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)

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Caiani EG, Massabuau P, Weinert L, Vaïda P, Lang RM. 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). J Appl Physiol 117: 624–632, 2014. First published July 31, 2014; doi:10.1152/japplphysiol.00122.2014.—This study examined cardiac remodeling and functional changes induced by 5 days of head-down (–6°) bed rest (HDBR) and the effectiveness of short-arm centrifugation (SAC) in preventing them in males. Twelve healthy men (mean age: 33 ± 7) were enrolled in a crossover design study (BR-AG1, European Space Agency), including one sedentary (CTRL) and two daily SAC countermeasures (SAC1, 30 min continuously; SAC2, 30 min intermittently) groups. Measurements included plasma and blood volume and left ventricular (LV) and atrial (LA) dimensions by transthoracic echocardiography (2- and 3-dimensional) and Doppler inflows. Results showed that 5 days of HDBR had a major impact on both the geometry and cardiac function in males. LV mass and volume decreased by 16 and 14%, respectively; LA volume was reduced by 36%; Doppler flow and tissue Doppler velocities were reduced during early filling by 18 and 12%, respectively; and aortic flow velocity time integral was decreased by 18% with a 3% shortening of LV ejection time. These modifications were presumably due to decreased physiological loading and dehydration, resulting in reduced plasma and blood volume. All these changes were fully reversed 3 days after termination of HDBR. Moreover, SAC was not able to counteract these changes, either when applied continuously or intermittently.

head-down bed rest; echocardiography; cardiac function; short-arm centrifugation

DURING SPACE FLIGHT, CHANGES in gravity (Gz; 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).

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 countering 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-Gz 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 $33 \pm 7$ (range, 21 to 41 yr; body mass index, $23.7 \pm 2.1$ kg/m$^2$; maximal oxygen uptake, $39 \pm 6$ 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 $\sim 1.5$ 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 Gz 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 acclimatized 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, VO$\textsubscript{2}$ max test, isometric maximum voluntary contraction and muscle fatigue test, dual energy X-ray absorptiometry for body composition, dynamic biax index and posturography 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 (SpitCo; 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 (Hb), 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:

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

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

Then blood volume was computed as:

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

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 (55 phased array).

In addition, real-time 3D echocardiography (RT3D) was obtained (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 generated using wide-angled acquisitions in which four to seven wedge-shaped subvolumes ($93 \times 21^\circ$) 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 ($V_{\text{max}}$) and mean ($V_{\text{mean}}$) velocity, velocity time integral ($V_{\text{TI}}$), time-to-$V_{\text{max}}$, 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
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 \( \pm SD \), 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, measurements 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, 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-

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

<table>
<thead>
<tr>
<th></th>
<th>CTRL</th>
<th>HDT5</th>
<th>SAC1</th>
<th>HDT5</th>
<th>SAC2</th>
<th>HDT5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>BCD-2: 76</td>
<td>74.7 ± 6.8*</td>
<td>BCD-2: 75.7</td>
<td>6.7</td>
<td>BCD-2: 75.4</td>
<td>6.7</td>
</tr>
</tbody>
</table>

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;
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. *P < 0.05 BCD-5 vs R + 0.](http://jap.physiology.org/).
mass \((P < 0.1)\) after only 2 wk of HDBR, with no change in cardiac output, 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. In keeping with the observed preload reduction, Doppler indexes of early diastolic filling were reduced with HDBR. In postdialysis, associated to reduced LV EDV and ESV, have been interpreted as secondary to a loss of cardiac interstitial 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 is 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-

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

<table>
<thead>
<tr>
<th></th>
<th>CTRL</th>
<th>SAC1</th>
<th>SAC2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCD-5</td>
<td>R + 0</td>
<td>R + 2</td>
</tr>
<tr>
<td>Peak E, cm/s</td>
<td>82 ± 23</td>
<td>62 ± 16*</td>
<td>88 ± 16†</td>
</tr>
<tr>
<td>Peak A, cm/s</td>
<td>50 ± 9</td>
<td>44 ± 6</td>
<td>49 ± 10</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.7 ± 0.6</td>
<td>1.4 ± 0.3</td>
<td>1.9 ± 0.6†</td>
</tr>
<tr>
<td>E integral, cm</td>
<td>14 ± 4</td>
<td>13 ± 3*</td>
<td>17 ± 3†</td>
</tr>
<tr>
<td>A integral, cm</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
<td>5 ± 1†</td>
</tr>
<tr>
<td>Time to peak E, ms</td>
<td>50 ± 4</td>
<td>49 ± 3</td>
<td>48 ± 2</td>
</tr>
<tr>
<td>E dec. time, sec</td>
<td>20 ± 2</td>
<td>27 ± 7*</td>
<td>21 ± 8</td>
</tr>
<tr>
<td>S peak, cm/s</td>
<td>57 ± 11</td>
<td>49 ± 7*</td>
<td>54 ± 9</td>
</tr>
<tr>
<td>D peak, cm/s</td>
<td>50 ± 11</td>
<td>49 ± 12</td>
<td>55 ± 1†</td>
</tr>
<tr>
<td>S/D ratio</td>
<td>1.2 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>1 ± 0.2</td>
</tr>
</tbody>
</table>

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.

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**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. *P < 0.05 BCD-5 vs R + 0; #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 Gz 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 Gz 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 Gz at the heart with 60-W exercise intensity, followed by 20 min with 0.3 Gz 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 Gz 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 VO$_2$ (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 Gz) 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 was included.
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.

However, in animal studies (10) it has been reported that the interstitial space in the heart tissue of in vivo nonnephrecto-

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

<table>
<thead>
<tr>
<th></th>
<th>CTRL</th>
<th>SAC1</th>
<th>SAC2</th>
<th>P Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass</td>
<td>-16% (-23, -15%)*</td>
<td>-15% (-19, -9%)*</td>
<td>-17% (-21, -10%)*</td>
<td>0.37</td>
</tr>
<tr>
<td>3D EDV</td>
<td>-14% (-19, -9%)*</td>
<td>-14% (-16, -9%)*</td>
<td>-11% (-15, -8%)*</td>
<td>0.32</td>
</tr>
<tr>
<td>3D ESV</td>
<td>-12% (-19, +3%)*</td>
<td>-11% (-13, -4%)*</td>
<td>-15% (-20, -11%)*</td>
<td>0.09</td>
</tr>
<tr>
<td>LA volume</td>
<td>-36% (-46, -28%)*</td>
<td>-27% (-45, -7%)*</td>
<td>-28% (-53, -19%)*</td>
<td>0.83</td>
</tr>
<tr>
<td>Peak E</td>
<td>-18% (-30, -13%)*</td>
<td>-19% (-25, -11%)*</td>
<td>-21% (-26, -15%)*</td>
<td>0.50</td>
</tr>
<tr>
<td>Peak A</td>
<td>-7% (-20,0%)*</td>
<td>-2% (-14, 8%)*</td>
<td>-5% (-19, 1%)*</td>
<td>0.78</td>
</tr>
<tr>
<td>E/A</td>
<td>-6% (-27, +7%)*</td>
<td>-13% (-27, -6%)*</td>
<td>-14% (-17, -6%)*</td>
<td>0.45</td>
</tr>
<tr>
<td>E integral</td>
<td>-1% (-19, +5%)*</td>
<td>-22% (-31, 10%)*</td>
<td>-12% (-16, -5%)*</td>
<td>0.20</td>
</tr>
<tr>
<td>A integral</td>
<td>-9% (-26, -4%)*</td>
<td>-9% (-16, +2%)*</td>
<td>-17% (-24, -3%)*</td>
<td>0.93</td>
</tr>
<tr>
<td>Time to peak E</td>
<td>-2% (-5, +5%)*</td>
<td>+1% (-3, 7%)*</td>
<td>+1% (-2, +3%)*</td>
<td>0.05*</td>
</tr>
<tr>
<td>E Deceleration time</td>
<td>+28% (+11,+5%)*</td>
<td>+13% (0, +25%)*</td>
<td>+22% (+1, +34%)*</td>
<td>0.39</td>
</tr>
<tr>
<td>S peak</td>
<td>-7% (-25,0%)*</td>
<td>-25% (-32, -12%)*</td>
<td>-6% (-10, +8%)*</td>
<td>0.08</td>
</tr>
<tr>
<td>D peak</td>
<td>-5% (-11, 4%)*</td>
<td>-15% (-20, -9%)*</td>
<td>-2% (-9, +4%)*</td>
<td>0.03*</td>
</tr>
<tr>
<td>Vmax</td>
<td>-13% (-22, -4%)*</td>
<td>-11% (-22, +7%)*</td>
<td>+3% (-8, +10%)*</td>
<td>0.66</td>
</tr>
<tr>
<td>Vr0</td>
<td>-18% (-19, -13%)*</td>
<td>-13% (-24, -9%)*</td>
<td>-14% (-23, -10%)*</td>
<td>0.66</td>
</tr>
<tr>
<td>Vmean</td>
<td>-10% (-13, -8%)*</td>
<td>-14% (-21, -11%)*</td>
<td>-13% (-15, -10%)*</td>
<td>0.40</td>
</tr>
<tr>
<td>Time to Vmax</td>
<td>0% (-16, 9%)*</td>
<td>0% (-10, +12%)*</td>
<td>-6% (-16, +46%)*</td>
<td>0.85</td>
</tr>
<tr>
<td>Ejection time</td>
<td>-3%(-14, -3%)*</td>
<td>-2% (-7, -3%)*</td>
<td>-8% (-12, -1%)*</td>
<td>0.11</td>
</tr>
<tr>
<td>E’ Sep</td>
<td>-12% (-31, -5%)*</td>
<td>-18% (-31, -12%)*</td>
<td>-20% (-27, -14%)*</td>
<td>0.62</td>
</tr>
<tr>
<td>E’ Lat</td>
<td>-10% (-25, 4%)*</td>
<td>-26% (-34, -18%)*</td>
<td>-27% (-33, -19%)*</td>
<td>0.20</td>
</tr>
<tr>
<td>S’ Sep</td>
<td>+1% (-2, +1%)*</td>
<td>+11% (+1, +34%)*</td>
<td>-6% (-11, +2%)*</td>
<td>0.74</td>
</tr>
<tr>
<td>S’ Lat</td>
<td>+3% (-14, +26%)*</td>
<td>-2% (-26, +18%)*</td>
<td>0% (-7, +17%)*</td>
<td>0.06</td>
</tr>
<tr>
<td>E/E’ Sep</td>
<td>+14% (+5, +48%)*</td>
<td>+5% (-15, +31%)*</td>
<td>-5% (-19, +21%)*</td>
<td>0.64</td>
</tr>
<tr>
<td>E/E’ Lat</td>
<td>-9% (-23, +4%)*</td>
<td>+13% (-4, +29%)*</td>
<td>+5% (-2, +23%)*</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

The P value of the interaction resulting from the ANOVA test with repeated measures on both factors is also reported. *P < 0.05 BCD-5 vs. R + 0.

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

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

1D and 2D, 1- and 2-dimensional; LBNP, lower body negative pressure; EDV and ESV, end-diastolic and end-systolic volumes; SV, stroke volume; EXE, exercise.
mized 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.

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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.M., L.W., P.V., and R.M.L. interpreted results of experiments; E.G.C. prepared manuscript; P.M. performed experiments; L.W. and R.M.L. revised manuscript; E.G.C., P.M., L.W., P.V., and R.M.L. approved final manuscript. E.G.C., P.M., L.W., P.V., and R.M.L. performed experiments; L.W. and R.M.L. analyzed data.

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