasma volume in response to the present protocol support the hypovolemia aspect of this model of space flight cardiovascular deconditioning.

Strength of the present, and its companion study, lies in the fact that all subjects were taken to the same physiological limit. This limit was then used to determine the AG protocol to be administered. As a result, all subjects received a 90-min AG protocol that, although not of the same absolute g levels, provoked a response that was individualized to each subject's orthostatic tolerance level. This is particularly important when making comparisons between genders and between subjects of different body heights, body compositions and gravity tolerances.

Limitations: Values of cardiac output, stroke volume and peripheral vascular resistance reported here are from Finometer estimates. To minimize effects of women's menstrual cycles, spacing between AG and HDBR studies was to have been 28 days. However, other experimental constraints dictated a spacing of 21 days. There were three women in the luteal, and four in the follicular phase on each of the two study days. Addition of a fourth factor to the statistical analysis indicated a significant first day effect for several variables. However, the fact that half of the subjects had AG and half HDBR first, and half of the men and women participated in studies on each day, allowed us to examine the results from all subjects on both days. We ascribe the first day effects to a shorter time (up to 30 min) between furosemide infusion and start of the day's protocol during the second set of studies. Men had higher average pre-furosemide blood pressure on their HDBR day. This difference was not significant and was gone soon after the furosemide infusion, however, it could have contributed to the blood pressure difference noted in the subsequent OTL results. The AG exposure was not completely passive; at higher Gz levels, subjects were advised to bend their toes as needed. During the first part of the AG exposure, all subjects were taken to presyncope, followed by a 10-min rest and then a period of AG at a lower level of Gz for the next 45 min. To maintain the gravity treatment vs control aspects of each study, subjects walked to the restroom and to the OTL test room following the AG exposure, but were taken by gurney to both the restroom and OTL room following the HDBR exposure. Therefore, differences that are ascribed to AG vs HDBR exposures also include these conditions, which may have contributed to the results we are reporting. The average age of men in this study was 37 while the women's average age was 30. In addition, men's body size was larger (both height and weight), but body mass indexes for men (26.5) and women (25.3) were not different. The contribution of these factors to the gender differences we are reporting was not determined and could, therefore, influence the results. Finally, the model we used, 20 mg intravenous furosemide plus voluntary salt restriction, is a first step toward establishing AG as a successful countermeasure to space flight cardiovascular deconditioning; bed rest studies should be conducted for further confirmation.

Conclusions

There were three major conclusions from our study: A short bout of artificial gravity improved the orthostatic tolerance of hypovolemic men and women and, after further testing, should be considered as a space flight countermeasure that could be applied to astronauts before reentry into a gravity environment. Men and women demonstrated different mechanisms for regulating their cardiovascular responses to OTL tests following AG and HDBR exposures; women appeared to regulate blood pressure while men did not. As presyncope developed, both men's and women's cardiac output (due to stroke volume) declined to the same level on both days, independent of the length of the tolerance test or which protocol preceded the OTL test, indicating that cardiac filling was a likely variable to trigger the presyncopal response.

Acknowledgments The authors thank the subjects who volunteered and complied with the demands of this study. From NASA Ames Research Center, we are grateful to Dr. Ralph Pelligra for medical monitoring, to Farid Haddad for centrifuge operation, to Julie Levri for subject recruitment and scheduling and to Dan Morgan for facilities readiness. From Wyle Houston, we are grateful to Susan Bourbonais for nursing care and Tim Caine for heart and blood vessel ultrasound measures. From SUNY Downstate Medical, we are grateful to Dr Luis Maracaja for analysis of ultrasound images. From Veterans Affairs New Jersey Health Care System, we thank Melissa Blatt for subject care. From the University of Kentucky, we are grateful to Rachel Moore for data collection and analysis and to Qishan Wu from the Applied Statistical Laboratory for statistical assistance. This study was supported by KY NASA EPSCoR, Grant NNX07AT58A, KY; KY State matching Grants; NASA Johnson Space Center Human Research Program; Supported by NASA and the War Related Illness & Injury Study Center, Veterans Administration. War Related Illnesses and Injury Study Center, NJ Veterans Affairs and NASA Ames Research Center, Division of Space Biosciences and Human Health Countermeasures Project Element.

Compliance with ethical standards

Conflict of interest The authors declare that this research was conducted in the absence of any financial interest that could be construed as posing a conflict of interest.

Ethical approval All subjects gave written informed consent to the protocol approved by the NASA Ames Research Center, and the University of Kentucky, Human Research Institutional Review Boards.

References

Buckey JC Jr, Lane LD, Levine BD, Watenpaugh DE, Wright SJ, Moore WE, Gaffney FA, Blomqvist CG (1996) Orthostatic intolerance after spaceflight. J Appl Physiol 81(1):7–18

Convertino VA (1998) Gender differences in autonomic functions associated with blood pressure regulation. Am J Physiol 275(6 Pt 2):R1909–R1920

Cote AT, Bredin SS, Phillips AA, Koehle MS, Warburton DE (2015) Greater autonomic modulation during post-exercise hypotension following high-intensity interval exercise in endurance-trained men and women. Eur J Appl Physiol 115(1):81–89. doi:10.1007/s00421-014-2996-5

Evans JM, Ziegler MG, Patwardhan AR, Ott JB, Kim CS, Leonelli FM, Knapp CF (2001) Gender differences in autonomic cardiovascular regulation: spectral, hormonal, and hemodynamic indexes. J Appl Physiol (1985) 91 (6):2611–2618

Evans JM, Stenger MB, Moore FB, Hinghofer-Szalkay H, Rossler A, Patwardhan AR, Brown DR, Ziegler MG, Knapp CF (2004) Centrifuge training increases presyncopal orthostatic tolerance in ambulatory men. Aviat Space Environ Med 75(10):850–858

Fu Q, Witkowski S, Okazaki K, Levine BD (2005) Effects of gender and hypovolemia on sympathetic neural responses to orthostatic stress. Am J Physiol Regul Integr Comp Physiol 289(1):R109–R116. doi:10.1152/ajpregu.00013.2005

Goswami N, Evans J, Schneider S, von der Wiesche M, Mulder E, Rossler A, Hinghofer-Szalkay H, Blaber AP (2015) Effects of individualized centrifugation training on orthostatic tolerance in men and women. PLoS One 10(5):e0125780. doi:10.1371/journal.pone.0125780

Greenleaf JE, Convertino VA, Mangseth GR (1979) Plasma volume during stress in man: osmolality and red cell volume. J Appl Physiol 47(5):1031–1038

Joyner MJ, Limberg JK (2014) Blood pressure regulation: every adaptation is an integration? Eur J Appl Physiol 114(3):445–450. doi:10.1007/s00421-013-2636-5

MacDonald JR (2002) Potential causes, mechanisms, and implications of post exercise hypotension. J Hum Hypertens 16(4):225–236. doi:10.1038/sj.jhh.1001377

Oberg B, White S (1970) The role of vagal cardiac nerves and arterial baroreceptors in the circulatory adjustments to hemorrhage in the cat. Acta Physiol Scand 80(3):395–403. doi:10.1111/j.1748-1716.1970.tb04803.x

Romero SA, Moralez G, Rickards CA, Ryan KL, Convertino VA, Fogt DL, Cooke WH (2011) Control of cerebral blood velocity with furosemide-induced hypovolemia and upright tilt. J Appl Physiol 110(2):492–498. doi:10.1152/japplphysiol.01060.2010

Stenger MB, Evans JM, Patwardhan AR, Moore FB, Hinghofer-Szalkay H, Rossler A, Ziegler MB, Knapp CF (2007) Artificial gravity training improves orthostatic tolerance in ambulatory men and women. Acta Astronaut 60:267–272

Stenger MB, Evans JM, Knapp CF, Lee SM, Phillips TR, Perez SA, Moore AD Jr, Paloski WH, Platts SH (2012) Artificial gravity training reduces bed rest-induced cardiovascular deconditioning. Eur J Appl Physiol 112(2):605–616. doi:10.1007/s00421-011-2005-1

Thoren P (1979) Role of cardiac vagal C-fibers in cardiovascular control. Rev Physiol Biochem Pharmacol 86:1–94

Vernikos J, Ludwig DA, Ertl AC, Wade CE, Keil L, O'Hara D (1996) Effect of standing or walking on physiological changes induced by head down bed rest: implications for spaceflight. Aviat Space Environ Med 67(11):1069–1079

Yang H, Cooke WH, Reed KS, Carter JR (2012) Sex differences in hemodynamic and sympathetic neural firing patterns during orthostatic challenge in humans. J Appl Physiol (1985) 112(10):1744–1751. doi:10.1152/japplphysiol.01407.2011