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Cardiac atrophy in women following bed rest

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¹Institute for Exercise and Environmental Medicine, Presbyterian Hospital of Dallas, and ²Division of Cardiology, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas; ³Division of Physical Performance and Development, University of New Mexico, Albuquerque, New Mexico; ⁴Department of Clinical, Technological and Morphological Sciences, and Division of Internal Medicine, University of Trieste, Trieste, Italy; and ⁵Department of Orthopaedic Surgery, University of California, San Diego, California

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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. First published March 22, 2007; doi:10.1152/jappphysiol.01162.2006.— Both chronic microgravity exposure and long-duration bed rest induce cardiac atrophy, which leads to reduced standing stroke volume and orthostatic intolerance. However, despite the fact that women appear to be more susceptible to postspaceflight presyncope and orthostatic hypotension than male astronauts, most previous high-resolution studies of cardiac morphology following microgravity have been performed only in men. Because female athletes have less physiological hypertrophy than male athletes, we reasoned that they also might have altered physiological cardiac atrophy after bed rest. Magnetic resonance imaging was performed in 24 healthy young women (32.1 ± 4 yr) to measure left ventricular (LV) and right ventricular (RV) mass, volumes, and morphology accurately before and after 60 days of 6° head-down tilt (HDT) bed rest. Subjects were matched and then randomly assigned to sedentary bed rest (controls, $n = 8$) or two treatment groups consisting of 1) exercise training using supine treadmill running within lower body negative pressure plus resistive training ($n = 8$), or 2) protein ($0.45 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ increase) plus branched-chain amino acid (BCAA) (7.2 g/day) supplementation ($n = 8$). After sedentary bed rest without nutritional supplementation, there were significant reductions in LV (96 ± 26 to $77 \pm 25 \text{ ml}$; $P = 0.03$) and RV volumes (104 ± 33 to $86 \pm 25 \text{ ml}$; $P = 0.02$), LV (2.2 ± 0.2 to $2.0 \pm 0.2 \text{ g/kg}$; $P = 0.003$) and RV masses (0.8 ± 0.1 to $0.6 \pm 0.1 \text{ g/kg}$; $P < 0.001$), and the length of the major axis of the LV (90 ± 6 to $84 \pm 7 \text{ mm}$; $P < 0.001$), similar to what has been observed previously in men (8.0%; Perhonen MA, Franco F, Lane LD, Buckley JC, Blomqvist Zerwekh JE, Peshock RM, Weatherall PT, Levine BD. *J Appl Physiol* 91: 645–653, 2001). In contrast, there were no significant reductions in LV or RV volumes in the exercise-trained group, and the length of the major axis was preserved. Moreover, there were significant increases in LV (1.9 ± 0.4 to $2.3 \pm 0.3 \text{ g/kg}$; $P < 0.001$) and RV masses (0.7 ± 0.1 to $0.8 \pm 0.2 \text{ g/kg}$; $P = 0.002$), as well as mean wall thickness (9 ± 2 to $11 \pm 1 \text{ mm}$; $P = 0.02$). The interaction between sedentary and exercise LV and RV masses was highly significant ($P < 0.0001$). Protein and BCAA supplementation led to an intermediate phenotype with no change in LV or RV mass after bed rest, but there remained a significant reduction in LV volume (103 ± 14 to $80 \pm 16 \text{ ml}$; $P = 0.02$) and major-axis length (91 ± 5 to $88 \pm 7 \text{ mm}$; $P = 0.003$). All subjects lost an equivalent amount of body mass ($3.4 \pm 0.2 \text{ kg}$ control; $3.1 \pm 0.04 \text{ kg}$ exercise; $2.8 \pm 0.1 \text{ kg}$ protein). Cardiac atrophy occurs in women similar to men following sedentary 60 days HDT bed rest. However, exercise training and, to a lesser extent, protein supplementation may be potential counter-

measures to the cardiac atrophy associated with chronic unloading conditions such as in spaceflight and prolonged bed rest.

sex differences; microgravity exposure; cardiac atrophy; exercise; protein supplementation; magnetic resonance imaging

BOTH CHRONIC MICROGRAVITY exposure and long-duration bed rest result in eccentric cardiac atrophy and impaired cardiac compliance, leading to a prominent reduction in upright stroke volume (SV) and orthostatic intolerance (13, 34, 45, 58, 68). Although women appear to be more susceptible to postspaceflight presyncope and orthostatic hypotension than men (25), there are relatively few high-resolution studies of female cardiac structure after gravitational unloading. Currently, women account for 20% of the astronauts in the United States. The increased incidence of orthostatic intolerance in women following gravitational unloading has been attributed to a hypoadrenergic sympathetic system (16, 65), a greater dependence on plasma volume (16, 65), and a smaller SV secondary to a smaller and less compliant left ventricle (LV) (16, 19, 20), but the impact of microgravity on cardiac morphology in women remains poorly understood.

Following head-down-tilt (HDT) bed rest, there is a cephalic and intrathoracic redistribution of intravascular volume and a transient increase in SV secondary to the loss of hydrostatic and gravitational gradients. A diuresis ensues, resulting in a new hemodynamic steady state that is halfway between the upright and supine positions (34) and a prominent drop in LV filling (34, 45, 47). With the restoration of gravitational gradients, orthostatic hypotension occurs, but the reduction in SV after gravitational unloading cannot be attributed entirely to hypovolemia (11, 21, 35, 47). In fact, cardiac atrophy occurs in men following as little as 14 days of bed rest, and this adaptive response likely contributes to the orthostatic intolerance associated with microgravity exposure by reducing cardiac distensibility and thereby SV at any given filling pressure (34, 45, 47). Previous reports show a reduction of LV mass in men of 4.7, 8.0, and 15.6% after 2, 6, and 12 wk respectively (14, 45).

Endurance exercise training clearly causes eccentric LV hypertrophy and improved cardiac compliance (33) in previous male bed rest models (4). Furthermore, male athletes have more physiological hypertrophy than female athletes (38, 44, 57). Whether sex similarly affects the magnitude of myocardial atrophy following cardiovascular unloading, however, is un-

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clear. The purpose of this study was to use magnetic resonance imaging (MRI) to measure precise changes in female cardiac morphology following bed rest deconditioning. We hypothesized that HDT bed rest induces cardiac atrophy in women and that exercise training in women prevents the adaptive cardiac remodeling associated with gravitational unloading.

METHODS

Subjects

Twenty-four healthy female subjects aged 32.1 ± 4 yr (range 25–40 yr) participated in this study, which was part of an international collaboration among the French, European, Canadian, and American space agencies. The subjects represented a wide range of nationalities including Czech, Dutch, French, English, Finish, Polish, Scottish, German, and Swiss. All subjects had to be physically active with at least “average” aerobic fitness (mean \pm SD maximal oxygen uptake 39 ± 4 ml \cdot kg $^{-1}\cdot$ min $^{-1}$), but competitive athletes were excluded. Subjects were 59 ± 4 kg and 1.66 ± 0.07 m. The study was performed in accordance with the ethical principles stated in the Declaration of Helsinki 1964 and all its applicable amendments, and each subject signed an informed consent form meeting the requirements of the Good Clinical Practice recommendations of the International Conference on Harmonisation, and approved by the Institutional Review Boards of NASA Johnson Space Center and University of California, San Diego.

Study Design (Bed Rest)

All twenty-four subjects completed 60 days of strict 6° HDT bed rest. The eight subjects randomized to exercise walked/ran for 40 min on a vertical treadmill in a lower body negative pressure (LBNP) chamber (Fig. 1) at 1–1.15 body weights (50–60 mmHg LBNP) followed by 10 min of resting LBNP three to four times per week depending on their graded oxygen uptake; exercise intensity varied from 40 to 80% pre-bed rest maximal oxygen uptake in an “interval” fashion over the course of each session as previously described (8, 12, 24, 56). This mode of exercise was chosen because supine treadmill

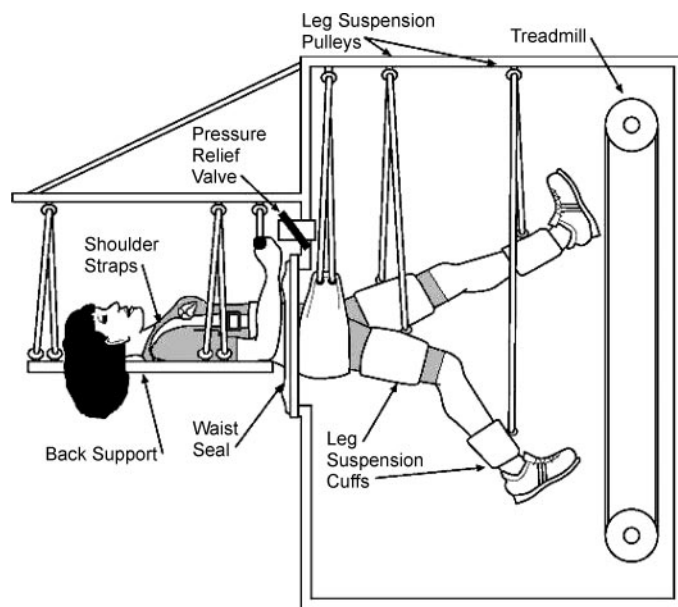


Fig. 1. Schematic depicting supine treadmill running in a lower body negative pressure (LBNP) chamber, and the inertial forces on the heart during supine treadmill running in a LBNP chamber are similar to the forces during upright endurance training on Earth.

exercise within LBNP provides weight bearing, muscle loads, and cardiovascular responses equivalent to upright treadmill exercise on Earth and simulates metabolic and kinetic features of upright exercise (8, 38). Furthermore, this exercise paradigm has maintained peak oxygen consumption in men exposed to 5 and 15 days of HDT bed rest (32, 64).

Resistive training of the knee extensor and plantar flexor muscle groups was conducted on a flywheel ergometer modified for bed rest every third day, and each training session included four sets of seven repetitions with each leg performed with maximal effort using both concentric and eccentric contractions with a 2-min resting period between each set (1, 52). Protein content in the diet was 1 g \cdot kg $^{-1}\cdot$ day $^{-1}$ in the control and exercise groups. In the nutrition group, dietary protein content was increased to 1.45 g/kg. Free branched-chain amino acids (BCAA) (3.6 g/day free leucine, 1.8 g/day free isoleucine, and 1.8 g/day free valine) were added as a supplement (Friliver, Bracco, Italy), which was given at the main meals. With the exception of the exercise session, all subjects were restricted to the HDT position at all times except for during meals when they were allowed to elevate on one elbow.

Subjects were housed and supervised in the Institut de Médecine et de Physiologie Spatiales (MEDES) Space Clinic located within the Toulouse Rangueil Hospital. Each subject was given a diet of 110% of their resting metabolic rate, which was determined before bed rest and adjusted every 15 days by indirect calorimetry and impedance measures of body composition. The maximum liquid intake of the sedentary and protein supplementation group was 60 ml \cdot kg $^{-1}\cdot$ day $^{-1}$, and the maximum liquid intake for the exercise group was 60 ml \cdot kg $^{-1}\cdot$ day $^{-1}$ on the days without training and 75 ml \cdot kg $^{-1}\cdot$ day $^{-1}$ on days with exercise training. Intake and urine output were recorded, and caloric intake was increased as needed for the exercise subjects with the goal of preventing changes in body composition. For all subjects, sodium intake was 1.2 to 1.6 mmol \cdot kg $^{-1}\cdot$ day $^{-1}$, potassium intake was 0.9 to 1.1 mmol \cdot kg $^{-1}\cdot$ day $^{-1}$, calcium intake was 1 g/day, and phosphorus intake was 1.2 to 1.6 mmol \cdot kg $^{-1}\cdot$ day $^{-1}$.

MRI Imaging

Parameters protocol. MRI was performed on all subjects immediately before and just before the end of bed rest at the Rangueil Hospital in Toulouse, France, and all images were acquired by a 1.5-T Phillips MRI scanner. After completing the standard imaging protocol for the assessment of mass and volume (23, 28, 30, 48), gradient echo, cine long- and short-axis MRI sequences with a temporal resolution of \sim 42 ms, a repetition time of \sim 4 ms, an echo time of \sim 2 ms, and a flip angle of 55° were obtained. The heart was sectioned in 6 mm slices with a gap of 4 mm spanning from the apex to the base, and the image resolution was 256 \times 256 with a 330-mm field of view.

Image analysis. One observer who was blinded to the group assignments read the cardiac MRI studies. Short axis slices were used for volume, mass, and mean wall thickness (MWT) calculations using the MRI-MR Analytical Software System (MEDIS, Leiden, The Netherlands) (41). LV end-diastolic volumes were determined by manually identifying the endocardial border at both end diastole and end systole. End diastole was the first frame in every sequence, and end systole was defined as the frame with the smallest endocardial (chamber) area. LV volumes were then calculated by summation using Simpson's rule (28, 48). The difference between endocardial and epicardial volumes were computed and multiplied by 1.05 g/ml, which is the density of cardiac muscle, to calculate the LV mass (30). This technique has been shown to be extremely accurate and reproducible in our laboratory (30, 34, 45) with an intraobserver variability for LV mass and right ventricular (RV) mass of $r = 0.98$ with a SE of estimate of 3.8 g and $r = 0.97$ with a SE of estimate of 2.1 g, respectively (45).

The RV end-diastolic volume was determined by manually identifying the endocardial border at end diastole, which was the first frame

in every sequence, and then it was computed by summation as described above for the calculation of LV end-diastolic volume (45). RV mass was calculated by manually outlining the endocardial and epicardial borders of the RV free wall in each slice at end diastole, and the sum of the differences between endocardial and epicardial areas was multiplied by the density of myocardial muscle, which is 1.05 g/ml (9, 43, 45). The moderator band and the interventricular septum were not included in the measurement of RV free wall mass (9, 43).

The MWT of the LV including papillary muscles was calculated by the MRI-MASS software system, which measures the distance between manually determined endocardial and epicardial borders of each segment at end diastole using the modified centerline method (26, 27). Calculations were performed from the base of the heart to the apex, and the slices at the valvular level as well as the lowest apical slice without blood volume at end systole were excluded (26). Using long-axis four-chamber slices, the major axis was measured from the mitral valve to the apex (50), and the minor axis was determined by measuring the widest distance from the interventricular septum to the lateral wall of the LV perpendicular to the major axis.

Statistical Analysis

The numerical data are presented as means \pm SD. The differences between before bed rest (pre) and end of bed rest (post) LV and RV end-diastolic volumes, masses, MWT, and linear dimensions for the sedentary, exercise, and protein supplementation groups were compared with a two-way, mixed-design ANOVA with pre-post bed rest as the paired comparison and sedentary vs. exercise vs. protein supplementation as the unpaired comparison. Significant *F* values were followed by Newman-Keuls post hoc test or Bonferroni correct *t*-tests for multiple comparisons. Because of the relatively small sample size, all individual *P* values are reported as per recommendation of the American Physiological Society (45, 47). LV and RV

masses (g) were adjusted for body size by dividing myocardial mass by body mass (kg) at the start of the study.

RESULTS

All subjects lost an equivalent amount of body mass (3.4 ± 0.2 kg control; 3.1 ± 0.04 kg exercise; 2.8 ± 0.1 kg protein).

LV End-Diastolic volume, LV Mass, and MWT

After sedentary prolonged bed rest without protein supplementation, there was a significant reduction in LV volume (96 ± 26 to 77 ± 25 ml; $P = 0.03$), LV mass (126 ± 14 to 113 ± 18 g; $P = 0.003$), and adjusted LV mass (2.2 ± 0.2 to 2.0 ± 0.2 g/kg; $P = 0.003$) (Fig. 2A), similar to what has been observed previously in men (Fig. 3). In contrast, when exercise was performed while the subjects were confined to bed rest, there was not a significant reduction in LV volume, and there was a significant increase in LV mass (115 ± 31 to 136 ± 27 g; $P < 0.001$), adjusted LV mass (1.9 ± 0.4 to 2.3 ± 0.3 g/kg; $P < 0.001$) (Fig. 2B), and MWT (9 ± 2 to 11 ± 1 mm; $P = 0.01$). After receiving protein supplementation during bed rest, LV mass and adjusted LV mass were preserved (Fig. 2C), but a significant reduction in LV volume (103 ± 14 to 80 ± 16 ml; $P = 0.02$) remained. The interaction among groups was highly significant for LV mass ($P < 0.0001$) although it did not achieve conventional significance for LV volume.

RV End-Diastolic Volume and RV Mass

At the end of HDT bed rest without protein supplementation, there was a significant reduction in RV volume (104 ± 33 to

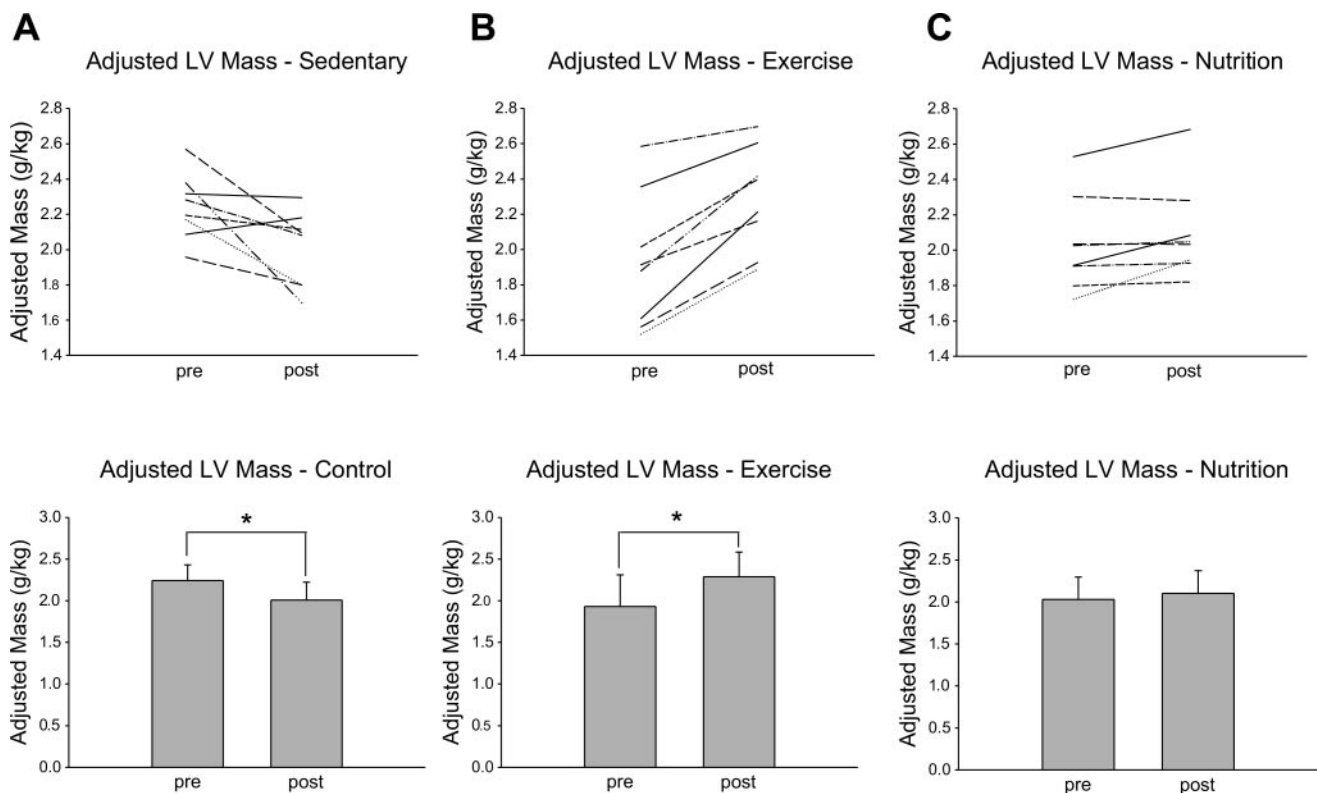


Fig. 2. A: adjusted left ventricular (LV) mass at baseline (pre) and after sedentary prolonged bed rest (post). B: adjusted LV mass at baseline and following exercise during bed rest. C: adjusted LV mass and protein supplementation during bed rest. * $P < 0.05$.

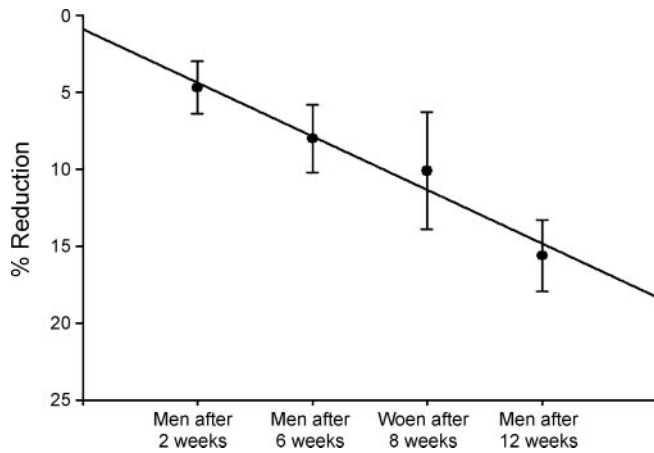


Fig. 3. LV mass measured by magnetic resonance imaging after 2, 6, 8, and 12 wk of sedentary head-down-tilt bed rest. Values are means \pm SE, and linear regressions are calculated from mean values of percent reduction in LV mass.

86 \pm 25 ml; $P = 0.02$), RV mass (44 \pm 8 to 34 \pm 6 g; $P < 0.001$), and adjusted RV mass (0.8 \pm 0.1 to 0.6 \pm 0.1 g/kg; $P < 0.001$) (Fig. 4A). In contrast, when exercise was performed during bed rest, there was not a significant reduction in RV volume, and there was a significant increase in RV mass (40 \pm 9 g to 47 \pm 10 g; $P = 0.005$; interaction $P < 0.0001$) as well as adjusted RV mass (0.7 \pm 0.1 to 0.8 \pm 0.2 g/kg; $P = 0.002$) (Fig. 4B). Furthermore, RV volume, RV mass, and adjusted RV mass (Fig. 4C) were preserved following protein supplementation during sedentary bed rest.

Changes in Cardiac Morphology

There was a significant reduction in the length of the major axis of the LV following sedentary bed rest with (91 \pm 5 to 88 \pm 7 mm, $P = 0.003$; Fig. 5C) or without protein supplementation (90 \pm 6 to 84 \pm 7 mm; $P < 0.001$; Fig. 5A). In contrast, the major axis of the LV was preserved after exercise while the subjects were confined to bed (Fig. 5B; interaction $P = 0.006$). The length of the minor axis was not statistically different following sedentary bed rest with or without exercise or protein supplementation. The LV mass-to-volume ratio did not change significantly at the end of strict bed rest without protein supplementation (1.4 \pm 0.2 to 1.6 \pm 0.5 g/ml; $P = 0.17$) consistent with an eccentric atrophy, but there were significant increases in the mass-to-volume ratio at the end of bed rest with protein supplementation (1.3 \pm 0.3 to 1.7 \pm 0.4 g/ml; $P = 0.02$) and exercise training (1.3 \pm 0.4 to 1.7 \pm 0.4 g/ml; $P = 0.009$).

DISCUSSION

The principal new findings of this study include the following: 1) sedentary HDT bed rest for 60 days results in cardiac atrophy in women to a degree that is similar to what has been observed previously in men (8.0%; Ref. 45); 2) exercise training and to a lesser extent protein supplementation prevent the adaptive cardiac atrophy associated with bed rest and preserve or even increase myocardial mass. As a result, exercise and to a lesser degree amino acid supplementation are effective countermeasures against the physiological cardiac remodeling following cardiovascular unloading.

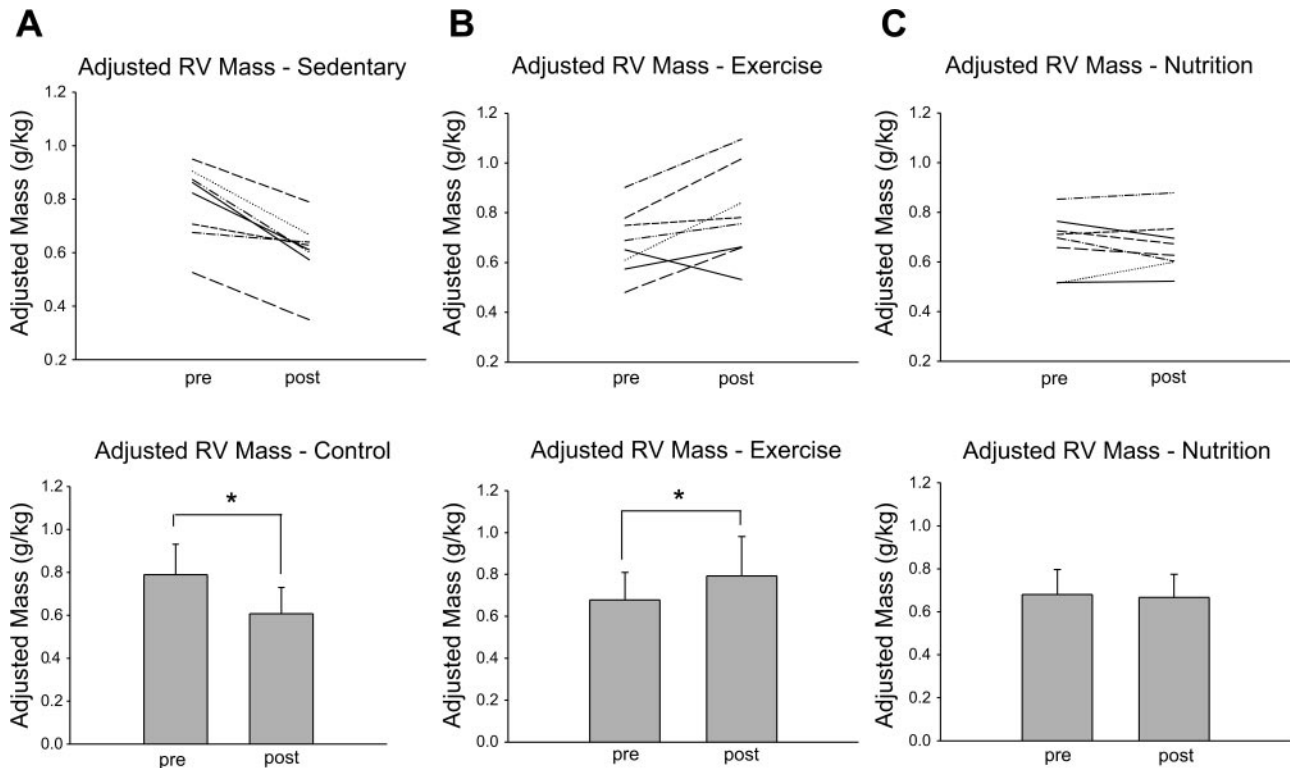


Fig. 4. A: adjusted right ventricular (RV) mass at baseline and after sedentary prolonged bed rest. B: adjusted RV mass at baseline and following exercise during bed rest. C: adjusted RV mass and protein supplementation during bed rest. * $P < 0.05$.

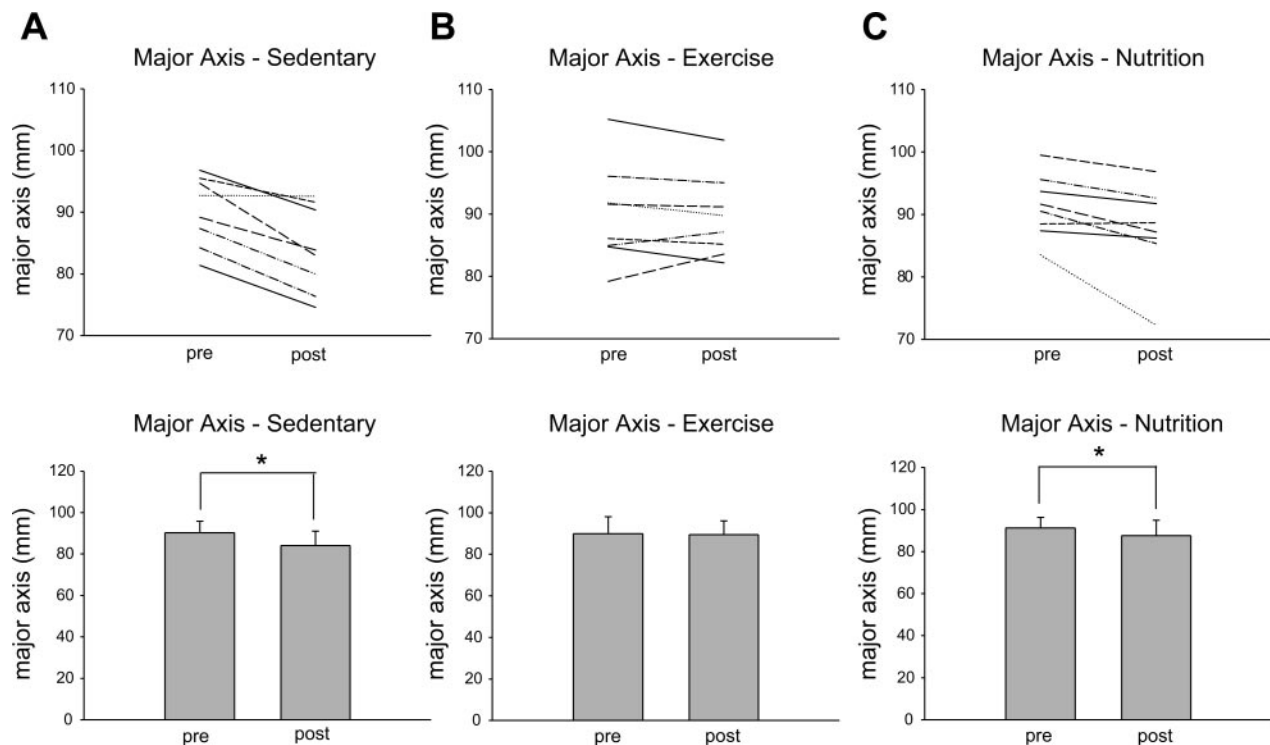


Fig. 5. A: length of the major axis of the LV at baseline and after sedentary prolonged bed rest. B: length of the major axis of the LV baseline and following exercise during bed rest. C: length of the major axis of the LV and protein supplementation during bed rest. * $P < 0.05$.

Sex and Orthostatic Intolerance

Women appear to be more susceptible than men to presyncope and symptomatic orthostatic hypotension following microgravity exposure (16, 18–20, 25, 65), but the mechanism remains unclear. Although some argue that there are sex differences in sympathetic responses following cardiovascular unloading, the data are controversial and inconclusive (16, 17, 19, 20, 65), and the impact of cardiac atrophy in this setting is poorly defined.

Sex-specific cardiovascular responses to gravitational stress have been described (17, 25). For example, some reports have suggested that women have a blunted increase in vascular resistance and a greater augmentation in heart rate during LBNP compared with men, which has been attributed to the vasodilatory effects of estrogen (17, 27, 65). Furthermore, sympathetic nerve activity is decreased in postmenopausal women following hormone replacement therapy (65). In contrast, Fu and coworkers (20) recently showed that men and women have similar increases in both total peripheral resistance and heart rate during head up tilt (20), and similar increases in total peripheral resistance during maximal LBNP to presyncope (19). Heart rate was elevated in this study because of a greater and more precipitous decline in SV and/or stroke index in women consistent with a primary difference in diastolic function. Furthermore, Fu and coworkers demonstrated that sex does not affect mean sympathetic nerve activity (bursts/min), plasma norepinephrine levels, systemic vascular resistance, or diastolic blood pressure following graded head-up tilt under both normovolemic or hypovolemic conditions. This study also showed that relative to men, women have significantly lower systolic blood pressures under both condi-

tions, suggesting that women have smaller SV and less cardiac filling during gravitational stress (19, 20, 65).

Although the majority of high-resolution studies on cardiac morphology after gravitational unloading have been performed on men, cardiovascular structural differences between sexes without unloading have been described (41, 50, 51). For example, LV end-diastolic volume, RV end-diastolic volume, LV mass, RV mass, and LV cavity length are all greater for men than women despite adjustments for body size (41, 50, 51). Moreover, women have a smaller SV secondary to a smaller and stiffer LV than men during orthostatic stress (19, 20), and female athletes have less physiological hypertrophy than male athletes (38, 44, 57). Given the sex-specific morphological differences, high-resolution studies on female cardiac structure after microgravity exposure are necessary to understand the mechanisms behind the increased incidence of orthostatic intolerance in women after spaceflight.

Cardiac Remodeling Following Bed Rest in Men

Cardiac atrophy occurs after as short as 10 days of spaceflight in men (45). Although small animal models have been controversial (49), there is a prominent reduction in LV mass after thoracic inferior vena caval constriction in canines (37) consistent with the concept that chronic unloading of the heart of large mammals leads to cardiac atrophy. A recent study in our laboratory demonstrated that there was a 5% reduction in LV mass following 2 wk of bed rest deconditioning in men (14) and that LV mass decreased by 8% after 6 wk with an additional reduction of 8% after 12 wk (45, 46). Similar to what has been observed previously in men, this study found a 10% reduction in LV mass following 8 wk of bed rest in

women (Fig. 3). Moreover, after 6 wk of HDT bed rest in men (45, 46), LV end-diastolic volume decreased by $\sim 14\%$ ($P = 0.002$), MWT decreased by $\sim 4\%$ ($P = 0.01$), RV end-diastolic volume decreased by $\sim 16\%$ ($P = 0.06$), and RV mass decreased by $\sim 10\%$ ($P = 0.06$), all qualitatively and quantitatively similar to the reductions observed in this study in healthy premenopausal women.

Implications for Exercise Training During Bed Rest

To prevent the physiological cardiac remodeling following microgravity exposure, effective countermeasures such as exercise must be implemented during spaceflight. Endurance exercise prevents the cardiac adaptations associated with gravitational unloading while preserving myocardial mass (14) and diastolic filling (34, 35, 39, 53). Not only does exercise prevent the reduction in LV compliance associated with aging (4, 34), LV end-diastolic volume, LV mass, and LV distensibility are all greater in Masters endurance athletes compared with age-matched sedentary seniors (4). Exercise training augments early filling rates, lowers peak atrial filling velocities (34, 53), and increases diastolic untwisting rates (14, 39). Moreover, preliminary reports using supine cycle ergometry alone (90 min/day, twice as long as the LBNP treadmill exercise in the present study) showed that while there was a significant reduction in LV mass (5%) after 18 days of sedentary -6° HDT bed rest, exercise training during bed actually resulted in a 4% increase in LV mass (14). Furthermore, endurance training prevents a decline in diastolic suction associated with HDT bed rest (14). The principal new findings of this study extend the previous research on the protective role for exercise during bed rest and demonstrate that despite a significant reduction in LV mass (10%) and RV mass (23%) following 60 days of HDT bed rest in women, there was a significant increase in LV mass (18%) and RV mass (18%) following exercise training during bed rest. Although the mechanisms behind the beneficial effects of exercise during gravitational unloading remain unclear, endurance exercise, with or without strength training certainly prevents cardiac atrophy, preserves myocardial viscoelastic properties, increases LV mass, optimizes LV chamber geometry (eccentric ventricular hypertrophy), and improves LV compliance (4, 35, 45).

Although the exercise protocol in this study consisted of dynamic treadmill running within an LBNP chamber and short episodes of resistive training on a flywheel ergometer, it is likely that any mode or combination of modes of exercise that increases cardiac work for an adequate duration and intensity by augmenting heart rate, preload, afterload, and/or contractility may cause physiological hypertrophy and prevent myocardial atrophy following bed rest or spaceflight. In fact, an earlier study that evaluated 947 elite athletes demonstrated that 27 different sports resulted in larger LV diastolic dimensions (57). However, cycling, rowing, and swimming are associated with larger LV dimensions and MWT than a purely static sport such as weightlifting, consistent with the eccentric physiological hypertrophy of endurance athletes (57).

Implications for Protein Supplementation During Bed Rest

This study was only a small part of a very large collaboration between the French, European, Canadian, and American space agencies, and the goal of the International Space Life Sciences

Working Group was to design a protocol that would ensure the safety and health of female astronauts both during and after spaceflight. As a result, protein supplementation was implemented as an additional and separate countermeasure to the peripheral muscle wasting (5, 7, 58, 59, 60) associated with spaceflight. However, interestingly, LV and RV mass were preserved in this study following a 0.45 g/kg daily increase in dietary protein, which was supplemented with 7.2 g/kg of BCAA. Although this finding was quite surprising, there is a plethora of data supporting the notion that protein intake and BCAA supplementation stimulate protein synthesis, suppress protein breakdown, and prevent nitrogen loss in skeletal muscle and myocardium (2, 3, 5, 15, 59, 61, 66). Despite an intravenous infusion of amino acids, a recent study showed that there is less synthesis of lean-body protein after 14 days of bed rest compared with ambulatory controls (5, 6), but a significant increase in protein intake might achieve the same postabsorptive anabolic effect during best rest as seen during ambulatory periods (7). Moreover protein stimulation is augmented by BCAA by enhancing the production and function of proteins involved in mRNA translation (2, 3), and a recent study demonstrated that a 30 mmol/day increase of BCAA attenuated nitrogen loss following 6 days of bed rest compared with the group receiving nonessential amino acids (59), providing further evidence that there maybe a beneficial effect of branched-chain amino acid supplementation during bed rest. It should be noted that virtually all of these studies have examined protein balance in skeletal muscle. To our knowledge, detailed studies of protein balance during unloading have not been performed in cardiac muscle.

Myocardial Atrophy vs. Interstitial Fluid Volume

Numerous male bed rest models have established that cardiac remodeling occurs following microgravity exposure (11, 21, 34, 45, 47), but controversy still exists. Critics maintain that the apparent reduction in myocardial mass following microgravity exposure is a function of a reduction in myocardial interstitial fluid volume rather than a true cardiac atrophy (62). These authors' argument was based on the assertion that the reduction in LV mass (9.1%) measured by echocardiography after spaceflight was normalized after a 3-day recovery period, and it was also based on the observation that there was a small reduction in LV mass (3.7%) after dialysis as assessed by MRI (29, 62). Several lines of evidence suggest that this hypothesis is not correct.

First, after HDT bed rest, there is a greater leftward shift in the pressure-volume relationship than after a similar degree of hypovolemia due to intravenous furosemide (47), and volume replacement alone does not prevent the orthostatic intolerance observed following cardiovascular unloading (11, 21, 34, 47). In fact, after HDT bed rest, there is a reduction in the equilibrium volume and a significant decline in diastolic untwisting rates, suggesting a reduction in diastolic suction from true cardiac remodeling rather than exclusively hypovolemia (14, 31).

Moreover, in previous spaceflight studies, ventricular mass was measured using a mixed combination of two-dimensional echocardiography and two-dimensional guided M-mode echocardiography based on the Teicholz formula, which calculates mass from a single dimension raised to the third power.

However, such echocardiographic measurements of myocardial mass are highly variable and imprecise (41, 50, 51), and thus echocardiography is not likely to be sensitive enough to detect small changes in cardiovascular structure (41, 50, 51). Although echocardiography is commonly used in clinical practice, its ability to measure cardiac mass is poorly reproducible and inaccurate (41, 50, 51). Two-dimensional echocardiography requires good acoustic windows for imaging and accurate delineation of endocardial borders, which is quite difficult given its limited resolution (41). Moreover, foreshortening is quite common with echo measurements (41), and echocardiography measurements of myocardial mass are dependent on ventricular volume. In fact, mass and volume calculations obtained from echocardiography are frequently discrepant from those values that were measured by cardiac MRI (40, 41, 51).

In comparison, MRI is very accurate and precise, and it is the gold standard for the assessment of LV mass and volume (45). Cardiac MRI does not rely on geometric assumptions, and it offers very high temporal and spatial resolution independent of patient size and operator skill (41, 50, 51). A recent study addressing the ability of MRI to assess LV and RV volume as well as LV and RV mass reported that intraobserver and interobserver variability ranged from 1.4 to 5.9% (51). In the previously mentioned dialysis study, LV mass only decreased by 3.7% despite an enormous 2- to 3-liter loss of volume after dialysis (29). In contrast, after losing only 200 ml of volume during spaceflight, the measured reduction in LV mass was three times greater than the reduction in LV mass after dialysis (29, 62). As a result, the authors of this study concluded that MRI measures LV mass both reliably and independently of LV loading (29). Hence, even extreme dehydration minimally impacts LV mass when assessed by appropriate techniques, although standard echocardiographic techniques may indeed be too sensitive to changes in LV volume to be reliably used to measure LV mass under these circumstances.

Furthermore, in this study LV volumes decreased similarly after bed rest with (22%) and without protein supplementation (20%), but these groups of subjects had drastically different changes in LV mass. Whereas LV mass decreased by 10% after sedentary bed rest, LV mass was preserved after subjects received protein supplementation during bed rest despite an equivalent loss of volume, providing further evidence that the apparent reduction in cardiac mass following microgravity exposure results from cardiac remodeling rather than hypovolemia.

Limitations

Perhaps, another limitation of our study is the fact that the subjects underwent only pre- and post-bed rest cardiac MRI studies, instead of a protocol with interval measurements of myocardial volumes and mass. Thus we cannot comment on the exact time course of the physiological atrophy associated with HDT bed rest in women. However, using a linear regression of LV mass after 2, 6, and 12 wk of bed rest in men, women appear to have the same degree of cardiac atrophy (Fig. 3).

Although this study did not directly address whether or not exercise and protein supplementation would decrease the incidence of orthostatic intolerance test associated with HDT bed

rest, it did clearly show that exercise and to a lesser extent protein supplementation prevent the cardiac atrophy observed during HDT bed rest.

Furthermore, sympathoactivation is an important trophic signal in the heart, and it is possible that LBNP-induced sympathetic activation may contribute to limiting cardiac atrophy independent of exercise. However, it is likely that exercise during LBNP would minimize the sympathetic activation produced by LBNP alone by virtue of the muscle pump.

Finally, the exercise intervention used in this study incorporated a combination of dynamic and static exercise rather than either type of exercise independently. This type of training is typical of competitive athletes but precludes us from attributing the prevention of cardiac atrophy to either the endurance or the strength component of the intervention by itself. Additional studies with a more direct comparison are necessary to answer this question explicitly.

In conclusion, using the gold standard for the measurement of cardiac mass, this study found that women have the same relative degree of cardiac atrophy as do men following 60 days of sedentary long-term HDT bed rest, and this myocardial atrophy likely contributes to the reduction in cardiac filling and the prominent drop in upright SV observed after microgravity exposure. However, a combined program of dynamic and static exercise training during bed rest prevents this adaptive cardiac remodeling and increases myocardial mass. Although dietary protein with BCAA supplementation is less effective than exercise training, this study suggested that an increased intake of protein enriched with BCAA preserved cardiac mass and suppressed the physiological cardiac atrophy associated with cardiovascular unloading. We speculate that an exercise regimen coupled with protein and BCAA supplementation might offer the best protection from the myocardial wasting associated with microgravity exposure.

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REFERENCES

1. Alkner BA, Tesch PA. Knee extensor and plantar flexor muscle size and function following 90 days of bed rest with or without resistance exercise. *Eur J Appl Physiol* 93: 294–305, 2004.

2. Anthony JC, Anthony TG, Kimball SR, Jefferson LS. Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. *J Nutr* 131: 856S–860S, 2001.
3. Anthony JC, Anthony TG, Kimball SR, Vary TC, Jefferson LS. Orally administered leucine stimulates protein synthesis in skeletal muscle of post-absorptive rats in association with increased eIF4f formation. *J Nutr* 130: 139–145, 2000.
4. Arbab-Zadeh A, Dijk E, Prasad A, Fu Q, Torres P, Zhang R, Thomas JD, Palmer D, Levine BD. Effect of aging and physical activity on left ventricular compliance. *Circulation* 110: 1799–1805, 2004.
5. Biolo G, Ciochi B, Lebenstedt M, Barazzoni R, Zanetti M, Platen P, Heer M, Guarnieri G. Short term bed rest impairs amino acid-induced protein anabolism in humans. *J Physiol* 558: 381–388, 2004.
6. Biolo G, Ciochi B, Lebenstedt M, Heer M, Guarnieri G. Sensitivity of whole body protein synthesis to amino acid administration during short-term bed rest. *J Gravit Physiol* 9: 197–198, 2002.
7. Biolo G, Ciochi B, Stulle M, Piccoli A, Lorenzon S, Dal Mas V, Barazzoni R, Zanetti M, Guarnieri G. Metabolic consequences of physical inactivity. *J Ren Nutr* 15: 49–53, 2005.
8. Boda WL, Watenpaugh DE, Ballard RE, Hargens AR. Supine lower body negative pressure exercise simulates metabolic and kinetic features of upright exercise. *J Appl Physiol* 89: 649–654, 2000.
9. Boxt LM, Katz J, Kolb T, Czegledy FP, Barst RJ. Direct quantification of right and left ventricular volumes with nuclear magnetic resonance imaging in patients with primary pulmonary hypertension. *J Am Coll Cardiol* 19: 1508–1515, 1992.
10. Buller VG, Van der Geest RJ, Kool MD, van der Wall EE, de Roos A, Reiber JH. Assessment of regional left ventricular wall parameters from short axis magnetic resonance imaging using a three-dimensional extension to the improved centerline method. *Invest Radiol* 32: 529–539, 1997.
11. Bungo MW, Charles JB, Johnson PC. Cardiovascular deconditioning during space flight and the use of saline as a countermeasure to orthostatic intolerance. *Aviat Space Environ Med* 56: 490–494, 1987.
12. Cao P, Kimura S, Macias BR, Ueno T, Watenpaugh DE, Hargens AR. Exercise within lower body negative pressure partially counteracts lumbar spine deconditioning associated with 28-day bed rest. *J Appl Physiol* 99: 39–44, 2005.
13. Cooper GIV, Kent RL, Mann DL. Load induction of cardiac hypertrophy. *J Mol Cell Cardiol* 21, Suppl 5: 11–30, 1989.
14. Dorfman TA, Rosen BD, Perhonen MA, McColl R, Peshock RM, Levine BD. Diastolic untwisting is impaired following bed rest (Abstract). *Med Sci Sports Exerc* 38: S325, 2006.
15. Escobar J, Frank JW, Suryawan A, Nguyen HV, Kimball SR, Jefferson LS, Davis TA. Regulation of cardiac and skeletal muscle protein synthesis by individual branched-chain amino acids in neonatal pigs. *Am J Physiol Endocrinol Metab* 290: E612–E621, 2006.
16. Foley CM, Mueller PJ, Hasser EM, Heesch CM. Hindlimb unloading and female gender attenuate baroreflex-mediated sympathoexcitation. *Am J Physiol Regul Integr Comp Physiol* 289: R1440–R1447, 2005.
17. Frey MA, Hoffer GW. Association of sex and age with responses to lower-body negative pressure. *J Appl Physiol* 65: 1752–1756, 1988.
18. Fritsch-Yelle JM, Charles JB, Jones MM, Wood ML. Microgravity decreases heart rate and arterial pressure in humans. *J Appl Physiol* 80: 910–914, 1996.
19. Fu Q, Arbab-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.
20. Fu Q, Witkowski S, Okazaki K, Levine BD. Effects of gender and hypovolemia on sympathetic neural responses to orthostatic stress. *Am J Physiol Regul Integr Comp Physiol* 289: R109–R116, 2005.
21. Gaffney FA, Buckley JC, Lane LD, Hillebrecht A, Schulz H, Meyer M, Baisch F, Beck L, Heer M, Maass H. The effects of a 10-day period of head-down tilt on the cardiovascular responses to intravenous saline loading. *Acta Physiol Scand Suppl* 604: 121–130, 1992.
22. Gaffney FA, Nixon JV, Karlsson ES, Campbell W, Dowdey AB, Blomqvist CG. Cardiovascular deconditioning produced by 20 hours of bed rest with head-down tilt (–5 degrees) in middle-aged healthy men. *Am J Cardiol* 56: 634–638, 1985.
23. Germain P, Roul G, Kastler B, Mossard JM, Bareiss P, Sacrez A. Inter-study variability in left ventricular mass measurement. Comparison between M-mode echocardiography and MRI. *Eur Heart J* 13: 1011–1019, 1992.
24. Hargens AR, Whalen RT, Watenpaugh DE, Schwandt DF, Krock LP. Lower body negative pressure to provide load bearing in space. *Aviat Space Environ Med* 62: 934–937, 1991.
25. Harm DL, Jennings RT, Meck JV, Powell MR, Putcha L, Sams CP, Schneider SM, Shackelford LC, Smith SM, Whitson PA. Invited Review: gender issues related to spaceflight: a NASA perspective. *J Appl Physiol* 91: 2374–2383, 2001.
26. Hees PS, Fleg JL, Lakatta EG, Shapiro EP. Left ventricular remodeling with age in normal men versus women. *Am J Cardiol* 90: 1231–1236, 2002.
27. Holman ER, Buller VG, De Roos A, van der Geest RJ, Baur LH, van der Laarse A, Bruschke AV, Reiber JH, van der Wall EE. Detection and quantification of dysfunctional myocardium by magnetic resonance imaging. A new three-dimensional method for quantitative wall-thickening analysis. *Circulation* 95: 924–931, 1997.
28. Hundley WG, Li HF, Willard JE, Landau C, Lange RA, Meshack BM, Hillis LD, Peshock RM. Magnetic resonance imaging assessment of the severity of mitral regurgitation. *Circulation* 92: 1151–1158, 1995.
29. 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.
30. Katz J, Milliken MC, Stray-Gundersen J, Buja LM, Parkey RW, Mitchell JH, Peshock RM. Estimation of human myocardial mass with MR imaging. *Radiology* 169: 495–498, 1988.
31. Kitzman DW, Scholz DG, Hagen PT, Ilstrup DM, Edwards WD. Age-related changes in normal human hearts during the first 10 decades of life. Part II (Maturity): a quantitative anatomic study of 765 specimens from subjects 20 to 99 years old. *Mayo Clin Proc* 63: 137–146, 1988.
32. Lee SMC, Bennett BS, Hargens AR, Watenpaugh DE, Ballard RE, Murthy G, Ford SR, Fortney SM. Upright exercise or supine lower body negative pressure exercise maintains exercise responses after bed rest. *Med Sci Sports Exerc* 29: 892–900, 1997.
33. Levine BD, Lane LD, Buckley JC, Friedman DB, Blomqvist CG. Left ventricular pressure-volume and Frank-Starling relations in endurance athletes. Implications for orthostatic tolerance and exercise performance. *Circulation* 84: 1016–1023, 1991.
34. Levine BD, Zuckerman JH, Pawelczyk JA. Cardiac atrophy after bed-rest deconditioning: a nonneural mechanism for orthostatic intolerance. *Circulation* 96: 517–525, 1997.
35. Levy WC, Cerqueira MD, Abrass IB, Schwartz RS, Stratton JR. Endurance exercise training augments diastolic filling at rest and during exercise in healthy young and older men. *Circulation* 88: 116–126, 1993.
36. MacKenna DA, Omens JH, McCulloch AD, Covell JW. Contribution of collagen matrix to passive left ventricular mechanics in isolated rat hearts. *Am J Physiol Heart Circ Physiol* 266: H1007–H1018, 1994.
37. McGowan BS, Scott CB, Mu A, McCormick RJ, Thomas DP, Margulie KB. Unloading-induced remodeling in the normal and hypertrophic left ventricle. *Am J Physiol Heart Circ Physiol* 284: H2061–H2068, 2003.
38. Murthy G, Watenpaugh DE, Ballard RE, Hargens AR. Supine exercise during lower body negative pressure effectively simulates upright exercise in normal gravity. *J Appl Physiol* 76: 2742–2748, 1994.
39. Myers J, Wagner D, Schertler T, Beer M, Luchinger R, Klein M, Rickli H, Muller P, Mayer K, Schwitzer J, Dubach P. Effects of exercise training on left ventricular volumes and function in patients with nonischemic cardiomyopathy; application of magnetic resonance myocardial tagging. *Am Heart J* 144: 719–725, 2002.
40. Myerson SG, Bellenger NG, Pennell DJ. Assessment of left ventricular mass by cardiovascular magnetic resonance. *Hypertension* 39: 750–755, 2002.
41. Nikitin NP, Loh PH, Silva R, Witte KK, Lukaschuk EI, Parker A, Farnsworth TA, Alamgir FM, Clark AL, Cleland JG. Left ventricular morphology, global and longitudinal function in normal older individuals: a cardiac magnetic resonance study. *Int J Cardiol* 108: 76–83, 2006.
42. Olivetti G, Giordano G, Corradi D, Melissari M, Lagrasta C, Gambert SR, Anversa P. Gender differences and aging: effects on the human heart. *J Am Coll Cardiol* 26: 1068–1079, 1995.
43. Pattynama PMT, Lamb HJ, Van der Velde EA, Van der Geest RJ, Van der Wall EE, De Roos A. Reproducibility of MRI-derived measurements of right ventricular volumes and myocardial mass. *Magn Reson Imaging* 13: 53–64, 1995.
44. Pelliccia A, Maron BJ, Culasso F, Spataro A, Caselli G. Athlete's heart in women. Echocardiographic characterization of highly trained elite female athletes. *JAMA* 276: 211–215, 1996.

45. **Perhonen MA, Franco F, Lane LD, Buckey JC, Blomqvist Zerwekh JE, Peshock RM, Weatherall PT, Levine BD.** Cardiac atrophy after bed rest and space flight. *J Appl Physiol* 91: 645–653, 2001.
46. **Perhonen MA, Zuckerman JH, Hinojosa JR, Zhang R, Iwasaki K, Levine BD.** Exercise training reverses cardiac atrophy during head-down tilt bed rest (Abstract). *Med Sci Sports Exerc* 93: S53, 2000.
47. **Perhonen MA, Zuckerman JH, Levine BD.** Deterioration of left ventricular chamber performance after bed rest: “cardiovascular deconditioning” or hypovolemia? *Circulation* 103: 1851–1857, 2001.
48. **Peshock RM, Willett DL, Sayad DE, Hundley WG, Chwialkowski MC, Clarke GD, Parkey RW.** Quantitative MR imaging of the heart. *Magn Reson Imaging Clin N Am* 4: 287–305, 1996.
49. **Ray CA, Vasques M, Miller TA, Wilkerson MK, Delp MD.** Effect of short-term microgravity and long-term hindlimb unloading on rat cardiac mass and function. *J Appl Physiol* 91: 1207–1213, 2001.
50. **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. *J Am Coll Cardiol* 39: 1055–1060, 2002.
51. **Sandstede J, Lipke C, Beer M, Hofmann S, Pabst T, Kenn W, Neubauer S, Hahn D.** Age and gender specific differences in left and right ventricular cardiac function and mass determined by cine magnetic resonance imaging. *Eur Radiol* 10: 438–442, 2000.
52. **Shackelford LC, LeBlanc AD, Driscoll TB, Evans HJ, Rianon NJ, Smith SM, Spector E, Feedback DL, Lai D.** Resistance exercise as a countermeasure to disuse-induced bone loss. *J Appl Physiol* 97: 119–129, 2004.
53. **Shapiro LM, Smith RG.** Effect of training on left ventricular structure and function: an echocardiographic study. *Br Heart J* 50: 534–539, 1983.
54. **Shoemaker JK, Hogeman CS, Khan M, Kimmerly DS, Sinoway LI.** Gender affects sympathetic and hemodynamic response to postural stress. *Am J Physiol Heart Circ Physiol* 281: H2028–H2035, 2001.
55. **Shub C, Klein AL, Zachariah PK, Bailey KR, Tajik AJ.** Determination of left ventricular mass by echocardiography in a normal population: effect of age and sex in addition to body size. *Mayo Clin Proc* 69: 205–211, 1994.
56. **Smith SM, Nillen JL, Davis-Street JE, DeKerlegand DE, Hargens AR.** Alendronate and resistive exercise countermeasures against bed rest-induced bone loss: biochemical markers of bone and calcium metabolism (Abstract). *FASEB J* 15: A1096, 2001.
57. **Spirito P, Pelliccia A, Proschan MA, Granata M, Spataro A, Bellone P, Caselli G, Biffi A, Vecchio C, Maron BJ.** Morphology of the “athlete’s heart” assessed by echocardiography in 947 elite athletes representing 27 sports. *Am J Cardiol* 74: 802–806, 1994.
58. **Stein TP, Schluter MD.** Human skeletal muscle protein breakdown during spaceflight. *Am J Physiol Endocrinol Metab* 272: E688–E695, 1997.
59. **Stein TP, Schluter MD, Leskiw MJ, Boden G.** Attenuation of the protein wasting associated with bed rest by branched-chain amino acids. *Nutrition* 15: 656–660, 1999.
60. **Stein TP, Schluter MD.** Plasma amino acids during human spaceflight. *Aviat Space Environ Med* 70: 250–255, 1999.
61. **Stuart CA, Shangraw RE, Peters EJ, Wolfe RR.** Effect of dietary protein on bed-rest related changes in whole-body-protein synthesis. *Am J Clin Nutr* 52: 509–514, 1990.
62. **Summers RL, Martin DS, Meck JV, Coleman TG.** Mechanisms of spaceflight-induced changes in left ventricular mass. *Am J Cardiol* 95: 1128–1130, 2005.
63. **Urabe Y, Mann DL, Kent RL, Nakano K, Tomanek RJ, Carabello BA, Cooper GIV.** Cellular and ventricular contractile dysfunction in experimental canine mitral regurgitation. *Circ Res* 70: 131–147, 1992.
64. **Watenpaugh DE, Ballard RE, Schneider SM, Lee SM, Ertl AC, William JM, Boda WL, Hutchinson KJ, Hargens AR.** Supine lower body negative pressure exercise during bed rest maintains upright exercise capacity. *J Appl Physiol* 89: 218–227, 2000.
65. **Waters WW, Ziegler MG, Meck JV.** Post-space flight orthostatic hypotension occurs mostly in women and is predicted by low vascular resistance. *J Appl Physiol* 92: 586–594, 2002.
66. **Young LH, McNulty PH, Morgan C, Deckelbaum LI, Zaret BL, Barrett EJ.** Myocardial protein turnover in patients with coronary artery disease. Effect of branched chain amino acid infusion. *J Clin Invest* 87: 554–560, 1991.
67. **Zemva A, Rogel P.** Gender differences in athlete’s heart: association with 24-h blood pressure. A study of pairs in sport dancing. *Int J Cardiol* 77: 49–54, 2001.
68. **Zile MR, Tomita M, Ishihara K, Nakano K, Lindroth J, Spinale F, Swindle M, Carabello BA.** Changes in diastolic function during development and correction of chronic LV volume overload produced by mitral regurgitation. *Circulation* 87: 1378–1388, 1993.