Effects of 5 days of head-down bed rest, with and without short-arm centrifugation as countermeasure, on cardiac function in males (BR-AG1 study)

E. G. Caiani,[1] P. Massabuau,[2] L. Weinert,[3] P. Vaïda,[4] and R. M. Lang[3]

[1]*Politecnico di Milano, Electronics, Information and Biomedical Engineering Department, Milano, Italy;* [2]*University Hospital of Rangueil, Department of Cardiology, Toulouse, France;* [3]*University of Chicago, Noninvasive Cardiac Imaging Laboratory, Chicago, Illinois; and* [4]*Université de Bordeaux, Bordeaux, France*

Submitted 10 February 2014; accepted in final form 24 July 2014

DURING SPACE FLIGHT, CHANGES in gravity (G_z; head-to-foot acceleration) affect the cardiovascular system by causing fluid shifts from the lower extremities toward the head and thorax, thus altering central filling volumes and pressures (8, 9, 23, 34). These hemodynamic alterations, which directly influence cardiac chamber dimensions and function, are responsible for many of the adverse effects associated with postflight orthostatic intolerance observed in astronauts representing an important clinical concern (16, 17, 22). An in-depth assessment of the cardiac adaptations to weightlessness is crucial for better understanding of both cardiac physiology in space and the appropriate design and testing of specific countermeasures (CM).

Address for reprint requests and other correspondence: E. G. Caiani, Politecnico di Milano, Electronics, Information and Biomedical Engineering Dept., P.zza L. da Vinci 32, 20133 Milano, Italy (e-mail: enrico.caiani @ polimi.it).

Ground-based studies represent an invaluable perspective to investigate human physiology during simulated microgravity conditions. Among them, the 6° head-down tilt bed rest (HDBR) model represents a unique opportunity for studying the effects of prolonged space flight on the cardiovascular system as well as testing the efficacy of CM. In fact, HDBR represents a model of chronic circulatory unloading, simulating sustained exposure to microgravity. Positioning head-down leads to fluid shift from the legs to the chest, causing an increase in left ventricular (LV) transmural pressure, end-diastolic volume (EDV), and stroke volume (SV) (15, 39). These changes activate short-term volume regulatory mechanisms that result in plasma volume loss, with the achievement of a new hemodynamic steady state within 48 h characterized by decreased volume loading of the heart similar to what reported during space-flight (1, 35).

As hemodynamic load is an important determinant of LV geometry and mass (11), this new steady state leads to LV remodeling, evidenced by a reduction in LV mass and EDV associated with reduced cardiac distensibility and SV at any given filling pressure, potentially contributing to orthostatic hypotension when the gravitational gradients are restored (35, 41).

Alterations in LV remodeling associated with changes in the pressure-volume relationship, as well as slowed ventricular relaxation and decreased diastolic suction, have been reported after prolonged (2–5 wk) HDBR (7, 13, 24, 35, 40, 46). In contrast, the effects of shorter periods of HDBR on LV dimensions and SV have been less thoroughly investigated (1, 3), and no data exist on the effects on LV mass and Doppler-derived valvular flows.

To reduce simulated human physiologic deconditioning associated with spaceflight, exposure to gravitational stimuli by short-arm centrifugation (SAC) is considered a promising CM (30, 37, 49) to maintain human performance and to prevent degradation of cardiovascular, neuromuscular and skeletal functions (4). However, the effectiveness of SAC in counteracting LV remodeling during short-term HDBR has not yet been tested. In addition, the majority of published HDBR studies aiming at evaluating CM effectiveness are limited by the inclusion of different subjects in the control and CM groups.

Accordingly, the aims of this study were *1*) to determine whether in males short periods of HDBR (5 days) are also associated with changes in LV and left atrial (LA) dimensions and diminished diastolic suction, previously only reported in prolonged HDBR studies; and *2*) to use a crossover experi-

mental design to test the effectiveness of daily 30-min doses of 1-G_z SAC training in preventing potential cardiac remodeling and functional changes.

MATERIALS AND METHODS

Subjects. As part of the European Space Agency (ESA) HDBR strategy, an only male population composed by 12 healthy volunteers aged 33 ± 7 (range, 21 to 41 yr; body mass index, 23.7 ± 2.1 kg/m^2; maximal oxygen uptake, 39 ± 6 ml·kg^{-1}·min^{-1}) were recruited after multiple screening and psychological tests. The choice of including only males was driven by the ESA standardization plan, in which it is determined that CM will undergo screening in males first, and only if efficient, they should be evaluated in females as well, before evaluation in long-term bed rest.

Subjects had no history of cardiovascular disease and were not taking medications of any kind. Each subject provided written, informed consent that was approved by the Institutional Review Board of the "Comité de Protection des Personnes Sud Ouest et Outre Mer I" and by the French Drug Agency (Agence Française de Sécurité Sanitaire pour le Produits de Santé).

Study design (BR-AG1 bed rest). Subjects were enrolled in a crossover design with a washout period of ~1.5 mo between two consecutive campaigns, with one sedentary control (CTRL) period and two treatment types receiving daily either SAC1, with short-arm centrifugation subministered for 30 min continuously, or SAC2, with short-arm centrifugation for 30 min intermittently (6 periods of 5 min each). For both treatment groups, the gravitational stimulus was obtained with a short-arm (radius 2.82 m) centrifuge (Verhaert Space) where the rate of rotation and distance along the centrifuge arm were adjusted such that treatment subjects received 1.0 G_z at the heart. Subjects did not perform any exercise during SAC treatment.

All subjects adhered to a strict 6° head-down tilt bed rest, 24 h a day for 5 days. They were admitted in the Institut de Médecine et de Physiologie Spatiales (MEDES) facility at the University Hospital of Rangueil, Toulouse, France. Subjects were acclimated to the bed rest unit for 5 days before initiating uninterrupted bed rest, monitored 24 h a day, and provided with strictly controlled diet aimed at preventing body weight changes. Subjects were awakened each day at 6:30 AM and prompted to start sleeping at 11:00 PM each day, with no napping allowed during the day. After completing the HDBR portion of the study, subjects remained in the facility for an additional 5 days for further testing. In the ambulatory periods preceding and following HDBR, lying in bed during the day was not allowed.

During bed rest, eight different independent research groups performed their experiments on several physiological systems. In addition, several ESA standardized measurements and tests (plasma volume, tilt test, $\dot{V}O_{2\,max}$ test, isometric maximum voluntary contraction and muscle fatigue test, dual energy X-ray absorptiometry for body composition, dynamic bait index and posturography, analysis urine and blood analyses, and psychological assessment by questionnaires) were performed by MEDES personnel, the results of which were made available first to the ESA nominated Artificial Gravity Experts Group for a specific publication, and then to all other requesting investigators. The daily schedule was carefully planned to exclude or minimize possible interaction among physiological effects induced by the different experiments.

Plasma volume assessment. Plasma volume was measured at 7:15 AM both before (BCD-2) and in the last day (HDT5) of HDBR, using the optimized cardiac output (CO)-rebreathing method (SpiCO; Blood tec GbR, Bayreuth, Germany). The CO-rebreathing method is one of the most accurate and least harmful dilution techniques to determine total hemoglobin mass (tHb), with a reported mean measurement error of 2.2% (90% confidence interval 1.4–3.5%) (21), together with hematocrit (Hct), from which plasma and blood volume (BV) is derived as follows: first, erythrocyte volume (EV) was computed as:

$$EV(l) = \frac{tHb(g)}{MCHC(g/dl) \cdot 10}$$

where MCHC is the mean corpuscular hemoglobin concentration, reflecting hemoglobin concentration-to-Hct ratio.

Then blood volume was computed as:

$$BV(l) = \frac{EV(l) \cdot 100}{Hct(\%) \cdot 0.91}$$

from which PV computed by subtracting EV to BV.

The "optimized CO-rebreathing method" (45) is reported to provide an even lower typical error which is between 1–2% when used by experienced personnel (20).

Echocardiographic imaging. A single expert operator performed all the transthoracic two- and three-dimensional (2D/3D) echocardiographic studies using an iE33 ultrasound equipment (Philips Medical System, Andover, MA). All participants underwent standard 2D (apical 2-, 3-, and 4-camber views, parasternal short and long axis views), pulsed and continuous wave and tissue Doppler echocardiography (S5 phased array).

In addition, real-time 3D echocardiography (RT3D) was performed (X3-1 matrix array). RT3D imaging was performed in the harmonic 3D full volume mode, whereby a pyramidal volume dataset was acquired from an apical window, taking particular care to include the entire left atrium and ventricle within the pyramidal 3D scan volume. RT3D datasets were acquired using wide-angled acquisitions in which four to seven wedge-shaped subvolumes (93 × 21°) were obtained over consecutive cardiac cycles during a breath-hold with ECG gating.

Image acquisition was performed before (BCD-5), within 3 h from the conclusion of HDBR (R + 0), and in the third day after conclusion of HDBR (R + 2), with the subject in supine left decubitus position (Fig. 1).

Image analysis. Analysis was performed by an investigator blinded to the subject identity, time of acquisition, and group assignment. 2D and Doppler measurements were obtained using Xcelera (Philips).

LV mass was computed using the area-length method from end-diastolic 2D images, while LV EDV and end-systolic volume (ESV) were computed semiautomatically (QLab 8; Philips) from 3D echo data (Fig. 2) from which ejection fraction (EF) was derived (33). LA volumes were measured at ventricular end-systole using the biplane method of disks.

Following current guidelines (43), the following parameters were measured from the mitral inflow pulsed Doppler tracings: peak early (E) and late (A) diastolic velocities, E/A ratio, E and A wave integrals, time-to-peak E, and E deceleration time. From the pulmonic vein Doppler, the systolic (S) and diastolic (D) peak flow velocities, as well as their ratio (S/D) were measured. From the continuous wave Doppler images of the LV outflow, the following parameters were measured: maximum (V_{max}) and mean (V_{mean}) velocity, velocity time integral (V_{TI}), time-to-V_{max}, and ejection time.

For the computation of cardiac output (CO), SV was determined as the product of the aortic valve cross-sectional area, wherein the

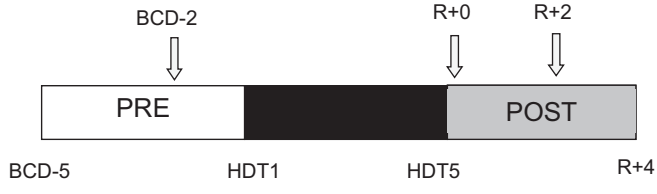

Fig. 1. Schematics of the timing (as evidenced by arrows) of the echocardiographic studies during the bed rest. BDC-2 and BCD-5, before head-down bed rest (HDBR); HDT1 and HDT5, 1st and last day of HDBR; R + 0, within 3 h from the conclusion of HDBR; R + 2, 3rd day after conclusion of HDBR.

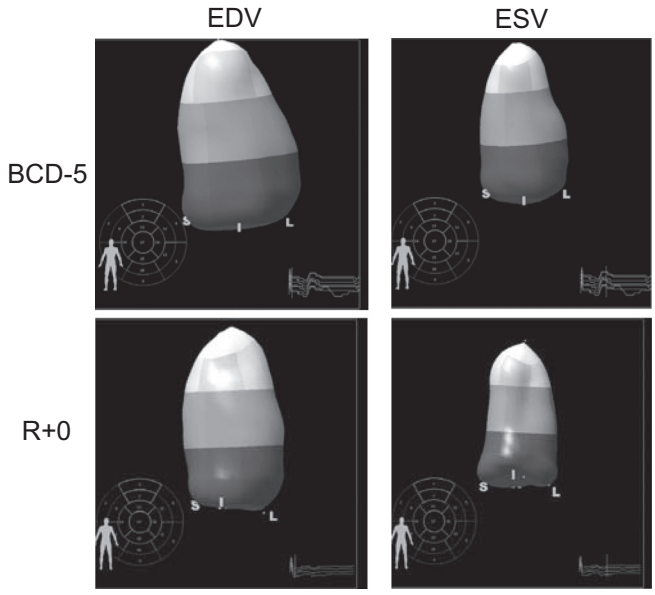

Fig. 2. Example of 3-dimensional (3D) left ventricular (LV) end-diastolic and end-systolic endocardial surfaces, obtained before (BCD-5) and at the end of HDBR (R + 0). The effect of reduction in LV volumes with bed rest is appreciable even by visual inspection. EDV and ESV, end-diastolic and -systolic volume.

diameter of the aortic annulus was measured from the 2D parasternal long-axis view, and the V_{TI} of the aortic Doppler outflow.

Tissue Doppler images were acquired with the sample volume positioned at the base of the septal and the lateral walls of the apical four-chamber view. From these images, the corresponding S', E', and A' velocities were measured and the E/E' ratio calculated.

Statistics. Data are expressed as means ± SD, unless otherwise specified. A two-way ANOVA with repeated measures was applied to test for differences among time points (BCD-5, R + 0, and R + 2), among groups (CTRL, SAC1, and SAC2), and for their possible interaction ($P < 0.05$ was considered as significant).

RESULTS

Two subjects were excluded from the analysis since for medical reasons they did not complete all the three study campaigns.

Body weight, plasma, and blood volumes. Table 1 shows the results of body weight, plasma and blood volumes in the different experimental conditions. Once in CTRL, compared with BCD-5, 5 days HDBR led to a significant reduction in plasma volume (median value 15%, 25th and 75th percentile: 7–16%), and in blood volume (10%, 4–13%), together with a 1.7% loss in body weight. When in SAC1, plasma volume decreased by (14%, 12–25%) together with blood volume (12%, 8–17%), and a 1.8% loss in body weight. Finally, when in SAC2, both plasma (16%, 6–20%) and blood (11%, 4–14%) volumes were reduced, with a 0.6% loss ($P = 0.06$) in body weight. No statistical difference was found among groups, together with no interaction effect, except for body weight ($P = 0.02$).

Cardiac dimensions and mass. Table 2 shows the absolute results of the 2D and 3D echocardiographic examinations regarding LV dimensions and mass, and LA volume; see Table 6 for a depiction of the percent change in each group between BCD-5 and R + 0.

A significant reduction in LV mass compared with PRE was measured at R + 0, in all groups with no interaction effects (Fig. 3). A similar decrease was found in EDV and ESV at R + 0 in all groups; as a result, EF did not change. Also, heart rate was not statistically different, with no significant changes in CO. LA volume was reduced in CTRL and SAC2, and with a trend towards a reduction in SAC1 ($P = 0.1$).

In all groups, at R + 2 did not differ from the respective control values.

Pulsed mitral and pulmonic Doppler inflow. Table 3 shows the results of the measurements from the mitral and pulmonic Doppler inflow velocities; see Table 6 for a depiction of the intergroup percent changes between BCD-5 and R + 0.

A significant reduction of both the peak and wave integral of the early E relaxation velocity (Fig. 4), but not of the late A atrial contraction velocity, was found in all groups at R + 0 compared with BCD-5. As a result, the E/A ratio showed a nonsignificant trend towards a decrease. With regard to the temporal indexes, time-to-peak E did not change, while the E deceleration time was prolonged in the CTRL period but not during SAC1 and SAC2.

When considering the pulmonic vein flows, a reduction in the magnitude of both the S and D waves at R + 0 compared with BCD-5 was noted, but not in their ratio, in CTRL and SAC1, but not in SAC2. Interestingly, for these parameters a strong interaction ($P = 0.03$ for D peak and $P = 0.08$ for S peak) between groups and time points was seen. In all groups, at R + 2 all parameters did not differ from the respective control values.

Continuous aortic Doppler outflow. Table 4 shows the results of the measurements computed from the aortic Doppler outflow velocity; see Table 6 for the relevant percent changes in each group between BCD-5 and R + 0.

Both V_{max}, V_{mean}, and V_{TI} were decreased in all groups at R + 0. While time-to-V_{max} did not change, the ejection time was shorter at R + 0 in all groups. In all groups, at R + 2 all parameters did not differ from their respective control values.

Tissue Doppler velocities. Table 5 shows the results of the measurements computed from the pulsed tissue Doppler ve-

Table 1. *Results of body weight, plasma, and blood volume obtained in the 10 subjects in the 3 campaigns*

	CTRL		SAC1		SAC2	
	BCD-2	HDT5	BCD-2	HDT5	BCD-2	HDT5
Body weight, kg	76 ± 7	74.7 ± 6.8*	75.7 ± 6.7	74.3 ± 6.8*	75.4 ± 6.7	75 + 6.8
Plasma volume, ml	3,690 ± 388	3,219 ± 238*	3,891 ± 410	3,117 ± 459*	3,755 ± 518	3,225 ± 433*
Blood volume, ml	6,083 ± 639	5,580 ± 376*	6,374 ± 632	5,421 ± 637*	6,180 ± 806	5,560 ± 545*

Values are means ± SD. CTRL, sedentary control; SAC, short-arm centrifugation; HDBR, head-down bed rest; BCD-2, before HDBR; HDT5, last day of HDBR. *$P <$ 0.05 vs. BCD-2;

Table 2. *Results of LV and LA dimensions obtained in the 10 subjects in the 3 campaigns*

	CTRL			SAC1			SAC2		
	BCD-5	R + 0	R + 2	BCD-5	R + 0	R + 2	BCD-5	R + 0	R + 2
Heart rate, beats/min	64 ± 6	64 ± 10	62 ± 10	61 ± 7	59 ± 8	61 + 4	61 ± 6	65 ± 7	62 ± 8
LV mass, g	141 ± 19	113 ± 19*	149 ± 21	154 ± 23	129 ± 26*	152 ± 20	141 ± 20	116 ± 22*	138 ± 23
3D EDV, ml	173 ± 22	145 ± 19*	171 ± 21†	178 ± 25	157 ± 20*	170 ± 24†	169 ± 19	148 ± 21*	161 ± 22
3D ESV, ml	63 ± 12	58 ± 11*	64 ± 6	68 ± 13	61 ± 8*	60 ± 14	66 ± 13	55 ± 11*	62 ± 12
3D EF, %	63 ± 4	61 ± 3	62 ± 3	62 ± 4	61 ± 3	65 ± 4	62 ± 4	61 ± 4	62 ± 4
CO, l/min	6.4 ± 1.3	5.6 ± 0.8	6.6 ± 1.3	6.6 ± 1.3	6.8 ± 0.8	7 ± 1	6.4 ± 0.9	5.9 ± 1.1	6.4 ± 1.4
LA volume, ml	38 + 12	25 ± 10*	34 ± 9†	37 ± 12	26 ± 9	38 ± 14	42 ± 14	27 ± 10*	39 ± 10†

Values are means ± SD. R + 0, 3 h from the conclusion of HDBR; R + 2, 3rd day after conclusion of HDBR; LV, left ventricular; 3D, 3-dimensional; EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction; LA, left atrial. *$P < 0.05$ vs. BCD-5; †$P < 0.05$ R + 0 vs. R + 2.

locities, while in Table 6 the relevant percent changes in each group between BCD-5 and R + 0 are reported.

While the peak systolic velocity S', both in the septal and lateral walls, did not show any change with bed rest, the peak early diastolic velocity E' resulted reduced at R + 0 in all groups when measured in the septum, and in SAC1 and SAC2 but not in CTRL ($P = 0.09$), when measured in the lateral wall. As expected, tissue velocity values obtained from the lateral wall were higher than the corresponding septal values. Interestingly, both the S' and the E/E' ratio measured from the lateral wall showed significant statistical interaction between time points and groups.

DISCUSSION

This is the first HDBR study in which LA volumes and pulmonic vein Doppler flows are also reported. We focused our attention on the LA based on our results during parabolic flights studies (5), in which we described greater changes in LA dimensions compared with the LV with weightlessness.

The novel findings of this study are *1*) short HDBR had a major impact on both LV and LA geometry and function in males (i.e., a decrease in LV mass and volume; reduction in LA volume; decrease in Doppler flow and tissue Doppler velocities during early filling as well as a decrease in aortic flow velocities associated with shortened LV ejection time), presumably secondary to decreased physiological loading and dehydration, both resulting in reduced plasma and blood volume; *2*) all changes were fully reversed 3 days after the conclusion of HDBR; and *3*) SAC applied as a CM during HDBR was not able to prevent cardiac changes, either when applied continuously or intermittently.

Compared with previous HDBR studies that used cardiac echocardiography (see Table 7), we tried to minimize the sources of errors that could influence our results. Firstly, we used an individual crossover design wherein each subject repeated the HDBR three times, and secondly, we employed contemporary echocardiographic imaging systems for data acquisition. Importantly, we followed the latest guidelines for chamber quantification (33) for acquisition and measurement of LV volumes using 3D images and LV mass using the area-length method.

As regards potential sex dependency in our results, it is known that cardiovascular structural differences between sexes without unloading exist, with LV EDV, mass, and cavity length greater for men than women despite adjustments for body size (44), together with increased incidence of orthostatic intolerance in women after spaceflight (18, 50). However, it is plausible to consider the hypothesis of similar LV remodeling processes, as women appear to have the same degree of cardiac atrophy than men after 60 days of sedentary HDBR (12).

Effects on LV mass and volumes. It is known that, after short-term activation of volume regulatory mechanisms by central fluid shifts induced by HDBR position, as well as during space flights, a loss of plasma volume and the establishment of a new hemodynamic steady state is reached within 24 to 48 h, resulting in decreased volume loading of the heart that could lead to cardiac atrophy. During bed rest, previous studies have verified this observation by reporting a decrease in LV mass and EDV. Kozakova et al. (31) used 2D echocardiography to report a reduction in LV mass and wall thickness, EDV, and SV after 5 wk of HDBR. Levine et al. (35) demonstrated using similar methods a decrease in LV EDV and LV

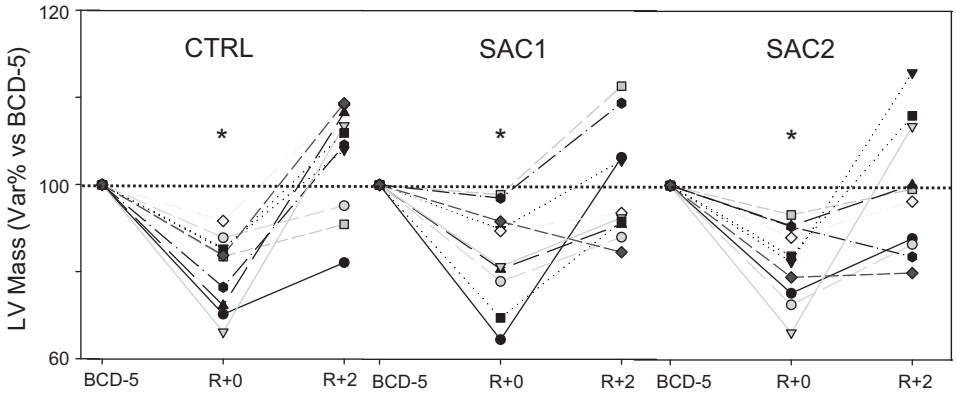

Fig. 3. Individual results for LV mass obtained in the 10 subjects while in control condition (CTRL) or in the short-arm centrifugation countermeasure (SAC1 and SAC2) groups. Values are reported as change from the pre (BCD-5)-bed rest evaluation.

Table 3. *Results of mitral and pulmonic pulsed-Doppler flow parameters obtained in the 10 subjects in the 3 campaigns*

	CTRL			SAC1			SAC2		
	BCD-5	R + 0	R + 2	BCD-5	R + 0	R + 2	BCD-5	R + 0	R + 2
Peak E, cm/s	82 ± 23	62 ± 16*	88 ± 16†	82 ± 20	67 ± 18*	87 ± 21†	86 ± 20	67 ± 19*	88 ± 21†
Peak A, cm/s	50 ± 9	44 ± 6	49 ± 10	47 ± 11	45 ± 6	52 ± 6	48 ± 12	45 ± 8	51 ± 9
E/A ratio	1.7 ± 0.6	1.4 ± 0.3	1.9 ± 0.6†	1.8 ± 0.5	1.5 ± 0.3	1.7 ± 0.5	1.9 ± 0.5	1.5 ± 0.5	1.8 ± 0.5
E integral, cm	14 ± 4	13 ± 3*	17 ± 3†	17 ± 4	14 ± 4*	17 ± 4†	15 ± 4	13 ± 3*	16 ± 3†
A integral, cm	5 ± 1	4 ± 1	5 ± 1†	4 ± 1	4 ± 1	5 ± 1†	5 ± 1	4 ± 1	5 ± 1†
Time to peak E, ms	50 ± 4	49 ± 3	48 ± 2	48 ± 4	49 ± 2	49 ± 3	50 ± 2	50 ± 3	49 ± 2
E dec. time, sec	20 ± 2	27 ± 7*	21 ± 8	23 ± 5	25 ± 5	22 ± 5	20 ± 2	24 ± 4	21 ± 5
S peak, cm/s	57 ± 11	49 ± 7*	54 ± 9	59 ± 12	46 ± 5*	56 ± 10†	54 ± 10	51 ± 8	54 ± 12
D peak, cm/s	50 ± 11	49 ± 12	55 ± 9†	57 ± 11	47 ± 11*	58 ± 12†	53 ± 11	51 ± 10	55 ± 10
S/D ratio	1.2 ± 0.2	1.1 ± 0.3	1 ± 0.2	1.1 ± 0.3	1 ± 0.3	1 ± 0.2	1.1 ± 0.3	1 ± 0.3	1 ± 0.2

Values are means ± SD. E and A, early and late diastolic velocities; dec., deceleration; S and D, systolic and diastolic flow velocities. *$P < 0.05$ vs. BCD-5; †$P < 0.05$ R + 0 vs. R + 2.

mass ($P < 0.1$) after only 2 wk of HDBR, with no change in cardiac outputs, and a parallel, leftward shift of the LV pressure-volume relationship with a decrease in equilibrium volume, suggesting decreased cardiac distensibility. This leftward shift did not occur after a similar degree of hypovolemia acutely induced by intravenous furosemide, compared with 18 days of HDBR in the same subjects. This study provided additional evidence of LV remodeling during bed rest, resulting from both hypovolemia and physical inactivity (41). Recently, both Hastings et al. (24) and Carrick-Ranson et al. (7) showed a reduction in LV mass and volumes in nine subjects after 21 days of HDBR with no CM. Interestingly, LV mass was increased in 18 subjects following daily rowing ergometry and biweekly strength training, with minimal changes in EDV. Differently, Stenger et al. (47) showed no changes in LV mass and a decrease in LV EDV in both control and CM interventions (SAC with 1 G_z at the heart applied 1 h/day, together with shallow squats and heel raises) groups for 21 days of HDBR. However, no intermediate earlier additional time points were reported in these studies regarding LV mass and volumes.

Perhonen et al. (40) showed in five subjects using MRI a decrease in LV mass after 6 wk of supine bed rest, but not after 2 wk, accompanied by a reduction in SV that was already noted after 2 wk. However, reported LV mass appeared to be higher (>220 g) than what should be assumed as fitting the normal range when computed by MRI (38), thus posing a question of accuracy on these results.

A possible physiologic interpretation for the reduction in LV mass that we noted after only 5 days of HDBR has been earlier proposed by Summer et al. (48), who studied 38 astronauts before and after 9–16 days of spaceflights. This study noted that the reduction of ~10% in LV mass and LV EDV measured immediately after landing was completely reversed 3 days later, a finding that is similar to that of our current short-term HDBR study. These authors hypothesized that the LV mass loss was not be primarily due to cardiac atrophy but related to dehydration, as it is unlikely that the cardiac muscle can remodel to such an extent in such a short period of time.

Similarly, the observations of an ~4% loss in LV mass postdialysis, associated to reduced LV EDV and ESV, have been interpreted as secondary to a loss of cardiac interstitial volume, thus corroborating the dehydration hypothesis (26).

Effects on LV and LA filling. As expected, HDBR resulted in a reduction in plasma and blood volume that were reflected in alterations in loading conditions. Changes in LV and LA volumes are interrelated (5, 36) and completely reversible after HDBR. In keeping with the observed preload reduction, Doppler ultrasound parameters are influenced by acute changes in preload in healthy individuals (6, 14), the Doppler and tissue-Doppler indexes of early diastolic filling were reduced with HDBR and restored to normal after 3 days, confirming the hypothesis of Carrick-Ranson et al. (7) that a reduction in LA pressure rather than changes in intrinsic ventricular function are likely to explain the effects of HDBR on Doppler measures of LV filling.

There are many physiological variables that affect the pulmonic flow velocities, with preload being one of them (25, 29). In particular, in patients with normal cardiac index, a signifi-

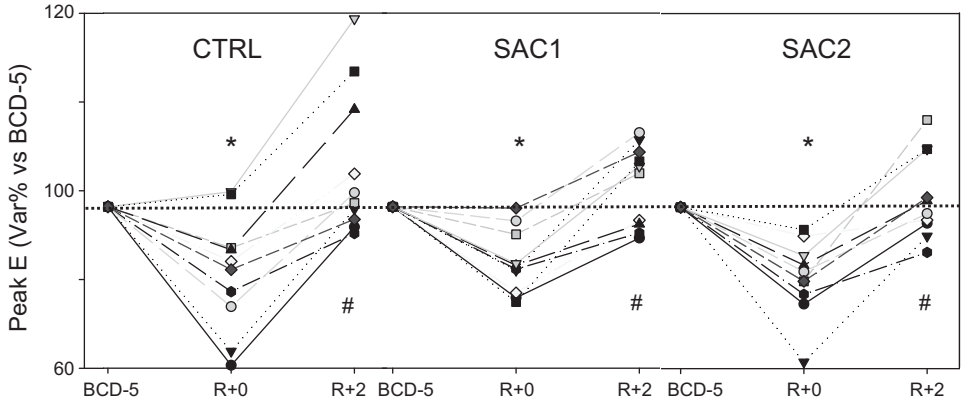

Fig. 4. Individual results for early mitral inflow velocity obtained in the 10 subjects while in control condition (CTRL) or in the short-arm centrifugation countermeasure (SAC1 and SAC2) groups. Values are reported as change from the pre (BCD-5) bed rest evaluation.

Table 4. *Results of aortic continuous Doppler flow parameters obtained in the 10 subjects in the 3 campaigns*

	CTRL			SAC1			SAC2		
	BCD-5	R + 0	R + 2	BCD-5	R + 0	R + 2	BCD-5	R + 0	R + 2
V_{max}, cm/s	124 ± 16	114 ± 15*	122 ± 15†	127 ± 13	111 ± 14*	131 ± 16†	128 ± 19	116 ± 13*	125 ± 18
V_{TI}, cm	27 ± 4	22 ± 2*	26 ± 2†	27 ± 4	23 ± 3*	28 ± 3†	27 ± 4	23 ± 4*	26 ± 4†
V_{mean}, cm/s	90 ± 11	82 ± 6*	89 ± 8†	91 ± 9	79 ± 10*	92 ± 7†	93 ± 14	82 ± 10*	89 ± 13
Time to V_{max}, ms	86 ± 25	83 ± 26	87 ± 12	92 ± 12	89 ± 21	92 ± 19	78 ± 19	85 ± 24	87 ± 19
Ejection time, ms	298 ± 15	274 ± 20*	293 ± 8†	295 ± 19	285 ± 18*	298 ± 14	293 ± 15	272 ± 15*	294 ± 18†

Values are means ± SD. V_{TI}, velocity time interval. *$P < 0.05$ vs. BCD-5; †$P < 0.05$ R + 0 vs. R + 2.

cant and positive relationship ($r = 0.69$) was found between the S component and the pulmonary wedge pressure (19). The significant reduction in S peak with HDBR was completely reversed after 3 days. This observation could be partially explained as a result of the decrease in pulmonary wedge pressure with bed rest, which has been previously demonstrated to occur after longer HDBR studies (7, 24, 46).

Effectiveness of SAC as CM. Gravitational stimulation induced by SAC represents a potential CM to prevent cardiovascular deconditioning, but the ideal combination of its magnitude, frequency, and duration required to be effective with or without exercise remains to be determined.

Our results obtained using SAC without exercise have shown that the applied CM during 5 days of HDBR was not effective in preventing loss in plasma and blood volumes and LV volume and mass. Similarly, it was ineffective in preventing a reduction in LA volume as well as early systolic filling flow and tissue velocities.

Both continuous and intermittent administration of daily SAC showed similar results, with very few parameters (time to peak E, D peak, and E/E' lat) showing potential statistical interaction (see Table 6) with the applied CM, thus not allowing for a clear explanation of the underlying phenomena.

In previous studies using SAC as CM during HDBR, exposure twice a day to 30 min with 2 G_z at the heart during a 4 days of HDBR in 10 male subjects prevented the negative effects on baroreflex function and plasma volume but could not reverse the decline in exercise capacity (27). Again, SAC for 30 min twice a day with intermittent (2-min period) switch between 1 and 2 G_z at the foot, in conjunction with ergometric 40-W exercise, during 4 days of HDBR was able to maintain in six males SV and CO to pre-HDBR levels (51).

Together with exercise training in alternate days (20 min with 0.8–1.4 G_z at the heart with 60-W exercise intensity, followed by 20 min with 0.3 G_z with 40–80% peak oxygen uptake exercise protocol), SAC was effective in maintaining respiratory and cardiovascular responses in five males during upright exercise after 20 days of HDBR (28). Also, daily 1 h at 1 G_z at the heart level associated with shallow squats and heel raises during centrifugation during 21 days of HDBR showed prolongation of tilt test tolerance time after bed rest in eight males and attenuation of decrease in peak $\dot{V}O_2$ (46) but did not prevent the reduction in EDV, SV, E wave velocity, and ejection time.

As in Shibata et al. (46), the absence of improvement in echocardiographic parameters of cardiac function we found both with continuous and intermittent SAC could be in part explained hypothesizing that the possible beneficial effect could manifest as alterations in peripheral vasculature in the lower body (subject to 2.5 G_z) without associated changes in cardiac function. Only longer application of higher gravity levels (27, 28) might result in positive effects on cardiac function.

Future studies will be required to better understand the specific effects of HDBR duration with different gravity profiles and loads, as well as the impact of exercise combined with SAC, on cardiac function during bed rest.

Study limitations. The relative short duration of the performed bed rest could be considered as a limitation. However, while there is an abundance of data for longer HDBR (4–6 wk), this study intentionally focused on a 5 days of duration to fill this gap. In addition, as the effects on the cardiovascular system are activated immediately after weightlessness exposure, 5 days of HDBR represent a model of cardiac unloading evidencing those changes. Consequently, the efficacy of CM specifically addressed to the cardiovascular system could be tested in short HDBR studies with crossover design, with a potential reduction in related costs.

The lack of data from female subjects constitutes a main limitation for this study, thus preventing gender comparisons that could reveal interesting differences. However, the inclusion of men only was decided a priori from the organizing agency and not related to expected poor significance in the results in our experiment if an additional source of variability

Table 5. *Results of tissue Doppler examinations*

	CTRL			SAC1			SAC2		
	BCD-5	R + 0	R + 2	BCD-5	R + 0	R + 2	BCD-5	R + 0	R + 2
E' Sep, cm/s	13 ± 2	11 ± 2*	13 ± 2	13 ± 2	11 ± 2*	14 ± 2†	13 ± 3	10 ± 2*	13 ± 2†
E' Lat, cm/s	18 ± 4	15 ± 3	16 ± 3	20 ± 4	15 ± 3*	18 ± 3†	19 ± 3	14 ± 3*	18 ± 3†
S' Sep, cm/s	10 ± 1	10 ± 2	10 ± 1	10 ± 1	10 ± 2	11 ± 2	10 ± 1	10 ± 1	10 ± 1
S' Lat, cm/s	13 ± 2	14 ± 3	12 ± 2	15 ± 4	13 ± 3	13 ± 2	13 ± 1	14 ± 2	14 ± 2
E/E' Sep	6.5 ± 2.7	7.6 ± 2.8	7.1 ± 2	6.4 ± 1.9	6.6 ± 2.6	6.3 ± 2	6.8 ± 2	6.7 ± 1.8	6.8 ± 1.7
E/E' Lat	4.9 ± 1.8	4.4 ± 1.4	5.6 ± 1.2	4.2±.9	4.7 ± 1.5	5 ± 1.5*	4.6±.9	4.9 ± 1.3	4.9 ± 1.2

Values are means ± SD. Sep, septal; Lat, lateral. *$P < 0.05$ vs. BCD-5; †$P < 0.05$ R + 0 vs. R + 2.

Table 6. *Summary of the variations, expressed as median (25th,75th percentile) of the percent change between BCD-5 and R + 0, measured in the 3 groups*

	CTRL	SAC1	SAC2	*P* Interaction
LV mass	−16% (−23,−15%)*	−15% (−19,−9%)*	−17% (−21,−10%)*	0.37
3D EDV	−14% (−19,−9%)*	−14% (−16,−9%)*	−11% (−15,−8%)*	0.32
3D ESV	−12% (−19,+3%)*	−11% (−13,−4%)*	−15% (−20,−11%)*	0.09
3D EF	−4% (−9,+2%)	−2% (−7,+6%)	0% (−2,+4%)	0.39
LA volume	−36% (−46,−28%)*	−27% (−45,−7%)	−28% (−53,−19%)*	0.83
Peak E	−18% (−30,−13%)*	−19% (−25,−11%)*	−21% (−26,−15%)*	0.50
Peak A	−7% (−20,0%)	−2% (−14,+8%)	−5% (−19,−1%)	0.78
E/A	−6% (−27,+7%)	−13% (−27,−6%)	−14% (−17,−6%)	0.45
E integral	−1% (−19,+5%)*	−22% (−31,−10%)*	−12% (−16,−5%)*	0.20
A integral	−9% (−26,−4%)	−9% (−16,+2%)	−17% (−24,−3%)	0.93
Time to peak E	−2% (−5,+5%)	+1% (−3,+7%)	+1% (−2,+3%)	0.05*
E Deceleration time	+28% (+11,+53%)*	+13% (0,+25%)	+22% (+1,+34%)	0.39
S peak	−7% (−25,0%)*	−25% (−32,−12%)*	−6% (−10,+8%)	0.08
D peak	−5% (−11,+4%)	−15% (−20,−9%)*	−2% (−9,+4%)	0.03*
S/D	−13% (−22,−4%)	−11% (−22,+7%)	+3% (−8,+10%)	0.66
V_{max}	−10% (−12,−2%)*	−15% (−22,−6%)*	−11%(−15,−7%)*	0.18
V_{TI}	−18% (−19,−13%)*	−13% (−24,−9%)*	−14% (−23,−10%)*	0.66
V_{mean}	−10% (−13,−8%)*	−14% (−21,−11%)*	−13% (−15,−10%)*	0.40
Time to V_{max}	0% (−16,+9%)	0% (−10,+12%)	−6% (−16,+46%)	0.85
Ejection time	−3%(−14,−3%)*	−2% (−7,−3%)*	−8% (−12,−1%)*	0.11
E' Sep	−12% (−31,−5%)*	−18% (−31,−12%)*	−20% (−27,−14%)*	0.62
E' Lat	−10% (−25,+4%)	−26% (−34,−18%)*	−27% (−33,−19%)*	0.20
S' Sep	+1% (−7,+1%)	+11% (+1,+34%)	−6% (−11,+2%)	0.74
S' Lat	+3% (−14,+26%)	−2% (−26,+18%)	0% (−7,+17%)	0.06
E/E' Sep	+14% (+5,+48%)	+5% (−15,+31%)	−5% (−19,+21%)	0.64
E/E' Lat	−9% (−23,+4%)	+13% (−4,+29%)	+5% (−2,+23%)	0.05*

The *P* value of the interaction resulting from the ANOVA test with repeated measures on both factors is also reported, as median (25th,75th percentile). *$P < 0.05$ BCD-5 vs. R + 0.

was introduced. In addition, for comparison purposes, to our knowledge all previous studies focusing on SAC as CM for the cardiovascular system were conducted in males. This underlines the need to explore possible sex-specific responses associated with SAC during HDBR.

Although echocardiography is commonly used in clinical practice, its ability to measure cardiac mass in a reproducible manner may not be optimal (32) and LV mass calculations obtained with 2D echocardiography are frequently discrepant from values measured with cardiac MRI, considered as the "gold standard" technique for LV mass assessment. These echocardiographic limitations could have been partially responsible for the "potential" overestimation of 16% drop in LV mass between subsequent measurements compared with previous investigations.

Table 7. *List of previous studied involving cardiac echocardiography during HDBR, with details on bed rest duration, number of subjects in sedentary (CTRL) or countermeasure groups, and methods applied to derive LV volume and mass from 1D or 2D images*

Author	Bed Rest Duration	No. Subjects	LV Volume	LV Mass
Arbeille et al. (2)	28 days (7,14,21,28)	CTRL (6), LBNP (6)	Teicholz	NO
Levine et al. (35)	2 wk	CTRL (12)	EDV from area-length or cylinder hemiellipsoid	Area-length or cylinder hemiellipsoid
Arbeille et al. (3)	7 days	Crossover design with 8 subjects: CTRL, tight cuffs	LV diameter from M-mode and Teicholz formula for EDV, ESV, SV	NO
Arbeille et al. (1)	10 h, 4, 5, 7, 30, 42 days	5–19	Teicholz	NO
Perhonen et al. (41)	2 wk, hypovolemia (same subjects)	CTRL (7)	Modified Simpson or area-length	NO
Platts et al. (42)	60, 90 days	CTRL (13)	From M-mode	From M-mode
Shibata et al. (46)	14–18 days	CTRL (7), supine cycle ergometer(14)	EDV by modified Simpson, area-length	MRI (Dorfman 2008)
Kozakova et al. (31)	5 wk	CTRL (10)	Biplane Simpson	From M-mode
Stenger et al. (47)	21 days	CTRL (7), SAC + EXE (8)	Echo (method not specified)	Echo (method not specified)
Hastings et al. (24)	5 wk	CTRL (9), daily rowing ergometry + biweekly strength training (18)	MRI +3D echo	MRI
Carrick-Ranson et al. (7)	21 days	CTRL (9), daily rowing ergometry + biweekly strength training (18)	3D echo	Not specified

1D and 2D, 1- and 2-dimensional; LBNP, lower body negative pressure; SV, stroke volume; EXE, exercise.

However, in animal studies (10) it has been reported that the interstitial space in the heart tissue of in vivo nonnephrectomized rats, represented by fluids and by intercellular substance, reaches up to 18.8% of the total space occupied by tissues, while plasma space represents up to 7.7%. As this space may dilate or shrink depending on hydration and on several other variables, it is possible that the combination of plasma reduction and dehydration caused by bed rest could have contributed to the observed reduction of the interstitial space, with a resultant higher packing of myocardial cell density, resulting in the observed drop in LV mass.

In conclusion, a significant reduction in LV mass and LV and LA volumes, as well as in Doppler indexes of early diastolic filling, was observed even after only 5 days of HDBR. All these changes were fully reversed shortly after discontinuation of HDBR. SAC as a CM resulted ineffective in preventing these changes, both when applied continuously or intermittently.

ACKNOWLEDGMENTS

We are extremely grateful to all the personnel of ESA and MEDES involved in the bed rest studies for the support to the realization of our experiment, as well as to the experimental subjects for their dedicated collaboration. Special thanks are addressed to Philips Medical Systems, which made the ultrasound equipment available for this study.

GRANTS

This research has been performed thanks to the contribution of the Italian Space Agency (contract nos. I/061/10/0 and 2013-032-R.0; to E. G. Caiani). P. Vaida research was funded by Centre National d'Études Spatiales (CNES) (contract CNES/4800000632).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: E.G.C. and P.V. conception and design of research; E.G.C., P.V., and R.M.L. interpreted results of experiments; E.G.C. prepared figures; E.G.C. drafted manuscript; E.G.C., P.V., and R.M.L. edited and revised manuscript; E.G.C., P.M., L.W., P.V., and R.M.L. approved final version of manuscript; P.M. performed experiments; L.W. and R.M.L. analyzed data.

REFERENCES

1. **Arbeille P, Fomina G, Roumy J, Alferova I, Tobal N, Herault S.** Adaptation of the left heart, cerebral and femoral arteries, and jugular and femoral veins during short- and long-term head-down tilt and spaceflights. *Eur J Appl Physiol* 86: 157–168, 2001.
2. **Arbeille P, Gauquelin G, Pottier JM, Pourcelot L, Guell A, Gharib C.** Results of a 4-week head-down tilt with and without LBNP countermeasure. 2. Cardiac and peripheral hemodynamics–comparison with a 25-day spaceflight. *Aviat Space Environ Med* 63: 9–13, 1992.
3. **Arbeille P, Herault S, Fomina G, Roumy J, Alferova I, Gharib C.** Influences of thigh cuffs on the cardiovascular system during 7-day head-down bed rest. *J Appl Physiol* 87: 2168–2176, 1999.
4. **Arya M, Paloski WH, Young LR.** Centrifugation protocol for the NASA artificial gravity-bed rest pilot study. *J Gravit Physiol* 14: 5–8, 2007.
5. **Caiani EG, Sugeng L, Weinert L, Capderou A, Lang RM, Vaida P.** Objective evaluation of changes in left ventricular and atrial volumes during parabolic flight using real-time three-dimensional echocardiography. *J Appl Physiol* 101: 460–468, 2006.
6. **Caiani EG, Weinert L, Takeuchi M, Veronesi F, Sugeng L, Corsi C, Capderou A, Cerutti S, Vaida P, Lang RM.** Evaluation of alterations on mitral annulus velocities, strain, and strain rates due to abrupt changes in preload elicited by parabolic flight. *J Appl Physiol* 103: 80–87, 2007.
7. **Carrick-Ranson G, Hastings JL, Bhella PS, Shibata S, Levine BD.** The effect of exercise training on left ventricular relaxation and diastolic suction at rest and during orthostatic stress after bed rest. *Exp Physiol* 98: 501–513, 2013.
8. **Charles JB, Bungo MW.** Cardiovascular physiology in space-flight. *Exp Gerontol* 26: 163–168, 1991.
9. **Charles JB, Lathers CM.** Cardiovascular adaptation to spaceflight. *J Clin Pharmacol* 31: 1010–1023, 1991.
10. **Cieslar J, Huang MT, Dobson GP.** Tissue spaces in rat heart, liver and skeletal muscle in vivo. *Am J Physiol Regul Integr Comp Physiol* 275: R1530–R1536, 1998.
11. **Devereux RB, Roman MJ, de SG, O'Grady MJ, Paranicas M, Yeh JL, Fabsitz RR, Howard BV.** Relations of left ventricular mass to demographic and hemodynamic variables in American Indians: the Strong Heart Study. *Circulation* 96: 1416–1423, 1997.
12. **Dorfman TA, Levine BD, Tillery T, Peshock RM, Hastings JL, Schneider SM, Macias BR, Biolo G, Hargens AR.** Cardiac atrophy in women following bed rest. *J Appl Physiol* 103: 8–16, 2007.
13. **Dorfman TA, Rosen BD, Perhonen MA, Tillery T, McColl R, Peshock RM, Levine BD.** Diastolic suction is impaired by bed rest: MRI tagging studies of diastolic untwisting. *J Appl Physiol* 104: 1037–1044, 2008.
14. **Firstenberg MS, Levine BD, Garcia MJ, Greenberg NL, Cardon L, Morehead AJ, Zuckerman J, Thomas JD.** Relationship of echocardiographic indices to pulmonary capillary wedge pressures in healthy volunteers. *J Am Coll Cardiol* 36: 1664–1669, 2000.
15. **Fortney SM, Schneider VS, Greenleaf JE.** The physiology of bed rest. In: *Handbook of Physiology. Environmental Physiology*. Bethesda, MD: Am Physiol Soc, 1996, sect 4, p. 889–939.
16. **Fritsch JM, Charles JB, Bennett BS, Jones MM, Eckberg DL.** Short-duration spaceflight impairs human carotid baroreceptor-cardiac reflex responses. *J Appl Physiol* 73: 664–671, 1992.
17. **Fritschyelle JM, Charles JB, Jones MM, Beightol LA, Eckberg DL.** Spaceflight alters autonomic regulation of arterial-pressure in humans. *J Appl Physiol* 77: 1776–1783, 1994.
18. **Fu Q, rbab-Zadeh A, Perhonen MA, Zhang R, Zuckerman JH, Levine BD.** Hemodynamics of orthostatic intolerance: implications for gender differences. *Am J Physiol Heart Circ Physiol* 286: H449–H457, 2004.
19. **Gentile F, Mantero A, Lippolis A, Ornaghi M, Azzollini M, Barbier P, Beretta L, Casazza F, Corno R, Faletra F, Giagnoni E, Gualtierotti C, Lombroso S, Mattioli R, Morabito A, Pepi M, Todd S, Pezzano A.** Pulmonary venous flow velocity patterns in 143 normal subjects aged 20 to 80 years old. An echo 2D colour Doppler cooperative study. *Eur Heart J* 18: 148–164, 1997.
20. **Gore CJ, Bourdon PC, Woolford SM, Ostler LM, Eastwood A, Scroop GC.** Time and sample site dependency of the optimized CO-rebreathing method. *Med Sci Sports Exerc* 38: 1187–1193, 2006.
21. **Gore CJ, Hopkins WG, Burge CM.** Errors of measurement for blood volume parameters: a meta-analysis. *J Appl Physiol* 99: 1745–1758, 2005.
22. **Guell A, Braak L.** Cardiovascular deconditioning syndrome during space flight. *Ann Cardiol Angeiol (Paris)* 38: 499–502, 1989.
23. **Hargens AR, Watenpaugh DE.** Cardiovascular adaptation to spaceflight. *Med Sci Sports Exerc* 28: 977–982, 1996.
24. **Hastings JL, Krainski F, Snell PG, Pacini EL, Jain M, Bhella PS, Shibata S, Fu Q, Palmer MD, Levine BD.** Effect of rowing ergometry and oral volume loading on cardiovascular structure and function during bed rest. *J Appl Physiol* 112: 1735–1743, 2012.
25. **Hoit BD, Shao Y, Gabel M, Walsh RA.** Influence of loading conditions and contractile state on pulmonary venous flow. Validation of Doppler velocimetry. *Circulation* 86: 651–659, 1992.
26. **Hunold P, Vogt FM, Heemann UW, Zimmermann U, Barkhausen J.** Myocardial mass and volume measurement of hypertrophic left ventricles by MRI–study in dialysis patients examined before and after dialysis. *J Cardiovasc Magn Reson* 5: 553–561, 2003.
27. **Iwasaki KI, Sasaki T, Hirayanagi K, Yajima K.** Usefulness of daily +2Gz load as a countermeasure against physiological problems during weightlessness. *Acta Astronaut* 49: 227–235, 2001.
28. **Katayama K, Sato K, Akima H, Ishida K, Takada H, Watanabe Y, Iwase M, Miyamura M, Iwase S.** Acceleration with exercise during head-down bed rest preserves upright exercise responses. *Aviat Space Environ Med* 75: 1029–1035, 2004.
29. **Keren G, Milner M, Lindsay J Jr, Goldstein S.** Load dependence of left atrial and left ventricular filling dynamics by transthoracic and transesophageal Doppler echocardiography. *Am J Card Imaging* 10: 108–116, 1996.
30. **Kotovskaya AR, Vil-Viliams IF.** The short-arm centrifuge: history and possible uses in cosmonautics and health care services. *Aviakosm Ekolog Med* 38: 3–11, 2004.

31. **Kozakova M, Malshi E, Morizzo C, Pedri S, Santini F, Biolo G, Pagani M, Palombo C.** Impact of prolonged cardiac unloading on left ventricular mass and longitudinal myocardial performance: an experimental bed rest study in humans. *J Hypertens* 29: 137–143, 2011.
32. **Kuhl HP, Franke A, Frielingsdorf J, Flaskamp C, Krebs W, Flachskampf FA, Hanrath P.** Determination of left ventricular mass and circumferential wall thickness by three-dimensional reconstruction: in vitro validation of a new method that uses a multiplane transesophageal transducer. *J Am Soc Echocardiogr* 10: 107–119, 1997.
33. **Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ.** Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr* 18: 1440–1463, 2005.
34. **Lathers CM, Charles JB, Elton KF, Holt TA, Mucai C, Bennet BS, Bungo MW.** Acute hemodynamic responses to weightlessness in humans. *J Clin Pharmacol* 29: 615–627, 1989.
35. **Levine BD, Zuckerman JH, Pawelczyk JA.** Cardiac atrophy after bed-rest deconditioning: a nonneural mechanism for orthostatic intolerance. *Circulation* 96: 517–525, 1997.
36. **Marsan NA, Maffessanti F, Tamborini G, Gripari P, Caiani E, Fusini L, Muratori M, Zanobini M, Alamanni F, Pepi M.** Left atrial reverse remodeling and functional improvement after mitral valve repair in degenerative mitral regurgitation: a real-time 3-dimensional echocardiography study. *Am Heart J* 161: 314–321, 2011.
37. **Moore ST, Diedrich A, Biaggioni I, Kaufmann H, Raphan T, Cohen B.** Artificial gravity: a possible countermeasure for post-flight orthostatic intolerance. *Acta Astronaut* 56: 867–876, 2005.
38. **Natori S, Lai S, Finn JP, Gomes AS, Hundley WG, Jerosch-Herold M, Pearson G, Sinha S, Arai A, Lima JA, Bluemke DA.** Cardiovascular function in multi-ethnic study of atherosclerosis: normal values by age, sex, and ethnicity. *AJR Am J Roentgenol* 186: S357–S365, 2006.
39. **Pavy-Le Traon A, Heer M, Narici MV, Rittweger J, Vernikos J.** From space to Earth: advances in human physiology from 20 years of bed rest studies (1986–2006). *Eur J Appl Physiol* 101: 143–194, 2007.
40. **Perhonen MA, Franco F, Lane LD, Buckey JC, Blomqvist CG, Zerwekh JE, Peshock RM, Weatherall PT, Levine BD.** Cardiac atrophy after bed rest and spaceflight. *J Appl Physiol* 91: 645–653, 2001.
41. **Perhonen MA, Zuckerman JH, Levine BD.** Deterioration of left ventricular chamber performance after bed rest: "cardiovascular deconditioning" or hypovolemia? *Circulation* 103: 1851–1857, 2001.
42. **Platts SH, Martin DS, Stenger MB, Perez SA, Ribeiro LC, Summers R, Meck JV.** Cardiovascular adaptations to long-duration head-down bed rest. *Aviat Space Environ Med* 80: A29–A36, 2009.
43. **Quinones MA, Otto CM, Stoddard M, Waggoner A, Zoghbi WA.** Recommendations for quantification of Doppler echocardiography: a report from the Doppler Quantification Task Force of the Nomenclature and Standards Committee of the American Society of Echocardiography. *J Am Soc Echocardiogr* 15: 167–184, 2002.
44. **Salton CJ, Chuang ML, O'Donnell CJ, Kupka MJ, Larson MG, Kissinger KV, Edelman RR, Levy D, Manning WJ.** Gender differences and normal left ventricular anatomy in an adult population free of hypertension. A cardiovascular magnetic resonance study of the Framingham Heart Study Offspring cohort. *J Am Coll Cardiol* 39: 1055–1060, 2002.
45. **Schmidt W, Prommer N.** The optimised CO-rebreathing method: a new tool to determine total haemoglobin mass routinely. *Eur J Appl Physiol* 95: 486–495, 2005.
46. **Shibata S, Perhonen M, Levine BD.** Supine cycling plus volume loading prevent cardiovascular deconditioning during bed rest. *J Appl Physiol* 108: 1177–1186, 2010.
47. **Stenger MB, Evans JM, Knapp CF, Lee SM, Phillips TR, Perez SA, Moore AD Jr,. Paloski WH, Platts SH.** Artificial gravity training reduces bed rest-induced cardiovascular deconditioning. *Eur J Appl Physiol* 112: 605–616, 2012.
48. **Summers RL, Martin DS, Meck JV, Coleman TG.** Mechanism of spaceflight-induced changes in left ventricular mass. *Am J Cardiol* 95: 1128–1130, 2005.
49. **Vil-Viliams IF, Kotovskaya AR, Lukjanuk VY.** Development of medical control of man in conditions of +Gz accelerations at short-arm centrifuge. *J Gravit Physiol* 11: 225–226, 2004.
50. **Waters WW, Ziegler MG, Meck JV.** Postspaceflight orthostatic hypotension occurs mostly in women and is predicted by low vascular resistance. *J Appl Physiol* 92: 586–594, 2002.
51. **Yang CB, Wang YC, Gao Y, Geng J, Wu YH, Zhang Y, Shi F, Sun XQ.** Artificial gravity with ergometric exercise preserves the cardiac, but not cerebrovascular, functions during 4 days of head-down bed rest. *Cytokine* 56: 648–655, 2011.