RESEARCH ARTICLE

Bed Rest Affects Ventricular and Arterial Elastances in Monkeys: Implications for Humans

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Methods: Experimental data were obtained from five chronically instrumented rhesus monkeys exposed to 96 h of 10° head-down bed rest (HDBR) and another 96 h of 80° upright control separated by 9 d of ambulatory recovery in a counter-balanced, crossover experiment design to test the hypotheses that: 1) headward and footward fluid shifts would increase systemic arterial (Eart) and left ventricular end-systolic (Ees) elastances; and 2) changes in Eart and Ees would be related in magnitude and direction. Ees and Eart were calculated from measurements taken during five observation periods for initial 2-h and 4-d exposures to HDBR that produced headward volume shifts, and acute exposure to graded levels of lower body negative pressure (LBNP) designed to produce orthostatic volume shifts. ***Results:*** There was no effect of HDBR on Ees and Eart for any observation period (initial 2-h, 4-d, or LBNP). Eart increased in a similar pattern during the 4-d exposure to both control and HDBR. Ees increased with increasing LBNP levels for both control and HDBR while Eart remained unchanged. ***Conclusion:*** Our data are consistent with the notion that elevated Eart may represent an adaptation to physical inactivity that is associated with cardiovascular deconditioning.

Keywords: arterial elastance, ventricular elastance, bed rest, lower body negative pressure.

ELASTANCE IS a mechanical property that is defined as a change in pressure divided by a change in volume. Specifically, left ventricular end-systolic elastance (Ees) represents the slope of the ventricular pressure-volume relationship and has been used as an index of myocardial contractility. It may also be viewed as an index of the 'stiffness' of the heart at end-systole. Systemic arterial elastance (Eart) represents the effective 'stiffness' of the entire systemic vasculature, including all vessels and branches that receive the ejected stroke volume from the heart. Relationships between the elastances of the left ventricle and the arterial system have been studied extensively. Sunagawa et al. (27) investigated the interaction between Ees and an effective arterial elastance (Ea) from which they developed a cardiovascular coupling model using the ratio of Ea/Ees. In subsequent studies, they investigated the effects of Ees on Ea (coupling ratio) in isolated heart preparations and with hydraulic models of the arterial system (27,28). Collectively, their work provided evidence of a link between ventricular elastance and arterial load. However, effective arterial elastance (Ea) is not a true arterial elastance (Eart) and relates more to changes in total peripheral resistance. Berger and Li (1) examined the temporal relationship between Ees and Eart over a single beat and found a direct mechanical link between changes in Ees and changes in Eart. Other investigators have examined the relationship between Ees and Eart over time periods of up to 2 h, but were unable to demonstrate a definitive relationship (17,20–21,23).

We are unaware of any previous investigations of the effects of acute or chronic headward or footward fluid shifts on Ees and Eart. Understanding the effects of fluid shifts on Ees and Eart may provide insight into adaptation and compromise of cardiovascular function induced by exposure to microgravity or confinement to bed rest. Previously, we reported that 4 d of exposure to head-down bed rest (10° HDBR) increased left ventricular diastolic compliance, i.e., reduced elastance (15). Although unrelated, this unique finding led us to speculate that Ees and/or Eart would also be reduced during the initial (2 h) or long-term (4 d) headward fluid shifts induced by HDBR. Furthermore, we were interested in the possibility that any changes in Ees might be related to changes in Eart, or vice versa. If a relationship exists between cardiac and arterial elastances, this may provide the first evidence of a long-term (4 d) coupling mechanism. We mined data recorded from five chronically instrumented rhesus monkeys exposed to 10° HDBR and 80° head-up tilt separated by 9 d of ambulatory recovery to examine Eart and Ees during initial 2-h and 4-d induction of headward fluid shifts that occur in bed-restricted patients or astronauts, and acute

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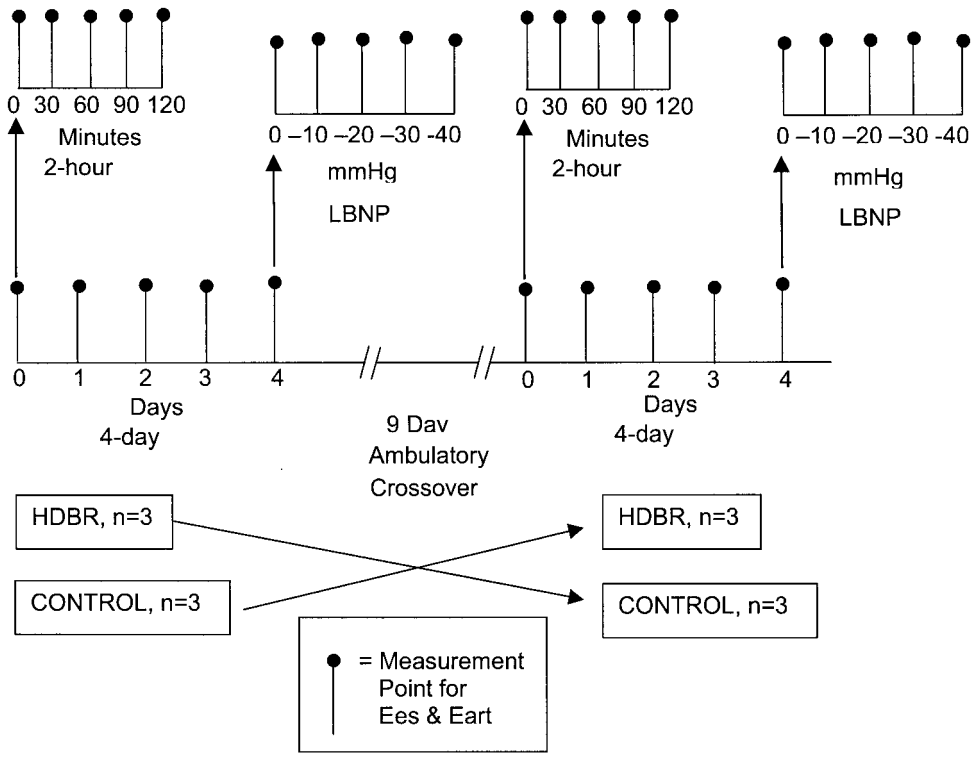

Fig. 1. Summary of experimental protocol. Note: One animal was excluded at the end of the study due to ventricular hypertrophy.

footward fluid shifts that occur during orthostatic challenge using graded levels of lower body negative pressure (LBNP). We used this retroactive analysis to test the hypotheses that: 1) headward and footward fluid shifts would increase systemic arterial (Eart) and left ventricular end-systolic (Ees) elastances; and 2) changes in Eart and Ees might be related in magnitude and direction.

METHODS

The test animals and portions of the experimental procedures presented in this manuscript have been previously reported (8,15). A brief description of the subjects and training protocol, experimental procedures, data acquisition, models and computer programs, and statistical analysis are presented.

Test animals and training protocol: There were six mature male rhesus monkeys (Macaca mulatta) averaging 4.5–8 kg in weight selected as candidates for this study. All experimental procedures and protocols were reviewed and approved by the Institutional Animal Care and Use Committee. The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council. Armstrong Laboratory has been fully accredited by the American Association for Accreditation of Laboratory Animal Care since 1967. The monkeys received 2 mo of tilt-table adaptation training prior to the start of the experiments. This training involved the following three phases: 1) preliminary caretaker handling, confinement jacket fitting, and light ketamine sedation; 2) approximately 2 h of confinement jacket and tilt-table acclimation training; and 3) confinement jacket and tilt-table adaptation training (up to 24 h). Test animals were trained and tested on custom-designed and fabricated head-down tilt tables that can be positioned at one of three settings: 10° HDBR, 0° prone, and 80° head-up tilt (15).

Experimental design: The experimental design, along with the testing and measurement schedule, is summarized in **Fig. 1**. A standard two-treatment crossover design was performed with each chronically instrumented monkey receiving both 10° prone HDBR and 80° prone head-up tilt (80° HUT). The 10° HDBR position was chosen since actual changes in cardiovascular responses induced by spaceflight have been closely simulated by this groundbased analog (4). The treatment order was randomized, but counterbalanced, so that three monkeys received 10° HDBR followed by the 80° HUT condition and three monkeys received the 80° HUT condition followed by 10° HDBR. Each treatment period lasted 96 h (4 d). The monkeys were kept unrestrained in their cages during the 9 d between treatment periods. Ees and Eart were evaluated during initial 2-h and 4-d exposure to 10° HDBR and LBNP stimuli. Hemodynamic measurements were recorded in the 0° prone posture for both 80° HUT and 10° HDBR test conditions to eliminate unwanted effects of posture so that only the cumulative effects of 10° HDBR were investigated.

Initial 2-h observation period: The initial 2-h condition consisted of 10° prone, 10° HDBR, or 80° prone head-up tilt on day 0. There were five 5-min hemodynamic recordings acquired for 80° HUT and 10° HDBR treatment conditions. These recordings were taken consecutively every 30 min following the start of each treat-

ment condition (day 0 at 0915 hours). Prior to each of the 5-min recordings, the experimental animal was tilted to the 0° prone position (80° HUT and 10° HDBR) allowing comparison of treatment effect on cardiovascular parameters in similar postures. An anesthetic agent (ketamine bolus of 0.3–0.5 ml) was administered to each experimental animal 3 min prior to each recording.

4-d observation period: The 80° HUT condition lasted 96 h (4 d) with each day consisting of 2 h of 80° prone head-up tilt (0700-0900 hours), up to 4 h of 0° prone during experimental measurements (0900-1300 hours), 10–12 h of 80° prone head-up tilt (1300-2300 hours), and 8 h of 0° prone to simulate sleeping posture (2300-0700 hours). A 5-min hemodynamic recording was collected at 0915 hours each day while the experimental animal was in the 0° prone posture. The 10° HDBR treatment condition also lasted 96 h and hemodynamic measurements were recorded in the 0° prone posture for a 5-min period. The 10° HDBR animals remained in the 0° prone posture for up to 4 additional h (0900-1300 hours, required for other studies). Immediately after these additional experiments were completed (up to 4 h in 0° prone position) the experimental animals were returned to the 10° prone 10° HDBR position. Ketamine was administered to each experimental animal 3 min prior to each recording.

LBNP observation period: An acute LBNP protocol was performed on day 4 of 10° HDBR and 80° HUT periods. Following daily hemodynamic recordings, experimental animals were fitted with inflatable skirts, and positioned inside a lower body pressure chamber placed on the tilt table at 0° prone position. Experimental animals were then anesthetized with ketamine infused at a constant rate (500 $\mu g \cdot kg^{-1} \cdot min^{-1}$) with a syringe pump (Baxter AS40A, Deerfield, IL). The sedation level was maintained throughout the duration of the LBNP test. Graded levels of negative pressure were applied to the chamber using a custom-built electro-mechanical control system. The pressure application sequence consisted of baseline (0 mmHg pressure) and graded steps from 0 mmHg down to −50 mmHg in −10 mmHg decrements with each pressure level lasting 3 min (total test time of 18 min). The first minute at each pressure level was allocated for experimental animal acclimation. Hemodynamic data were then recorded for 30 s. The LBNP test was subject to termination at the discretion of the attending medical monitor. The criterion for termination of the test was a mean aortic pressure below 50 mmHg.

Instrumentation: After verification that monkeys were able to adapt to the tilt table during all phases of training, they were instrumented with chronically implanted right atrial and left ventricular access catheters, a transit-time flow probe encircling the ascending aorta, and pericardial leads (15). The left ventricular catheter provided an access site for insertion of a dual-tip 3F micromanometer (Millar Instruments, Houston, TX) for simultaneous measurement of left ventricular and aortic pressures. The flow probe was used for continuous measurement of volumetric aortic flow. Chronic implantation of transducers was accomplished by midsternal thoracotomy. Experimental animals were given approximately one month of postoperative recovery before the start of the test protocol. This was the amount of time required for comprehensive return to normal physical status, including healing of surgical incisions, return of normal appetite, and resumption of normal species-specific behaviors.

The dual-sensor aortic and left ventricular micromanometers were pre- and post-calibrated at the start and end of each 4-d treatment period (15). Electrical DC equivalent voltage calibrations were performed for aortic flow (0–8 $L \cdot min^{-1}$) (15). Pressure transducer outputs were amplified with fixed-gain differential DC amplifiers (Ectron, San Diego, CA). Aortic flow signal conditioning was accomplished using a commercial transit-time module (Triton Technologies, San Diego, CA). These conditioned analog signals were then fed into a custom-designed and fabricated signal distribution unit for channeling outputs to multiple data acquisition units. The primary data acquisition unit was an A/D station comprised of antialaising filters (Precision Filters, Phoenix, AZ), A/D board (National Instruments, Austin, TX), desktop computer (Zeos, Nampa, ID), and A/D support software (National Instruments, Austin, TX). Data were low-pass filtered at 60 Hz and sampled at 250 Hz.

Models and computer programs: Hemodynamic data were analyzed using custom-designed software developed in Matlab™ (MathWorks, Natick, MA). Ees was defined as the difference between the maximum isovolumic pressure (P_{max}) and end-systolic pressure divided by the stroke volume. P_{max} was calculated using a single-beat left ventricular pressure waveform curve-fitting estimation technique (12,26). Eart was estimated using a 4-element Windkessel model (24) and Essler's frequency domain analysis technique (11). The specific details, models, equations, sample waveforms, and coefficient of variation in calculating these parameters are presented in **Appendix A**.

All parameters were estimated on a beat-to-beat basis using all beats within each data set. The mean value of all calculated beats for each parameter within each data set for all experimental conditions (initial 2 h, 4 d, or LBNP) during 10° HDBR and 80° HUT treatments was computed.

Statistical Analysis: A standard two-treatment condition (80° HUT, 10° HDBR) each with five time periods (minutes for initial 2-h test, days for 4-d test, and pressure levels for LBNP test) within subjects repeated-measures ANOVA was used to test differences in the two dependent variables (Ees, Eart). This model evaluated the main effect of treatment condition, the main effect of time period, and the time period by treatment condition interaction. Exact p values were calculated for each independent effect and reflect the probability of observing the effect given a noise-only system. Orthogonal polynomials were used to describe the general effect over time periods when time period-to-time period differences were detected. When statistical differences were detected across time periods, the time period error term was partitioned to account for the serial nature of the repeated measures and to generate exact

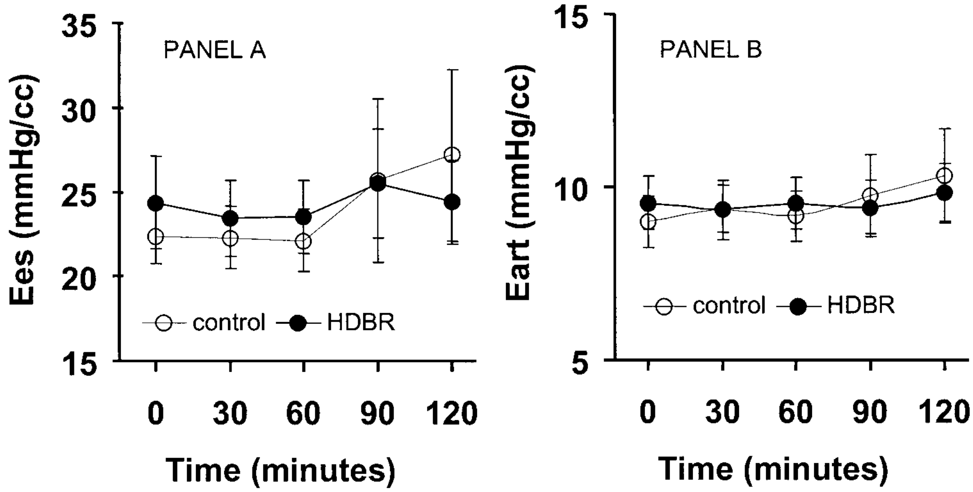

Fig. 2. Means and SDs for Panel A: Ees, and Panel B: Eart responses to 2-h timecourse for control and HDBR.

statistical tests. Raw (time specific) SDs are given graphically, but do not reflect variation specific to the experimental design or the variability associated with the statistical tests. There was one of the six animals that completed the experiment that was excluded due to ventricular hypertrophy. Of the remaining five animals, two animals did not complete the last pressure stage of the LBNP test. All statistical computation was performed using JMP Statistical Discovery Software (SAS Institute, Cary, NC).

RESULTS

Initial 2-h time course: The results for the initial 2-h time course are given in **Fig. 2**, Panels A and B, for Ees and Eart, respectively. No statistical differences were found for any of the independent effects (treatment, time, or interaction) for either of the dependent variables (F's $\leq$ 1.82, p's $\geq$ 0.15). The trend over time was flat with no overall height differences between the two levels of the treatment (80° HUT and 10° HDBR).

4-d time course: The results for the 4-d time course are given in **Fig. 3**, Panels A and B, for Ees and Eart, respectively. Although the overall time profile was slightly higher in the 10° HDBR treatment condition compared with 80° HUT (i.e., treatment main effect) for both Ees and Eart, this observed height difference between the time profiles was not statistically discernible (F (1,4) $\leq$ 1.25, p $\geq$ 0.327). Overall, Eart increased over time in a linear fashion (F (1,8) = 17.18, p = 0.0001), while Ees remained relatively flat (F (4,32) = 1.52, p = 0.220). There were no interactions between time and treatment for either Ees or Eart (F's (4,32) $\leq$ 0.52, p $\geq$ 0.716).

Lower body negative pressure: Responses for Ees (Panel A) and Eart (Panel B) during graded levels of LBNP are presented in **Fig. 4**. The time profiles for Eart were flat (F (4,30) = 0.203, p = 0.9347), with no overall height difference between treatment conditions (F (1,4) = 2.26, p = 0.207). The time profiles for Eart did depart at −40 mmHg ($F_{interaction}$ (4,30) = 2.19, p = 0.0944). However, this may have been caused by a slight survivor effect since two animals could not complete the −40 mmHg LBNP test after 10° HDBR. Overall, Ees increased in a quadratic fashion over the five LBNP pressure levels (F (1,8) = 5.65, p = 0.0448). All other effects (i.e., treatment and interaction) for Ees were small (F's $\leq$ 0.636, p's $\geq$ 0.6001.) The missing data toward the end of the LBNP test (−40 mmHg level for two experimental animals) was minimal and did not in any way bias the overall conclusions. Modern statistical

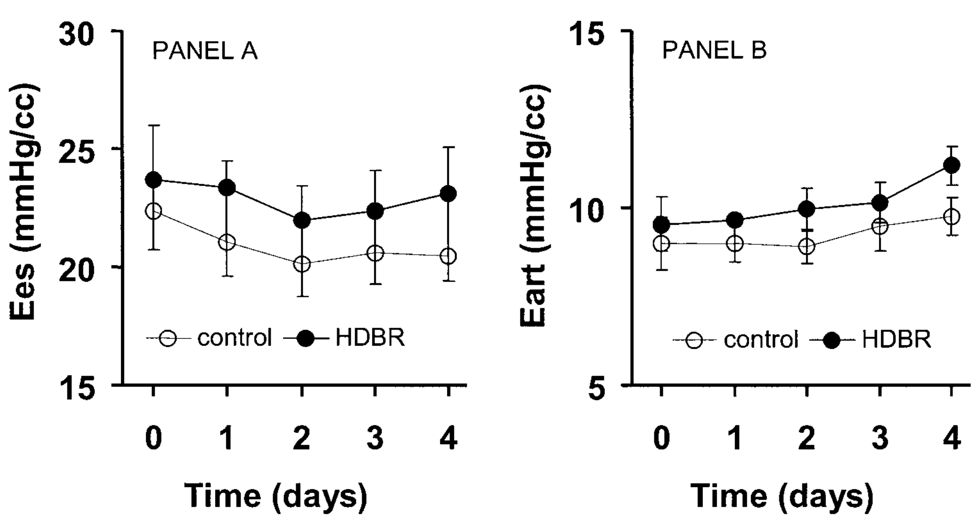

Fig. 3. Means and SDs for Panel A: Ees, and Panel B: Eart responses to 4-d timecourse for control and HDBR.

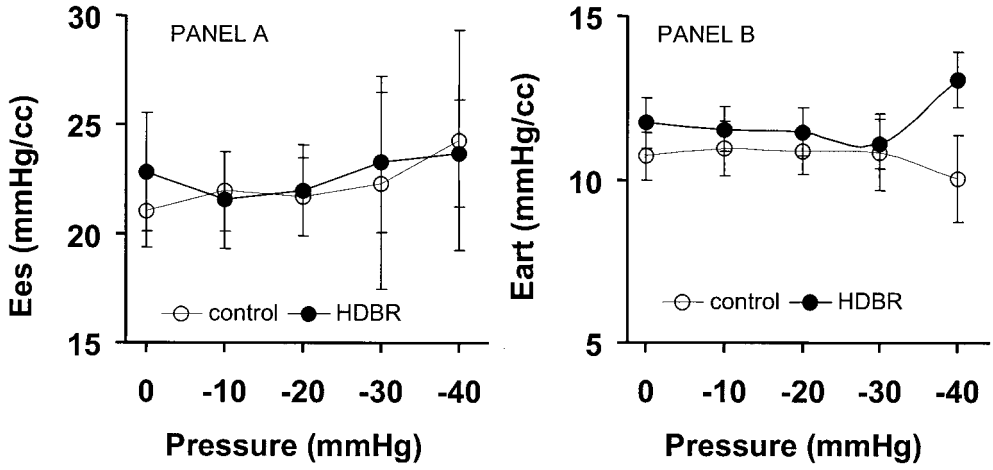

Fig. 4. Means and SDs for Panel A: Ees, and Panel B: Eart responses to increasing levels of LBNP for control and HDBR.

models efficiently correct for missing data via least squares adjustment and maximum likelihood estimation.

Of the six experimental animals, one was excluded from analysis due to hypertrophy, which was not seen during surgical implantation. Hypertrophy was determined at necropsy using an interventricular septal wall thickness of greater than 9 mm as the criterion. This definition for hypertrophy was historically applied to all monkeys at the study facility. By comparison, the other study monkeys all had septal wall thicknesses of 3–5 mm. The single case of hypertrophy was interpreted by the board-certified pathologist who made the diagnosis as being a congenital condition not related to the surgical instrumentation in this study.

DISCUSSION

Since HDBR decreased baseline plasma volume (8) and increased baseline total peripheral resistance (15) in our monkeys, we hypothesized that Eart and Ees would increase. Contrary to our hypothesis, we found no effect of HDBR on either Eart or Ees across any of the observation periods (initial 2 h, 4 d, and LBNP). Thus, our data does not support the notion that elastance of the heart and arteries are affected in the initial time period of exposure to headward fluid shifts.

Ees increased for both HDBR and control treatments with increasing graded levels of LBNP (Fig. 4, Panel A). Since increased Ees can be one indicator of increased myocardial contractility (14), baroreflex-mediated stimulation of the myocardium may represent a possible mechanism underlying the elevated Ees during graded central hypovolemia in our animals. However, previous studies have demonstrated that cardiac baroreflex sensitivity was reduced in monkeys following 14 d of restraint using body casts (2,9). In contrast, there would have to be no difference in cardiac baroreflex sensitivity between HDBR and control treatments in our animals in order for Ees to increase in a similar magnitude across LBNP. Although we did not measure cardiac baroreflex function directly in our monkeys, hemodynamic results from our experiments demonstrated similar maximal elevations in heart rate to similar maximal reductions in arterial pressure for HDBR ($\Delta HR/\Delta MAP = 1.4$ bpm $\cdot$ mmHg^{-1}) and control ($\Delta HR/\Delta MAP = 1.1$ bpm $\cdot$ mmHg^{-1}) conditions (15). These results are consistent with the absence of change in cardiac baroreflex sensitivity following only 3 d of exposure to HDBR in humans (5) and suggest that 4 d of HDBR was probably not long enough to cause significant alterations in cardiac baroreflex responsiveness in our monkeys. Thus, it is possible that increased Ees during exposure to graded central hypovolemia in both HDBR and control conditions represented cardiac baroreflex-mediated responses that were unaffected by short-term exposure to HDBR.

When two arteries of identical length and wall mechanical properties have an identical volume injected into them, the pressure rise of the artery with a smaller diameter will be greater than that of the artery with a larger diameter (i.e., the artery with a smaller diameter will have greater elastance even though the wall mechanical properties are identical). During LBNP, vessel diameters of the arterial system decrease as reductions in central venous pressure initiate baroreflex-mediated vasoconstriction (6). We, therefore, hypothesized that Eart would increase during LBNP with elevations in total peripheral resistance (15). However, against expectations, we found no change in Eart during LBNP for either control or HDBR treatments (Fig. 4, Panel B). For Eart to remain constant during graded levels of central hypovolemia (LBNP), the arterial system pressure-volume relationship must have remained linear over the range of blood volume shifts, or a control mechanism caused changes in wall mechanical properties. The latter mechanism for altering wall mechanical property is attractive since regulating Eart is important in determining arterial pressure and flow-wave velocities and subsequent maintenance of favorable cardiovascular energetics (18,19).

A major finding of our investigation was the observation that Eart was increased over the 4-d confinement periods during both HDBR and control experimental treatments (Fig. 3, Panel B). A common stimulus for elevation of Eart independent of fluid shift effects associated with HDBR was the lower physical activity introduced by confinement to the tilt table during both experimental conditions. The confinement system ap-

plied during both control and HDBR treatments prevented 'normal' daily physical activities such as body mobility and routine change in orientation to gravitational fields. The absence of regular daily physical activity removes the stimulus of normal arterial BP variations associated with rapid and frequent postural changes (e.g., standing, sitting, laying, and swinging) and is associated with the cardiovascular deconditioning state (3,29). Subsequently, the need for a compliant arterial system to accommodate relatively large fluctuations in arterial BP would be diminished, leading to a cardiovascular adaptation that was reflected in the gradual elevation in Eart over the time course of the present study. The notion that confinement and subsequent physical inactivity initiated an increase in Eart associated with cardiovascular deconditioning was further supported by the observation that Eart returned to pretreatment levels following the 9-d recovery period between control and HDBR treatments.

Sunagawa and co-workers have proposed that the integrity of cardiovascular function during rest and exercise is dependent on a mechanical coupling between the elastance properties of the myocardium (Ees) and the arterial system (12,17,26–28). If this hypothesis were true, in the present investigation we should have observed parallel alterations in Ees and Eart induced by exposures to physical confinement and LBNP. In contrast, we found no consistent relationship between changes in Ees and Eart. Increased Eart following 4 d of confinement were not accompanied by corresponding elevations in Ees. Similarly, increases in Ees induced by exposure to graded levels of LBNP were not accompanied by elevations in Eart. Thus, our experimental manipulations are unique in providing evidence that support the notion that specific physiological perturbations exist that disrupt mechanical matching between the left ventricle and arterial system.

Limitations: Interpretation of the alterations in Ees and Eart induced by acute or chronic exposure to HDBR, inactivity, and LBNP in the present investigation are made with an understanding of certain limitations in the methods applied for determination of myocardial and arterial elastance. Our calculation of Ees relied on the determination of P_{max} from the extrapolation of beginning and ending portions on the left ventricular isovolumic pressure relationship. We recognize the existence of other techniques for determination of Ees (13), and that each methodology has limitations. We selected the P_{max} estimation technique because of its high accuracy (>90%) when performed by the same investigator (12,26). Likewise, we chose a four-element Windkessel arterial model (24) and frequency-based parameter estimation technique (11) to calculate Eart. This technique is based on the assumption that Eart remained constant (i.e., linear) over a beat. Ignoring the possibility of non-linear characteristics could alter the absolute value of Eart, but probably did not influence the direction of change. Although use of an anesthetic agent (ketamine) and/or insertion of a transesophageal echocardiography probe could impact experimental outcomes, our preliminary investigations demonstrated no effect of these perturbations on Eart or Ees (16). However, most fundamental to our experimental approach and interpretations is that influences of these methodological limitations should prove minimal since all techniques were applied to all animals who acted as their own controls in a counterbalanced crossover experimental design.

Since human experiments have demonstrated restoration of cardiovascular functions within 14 d following HDBR (7,25), a period of 9 d between experimental treatments was chosen in the present experiment in order to conduct the 96-h protocols between Monday and Friday while allowing adequate recovery time. One of the assumptions of the crossover design is that the crossover period is sufficiently long so that the animals can recover from the previous treatment condition. For this size of an experiment, it is not possible to estimate true residual carryover between treatments. However, we did examine baseline heart rate and BP prior to the entry of each animal into their perspective treatment conditions. For the control condition, baseline heart rate and mean arterial pressure were 158 bpm and 95 mmHg, respectively. In comparison, baseline heart rate and mean arterial pressure prior to the HDT condition were 152 bpm and 98 mmHg. Neither heart rate nor BP was statistically different prior to the start of each respective treatment condition (t_{paired} (4) $\leq$ 1.27, $p \geq$ 0.274). Although the logistics of the experiment along with the health and welfare of the animals to some extent dictated the length of the crossover period, we believe the 9-d crossover interval was sufficiently long to allow for the washout of the majority of the residual effects. In the event of any lingering effects, the use of the counterbalanced crossover design insures that these effects do not bias the treatment comparisons.

The extrapolation of our results to human health and spaceflight are limited by the use of non-human primates for our subject population. We recognize that this (as any) animal model is not a perfect surrogate of the human response, particularly when sedation is required. Our monkeys exhibited greater heart rate, and their stroke volume, cardiac output, and peripheral resistance were smaller simply because they are anthropometrically smaller than humans. However, reduction in stroke volume and cardiac output and increases in heart rate and total peripheral resistance are qualitatively similar in our monkeys and human subjects (10,15). Therefore, our data support the notion that our non-human primate model provides a comparable surrogate for human cardiovascular adaptations to HDBR and LBNP and that our results may have direct implications for human health and spaceflight.

CONCLUSIONS

HDBR had no effect on the response of Ees and Eart to acute headward blood volume shifts (initial 2-h exposure), reduced plasma volume (4-d exposure), or rapid footward blood volume shifts (LBNP). Eart increased without changes in Ees for both control and HDBR treatments during 4 d of confinement to the restraint system, suggesting a cardiovascular deconditioning associated with physical inactivity that is reflected by an uncoupling between ventricular and arte-

rial elastances. Modest elevations in arterial elastance during only 4 d of restraint may have important clinical implications for bedridden patients, astronauts exposed to long-term space missions, the elderly, and individuals with restricted ambulatory lifestyles. The results of the present investigation introduce the possibility that extended periods of physical inactivity can lead to progressive stiffening of the vasculature (elevated Eart). A stiffer vasculature can produce early return of reflected pressure waveforms during systole, with the potential adverse effects of increasing ventricular workload, reducing diastolic myocardial perfusion, and causing ischemic damage to the myocardium (18,19). Thus, our results provide a possible mechanism that links sedentary lifestyle with progression of cardiac disease.

APPENDIX A. MODELS, EQUATIONS, SAMPLE WAVEFORMS, AND COEFFICIENT OF VARIATION USED IN CALCULATING Ees AND Eart.

Estimation of ventricular end-systolic elastance (Ees): Left ventricular end-systolic elastance (Ees) was defined as the difference between the maximum isovolumic pressure (P_{max}) and left ventricular end-systolic pressure divided by the stroke volume. Stroke volume was calculated by integrating the aortic root flow on a beat-to-beat basis. Maximum isovolumic pressure (P_{max}) was calculated using a modified single-beat left ventricular pressure waveform curve-fitting estimation technique developed by Sunagawa et al. (11,26). The isovolumic pressure waveform (P_{iso}) was derived by fitting the isovolumic contraction and relaxation portions of the left ventricular waveform using equation A1, where P_{max} is the maximum isovolumic pressure, P_{ed} is the left ventricular end-diastolic pressure, $\omega = 2\pi/T$, and T is the time period of the contraction. Sunagawa's technique assumes symmetry between isovolumic contraction and relaxation phases. However, this assumption is not necessarily the case since the isovolumic relaxation phase tends to be longer than the contraction phase. Therefore, we chose to use a modified version of the Sunagawa technique. P_{iso} waveforms were estimated for each beat of data for every data set, and calculated P_{max} values for each beat within each data set were averaged to obtain one mean value and a standard deviation.

$$Piso = 0.5Pmax(1 - \cos\omega t) + Ped \qquad A1$$

Examples of left ventricular pressure overlaid with estimated isovolumic pressure waveforms using this technique for each of the experimental test conditions is shown in **Fig. A1**. The coefficient of variation (12), defined as the standard deviation divided by the mean, for estimates of P_{max} was 5 ± 1% and for estimates of Ees was 7 ± 2% for each experimental test condition demonstrating minimal beat-to-beat variation.

An accurate assessment of stroke volume is a fundamental requirement for determination of Ees. A primary advantage of the Sunagawa technique to estimate P_{max} and derive Ees is that it does not rely on accurate left ventricular volume measurements that can be difficult to obtain by echocardiography in our experimental model and conditions. Therefore, although we were unable to obtain echocardiogarphic measures of end-diastolic volume (EDV) and end-systolic volume (ESV), we obtained accurate stroke volumes directly with the measurement of aortic flow.

Estimation of arterial elastance (Eart): Effective arterial elastance (Ea) has been used as a coupling parameter to characterize the vascular afterload. The derivation of Ea originally proposed by Sunagawa and co-workers included resistance and heart rate (R/T) (27). Recently, Segers and colleagues included an elastance term (1/C) into the calculation, providing a more robust assessment of subtle changes in Ea (22). The latter approach, however, is based on the assumption that a monotonic diastolic decay in aortic pressure exists. Since a monotonic diastolic decay in aortic pressure was not observed in a subset of our test subjects, we chose to investigate the relationship between Ees and Eart rather than Ea. Adequate sensitivity for measuring real alterations in arterial elastances by our technique was demonstrated by our ability to detect statistical differences in Eart during LBNP and 4 d confinement.

Eart was estimated using a four-element Windkessel model (24), where Eart is the inverse of C (Eart = 1/C), as shown in **Fig. A2**. Each of the four parameters were estimated using Essler's frequency domain analysis technique (11). Briefly, the experimental systemic input impedance (Z_{exp}) is calculated for up to 10 harmonics using experimental aortic pressure and flow waveforms. An impedance equation for the 4-element Windkessel model is derived as a function of frequency, which can be used to calculate the model input impedance (Z_{mod}). Each of the model parameters (Z, L, R, and C) are then iteratively adjusted until the error between Z_{exp} and Z_{mod} is minimized. Each of these parameters was estimated on a beat-to-beat basis, and a single mean value and standard deviation for every beat within each data set was calculated. The coefficient of variation (12), defined as the standard deviation divided by the mean, for estimates of Eart for each experimental test condition was 4 ± 2% demonstrating minimal beat-to-beat variation.

$$Z(s) = \frac{P(s)}{Q(s)} = \frac{sL(Z)}{sL + Z} + \frac{R\left(\frac{1}{sC}\right)}{R + \frac{1}{sC}} \qquad A2$$

$$Z(s) = \frac{s^2(LZRC) + s(LZ + LR) + ZR}{s^2(LRC) + s(L + ZRC) + Z} \qquad A3$$

$$Z(j\omega) = \frac{[-\omega^2 LZRC + ZR] + j\omega[LZ + LR]}{[-\omega^2 LRC + Z] + j\omega[L + ZRC]} \qquad A4$$

$$Re(j\omega) = \frac{\omega^2[(LZ + LR)(Z - \omega^2 LRC)] + [(ZR - \omega^2 LZoRC) + (Z - \omega^2 LRC)]}{\omega^2(L + ZRC)^2 + (Z - \omega^2 LRC)^2} \qquad A5$$

$$Im(j\omega) = \frac{j\omega[(\omega^2 LZRC - ZoR)(L + ZRC)] + [(LZ + LR)(Z - \omega^2 LRC)]}{\omega^2(L + ZRC)^2 + (Z - \omega^2 LRC)^2} \qquad A6$$

Ea has been used by others as a coupling parameter to characterize the vascular afterload. The derivation of Ea originally proposed by Sunagawa and co-workers

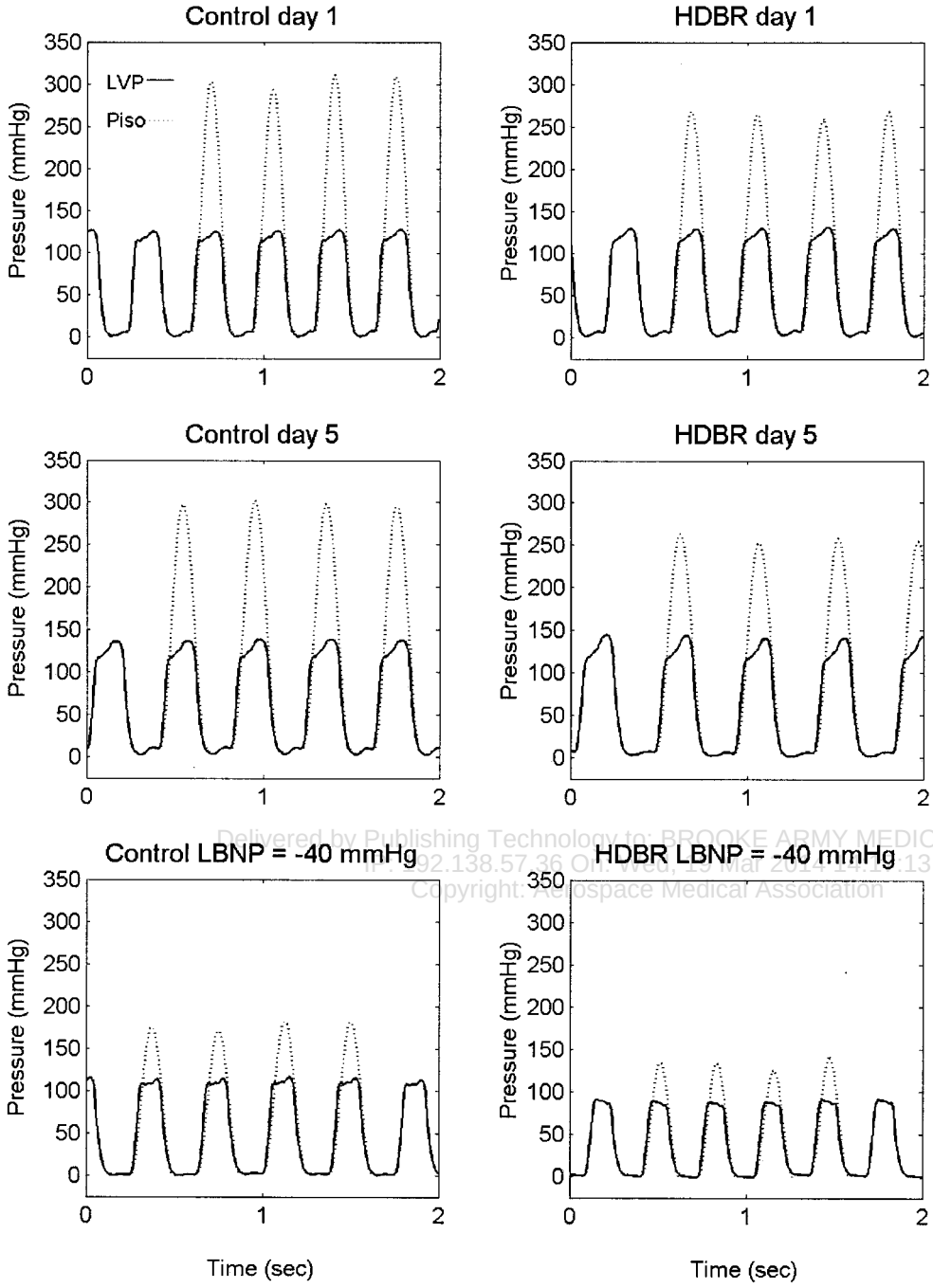

Fig. A1. Sample left ventricular pressure (LVP, solid line) and estimated isovolumic pressure (P_{iso}, dotted line) waveforms used to calculate Ees for baseline (day 1), chronic (day 5), and LBNP (−40 mmHg) test conditions for control and HDBR.

included resistance and heart rate (R/T) (27). Recently, Segers and colleagues included an elastance term (1/C) into the calculation, providing a more robust assessment of subtle changes in Ea (22). The latter approach, however, is based on the assumption that a monotonic diastolic decay in aortic pressure exists. Since a monotonic diastolic decay in aortic pressure was not observed in a subset of our test subjects, we chose to investigate the relationship between Ees and Eart rather than examine Ea. Adequate sensitivity for measuring real alterations in ventricular and arterial elastances by our technique was demonstrated by our ability to detect statistical differences in Ees and Eart during LBNP and 4 d confinement.

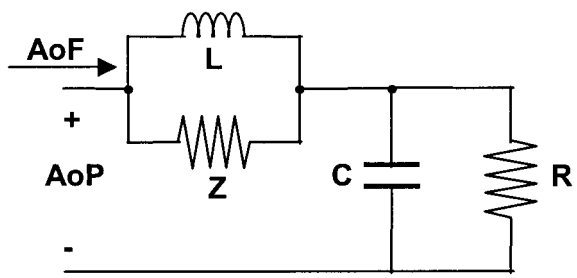

Fig. A2. The four-element Windkessel model used to estimate total peripheral resistance (R), systemic arterial compliance (C), inertance (L), and characteristic impedance (Z) from vascular input impedance data (AoP = aortic pressure and AoF = aortic flow). Eart is the inverse of systemic arterial compliance (Eart = 1/C).

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