Intermittent Gravity: How Much, How Often, How Long?

Joan Vernikos and David A. Ludwig

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Joan Vernikos, Ames Research Center, Moffett Field, California
David A. Ludwig, University of North Carolina, Greensboro, North Carolina

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Preface

This technical memorandum is a compilation of the results of a 4-day head-down bed rest (HDBR) study. This study was designed to investigate the effects of 4 days of HDBR alone or with 2 or 4 hours of standing or walking intermittently during each day of HDBR on orthostatic tolerance, maintenance of working capacity (peak oxygen uptake), calcium excretion, and endocrine and fluid and electrolyte responses. The following investigators participated: A. C. Ertl, Ph.D., C. E. Wade, Ph.D., L. Keil, Ph.D., J. E. Greenleaf, Ph.D., and D. O’Hara, R.N., NASA Ames Research Center, Moffett Field, CA 94035-1000. In addition, M. R. Duvoisin, Ph.D., and J. L. Stinn, Ph.D., Biomedical Operations and Research Office, NASA Kennedy Space Center, FL 32899, were instrumental in developing the software for the monitoring and recording of continuous cardiovascular data during the orthostatic tests and St. J. Maloney and V. Reyna for data analyses and compilation of this document. Portions of this report were funded by NASA Ames Research Center Consortium Agreement #NCA2-629.

Joan Vernikos, Ph.D.
Chief (Acting)
Life Science Division
NASA Ames Research Center
Moffett Field, California

David A. Ludwig, Ph.D.
Associate Professor of
Mathematics and Statistics
University of North Carolina
Greensboro, North Carolina
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<td>Human Research Facility</td>
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<td>ISE</td>
<td>ion specific electrode</td>
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<td>ANP</td>
<td>K</td>
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<tr>
<td>APA</td>
<td>LBNP</td>
<td>lower body negative pressure</td>
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<td>AVP</td>
<td>LDL</td>
<td>low density lipoprotein</td>
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<tr>
<td>BP</td>
<td>MAP</td>
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<tr>
<td>bpm</td>
<td>Na</td>
<td>sodium</td>
</tr>
<tr>
<td>BR</td>
<td>NE</td>
<td>norepinephrine</td>
</tr>
<tr>
<td>BV</td>
<td>NF</td>
<td>non-fainter during tilt test</td>
</tr>
<tr>
<td>C</td>
<td>P</td>
<td>probability value</td>
</tr>
<tr>
<td>Ca</td>
<td>PRA</td>
<td>plasma renin activity</td>
</tr>
<tr>
<td>DBP</td>
<td>PV</td>
<td>plasma volume</td>
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<td>E</td>
<td>RIA</td>
<td>radioimmunoassay</td>
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<tr>
<td>F</td>
<td>S</td>
<td>standing condition (e.g., S4, standing for 4 hours)</td>
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<tr>
<td>G</td>
<td>SBP</td>
<td>systolic blood pressure</td>
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<td>Gz</td>
<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>GFR</td>
<td>SE</td>
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<tr>
<td>Hct</td>
<td>VO₂peak</td>
<td>peak oxygen consumption</td>
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<tr>
<td>HDBR</td>
<td>W</td>
<td>walking condition (e.g., W4, walking for 4 hours)</td>
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<td>HDL</td>
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<td>high density lipoprotein</td>
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Intermittent Gravity: How Much, How Often, How Long?

JOAN VERNIKOS AND DAVID A. LUDWIG*

Ames Research Center

Summary

Continuous exposure to gravity may not be necessary to prevent the deconditioning effects of microgravity. It is not known, however, what the minimum gravity (G) exposure requirements are, whether they vary for different physiological systems, or whether passive Gz (gravity in the head-to-toe vector) or activity in a G field is more effective in preventing deconditioning. It is also not known what the optimal characteristics of the G stimulus should be in terms of amplitude, duration, and frequency. To begin to address these questions, we conducted a 4-day -6° head-down bed rest (HDBR) study. Nine males (aged 30-50 yr) were subjected, over a period of seven months, to four different +1 Gz exposure protocols (periodic standing or controlled walking each for a total of 2 or 4 hr/day in individual 15-min doses), plus a control (0 Gz) of continuous HDBR.

The study consisted of one ambulatory control day, 4 full days of -6° HDBR, and a recovery day when subjects were released at the end of HDBR after completion of tests. A battery of tests was selected and standardized in order to evaluate the known early responses to HDBR. Dependent variables of interest included orthostatic tolerance (30 min at 60° head-up tilt) and hemodynamics during head-up tilt, peak oxygen consumption (VO2peak) plasma volume (PV), and urinary calcium (Ca).

The results were as follows: (1) 4 hr standing completely prevented and 2 hr walking partially prevented post-HDBR orthostatic intolerance. Walking at 3 mi/hr for 4 hr/day provided no additional benefit. (2) Intermittent walking attenuated, but did not prevent, the decrease in VO2peak. (3) Both 4 hr conditions showed less PV loss by the end of HDBR; both 2 hr conditions were without effect. (4) Both 2 and 4 hr walking essentially prevented urinary Ca excretion and were more effective than standing. It is concluded that different physiological systems benefit differentially from passive +1 Gz or activity in +1 Gz, and the intensity of the stimulus may be an important contributing factor.

Introduction

All terrestrial living systems, including humans, have evolved over millions of years in the continuous presence of Earth's gravitational force, gradually developing, adapting, and migrating from sea to land. It is therefore not too surprising that animals and humans physiologically decondition very rapidly in microgravity, suggesting that chronic exposure to gravity (G) is important in maintaining a gravity-adapted state. Whether this chronic exposure must be continuous or can take the form of periodic "vaccinations" has been the subject of a few studies conducted mostly in the sixties and of much debate and speculation over the ensuing years. In the meantime, the search in the last 30 years for effective countermeasures to the effects of spaceflight has met with limited success. Concern is growing, especially as longer duration missions are being planned, that either comprehensive countermeasures will not be developed in time or that effective countermeasure procedures will be so time consuming and cumbersome that it would be unrealistic to expect crews to devote 2-4 hr/day, as some Russian crews have done (Garshnek et al., 1989), in order to maintain adequate health, fitness, and productivity.

Although Soviet cosmonaunts have been in space for as long as a year and have performed an extensive and intensive exercise and lower body negative pressure (LBNP) countermeasure program, they cannot walk unassisted for at least 48 hr after landing. They have also not been required to maintain or restore skilled physical performance in order to land their spacecraft as U.S. astronauts must, be immediately able to egress in an emergency, or perform adequately and consistently on landing in another gravitational field such as Mars. It appears, then, that exposure to some effective G level is probably essential to restore vascular hydrostatic pressures, enhance the effectiveness of exercise and activity on muscular strength and endurance, aerobic work capacity, and bone integrity and strength, and provide afferent input to maintain integrity of neural regulatory functions.

*University of North Carolina.
Head-down bed rest (HDBR) and horizontal bed rest, to a lesser extent, as ground simulation analogues of microgravity, have been used extensively to induce most of the physiological effects also observed during deconditioning in microgravity. This indicates that at least three aspects of a G condition are involved in maintaining normal health and fitness: the pull of the G force in the Gz (head-to-toe) direction, exertion against the G force, and the element of "change" provided by postural and other movement/orientation cues only possible in a gravitational field.

Normal, ambulatory people spend approximately 8 hr/day sleeping (horizontally) and 16 hr/day in the upright sitting or ambulatory mode. Whether all of the 16 hr/day at +1 Gz are needed to maintain overall normal health and fitness is not known. The results of classic, though not definitive, studies (because of the small number of subjects used) have come from bed rest (BR) research studies conducted at the Lankenau Hospital in Philadelphia in the 1960s. These studies show that:

(a) Eight hours daily of quiet sitting plus 16 hr/day of horizontal BR resulted in only minor decreases in aerobic capacity (maximal oxygen uptake); tilt tolerance was maintained in 3 of 4 subjects (Birkhead et al., 1964a).

(b) Cycle leg exercise for 2-4 hr/day in the supine position for 18 days was ineffective in reducing tilt intolerance or hypercalciuria. Quiet standing for 3 hr/day with 21 hr/day of horizontal BR decreased tilt intolerance in 3 of 5 subjects and decreased urinary calcium (Ca) excretion in 4 of 5 subjects. Application of intermittent LBNP in one subject attenuated development of tilt intolerance but increased urinary Ca excretion (Birkhead et al., 1966).

(c) One hour daily of supine or sitting leg exercise at 600 kpm/min for 24-42 days maintained aerobic capacity, but did not prevent tilt intolerance or increased urinary Ca loss in subjects (Birkhead et al., 1963; 1964b).

Rodahl et al. (1967) summarized the findings from these Lankenau experiments:

(a) Supine leg cycle exercise training for 2-4 hr/day during BR
   1. may maintain muscular strength and aerobic capacity.
   2. has no effect on urinary Ca loss.
   3. has no effect on tilt intolerance.

(b) Quiet sitting for 8 hr/day during BR
   1. has minimal effect on maintenance of muscular strength and aerobic capacity.
   2. has no effect on urinary Ca loss.

(c) Quiet standing for 3 hr/day during BR
   1. (muscular strength or aerobic capacity was not measured).
   2. reduces increased urinary Ca excretion to ambulatory levels.
   3. reduces tilt intolerance.

More recent evidence indicates that exposure to three 20-min sessions of LBNP (at -35 mmHg) daily virtually eliminates orthostatic (tilt) intolerance after 30 days of -6° HDBR (Guell, 1990). Kakurin et al. (1978) reported that an exercise protocol, performed for 58-67 min/day, consisting of leg cycling, rowing, inertial-impact loading on the legs, and breathing exercises, maintained the muscular strength and aerobic capacity of six men during 49 days of -4° HDBR. In addition, we have found that, after 30 days of -6° HDBR, high intensity and short duration aerobic exercise (leg cycle ergometry at 40% max) for 1 hr/day maintains aerobic capacity, and high intensity, intermittent, short duration isokinetic leg exercise maintains strength and endurance (Greenleaf et al., 1989; 1994). Whereas it appears possible to maintain aerobic capacity, muscle strength, and endurance with a variety of supine exercise procedures, some form of orthostatic stimulus seems to be necessary to prevent orthostatic intolerance. The magnitude and duration of such a stimulus, however, are unknown.

The most effective time of day to expose subjects to a +1 Gz stimulus is also important. During the night hours many physiological systems are unresponsive. Diuresis, natriuresis, and calciuresis, for example, do not occur in response to immersion or BR (Shiraki et al., 1986). This blunting of responses is independent of sleep or posture and appears to be a function of the circadian trough. How pervasive this nocturnal blunting to gravitational and other stimuli is across physiological systems has not received much attention. Nevertheless, it would appear that there is an optimal time in the diurnal cycle when the effectiveness of Gz stimuli are at their greatest. This optimal time does not appear to be during the circadian nadir or the nocturnal part of the cycle. It could therefore be inferred that exposure to a Gz force by centrifugation during sleep, as has been proposed by some (Cardus et al., 1990), would be of no substantial benefit.

Whether it is passive exposure to +1 Gz or the activity we carry out normally in +1 Gz that is the optimum stimulus in health maintenance is another question. It has been proposed (Whalen et al., 1988; Whalen, 1993) that, for maintenance of bone strength, it is the activity we conduct in a G field that is most effective. Whalen (1993) has
proposed that the apparent bone loss in spaceflight is secondary to the reduction in gravitational forces on the skeleton. This implies that exercise regimens that are selected as countermeasures must, in concert with the G force, replace absent mechanical stimuli and stresses.

Periodic hypergravity exposures during BR have been used in a few studies (Murray et al., 1965; White, 1965; Nyberg et al., 1966; Piemme et al., 1966; Dowell et al., 1968; Kotovskaya et al., 1977; Grigor'ev and Shulzhenko, 1979; Shulzhenko et al., 1979; Shulzhenko and Vil-Viliams, 1992). These studies have focused primarily on preventing post-bedrest or post-dry immersion orthostatic intolerance by exposing subjects 2–3 times a day for short periods of time to gravity levels up to +2 Gz. Some of these studies have suggested that such intermittent hypergravity exposures also induce fluid and sodium (Na) retention and prevent changes in plasma volume (PV). The fundamental flaw in these studies is that they have not been comprehensive, and tests and measurements vary, so that comparisons across regimens and across physiological systems are near impossible.

Exposure of animals to continuous gravity in flight provided by an onboard short radius centrifuge on the Cosmos-782 and Cosmos-936 Biosatellites indicated that this exposure protected animals from the physiological responses to spaceflight. In ground studies, using a 2 m radius centrifuge, Shulzhenko and Vil-Viliams (1992) exposed human subjects to periods of 40–60 min time blocks of continuous gravity during 3-day or 28-day dry immersions; exposure was 2–3 times a day to 1.3, 1.6, and 1.9 G either alone or with exercise or salt loading. The beneficial effects of these exposures were very encouraging. More studies are needed, but must be approached in a systematic fashion so that specific requirements for the most effective periodic centrifugation protocol may be developed.

Many questions remain unanswered regarding maintenance of health and fitness in spaceflight: What is the minimum exposure per 24 hr of +1 Gz? Does exposure have to be presented in a block of time or in divided doses? Is it the total duration of exposure per 24 hr or the number of bouts that is the crucial variable? What is the most effective time of day to expose subjects? Is it passive exposure to +1 Gz or the activity we carry out normally in +1 Gz that is the optimum stimulus in health maintenance? Does increasing the intensity of the +Gz stimulus reduce the time of exposure needed (i.e., does hypergravity increase the efficacy of +Gz exposure)? Do all physiological systems respond to gravitational treatment in a similar way? What are the side effects of short arm centrifugation? Can you stress the body by providing "weightbearing" by other means such as LBNP (Hargens et al., 1991; 1992)?

In order to address systematically the many questions that must be answered so that artificial G requirements for prolonged missions may be determined, it was important to develop the simplest ground simulation model possible whose cost would not be prohibitive and that: 1) would give reliable, measurable, and consistent changes within the briefest period of time; 2) could be repeated frequently, preferably in the same individuals; and, 3) would be based on an experimental design that could be randomized to enable relatively easy screening of various permutations of G stimulus presentation. It was also important to select the most sensitive tests available and standardize them in order to assess the extent of the deconditioning effects.

The value of −6° HDBR as a ground simulation analog of the effects of microgravity has been well documented (Grigor'ev et al., 1986; Convertino, 1994). Although we and others (Dallman et al., 1984; Baisch et al., 1992; Vernikos et al., 1993) have conducted numerous HDBR studies of 7 days or longer, the artificial gravity working group that met at NASA Ames Research Center (Artificial Gravity Workshop, NASA Ames Research Center, Galileo Room, April 11–13, 1989) indicated that 3 or 4 days of HDBR could serve as a reliable model for the rapid screening of preventive or therapeutic treatments, in large part because of the sufficiently large, consistent, and reproducible physiological responses induced during this time. Orthostatic intolerance becomes apparent within a few hours of −6° HDBR (Loellgen et al., 1984; Butler et al., 1991), and, although PV is somewhat reduced by 24 hr, reduction is essentially maximal by 3 days (Dallman et al., 1984; Heer et al., 1992). Diuresis and natriuresis occur mostly within the first 48 hr (Heer et al., 1992; Vernikos et al., 1993). Maximal oxygen consumption changes have also been reported evident within 48 hr of HDBR (Nixon et al., 1979). We therefore selected 4 days of HDBR as an appropriate time frame to acquire significant early adaptive responses and screen various dose, time, and frequency permutations of the +Gz stimulus. A 4-day HDBR study also had the advantage of being both practical and cost effective as it required, including control periods before and after HDBR, only a week to complete.

Methods

Subjects

Nine healthy, nonsmoking, normotensive men (paid volunteers), with a mean (SD) age of 37.9 (4.5) yr, a mean
height of 181.7 (5.1) cm, and a mean weight of 84.4 (7.8) kg, gave written consent to participate in this study. Participation was contingent on a positive medical screening that consisted of a detailed medical history and a physical examination. Subjects were recruited from the San Francisco Bay Area and were of average fitness for their age [mean peak oxygen consumption (VO2peak) = 32.1 ml/kg/min]. Prior to testing and subject recruitment, the study protocol was approved by the NASA Ames Human Research Experiments Review Board. Subjects were thoroughly briefed on all aspects of the experimental protocol before consenting to participate. One of the nine subjects withdrew from the study and did not participate in the 2 hr walking and standing conditions. Table 1 shows the subjects’ control test data and body weights over the seven months of testing.

**Treatments and Experimental Protocol**

Each of the nine subjects was tested across five treatment conditions to evaluate the effects of periodic +1 Gz gravity during 4 days of continuous −6° HDBR (each subject received each treatment during five 4-day HDBR exposures). The order in which the subjects received the treatments was counterbalanced as much as possible but was restricted due to the size and availability of the Human Research Facility (HRF) at NASA Ames Research Center.

Table 1. Control data from tests on the same subjects during their five visits before each HDBR exposure. N = number of subjects per group. Data are shown as mean ± standard error. Tilt tolerance is the percentage of subjects completing a 30-min 60° head-up tilt test

<table>
<thead>
<tr>
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<th>N = 9</th>
<th>N = 8</th>
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<th>N = 8</th>
<th>N = 9</th>
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<tr>
<td>Body weight (kg)</td>
<td>82.72 ± 2.58</td>
<td>84.21 ± 3.44</td>
<td>83.49 ± 2.71</td>
<td>84.88 ± 3.30</td>
<td>83.50 ± 2.50</td>
</tr>
<tr>
<td>Tilt tolerance (%)</td>
<td>75% (6 of 8)</td>
<td>100% (8 of 8)</td>
<td>100% (9 of 9)</td>
<td>75% (6 of 8)</td>
<td>77.8% (7 of 9)</td>
</tr>
<tr>
<td>Peak VO2 (L/min)</td>
<td>2.66 ± 0.15</td>
<td>2.64 ± 0.15</td>
<td>2.70 ± 0.13</td>
<td>2.66 ± 0.18</td>
<td>2.70 ± 0.15</td>
</tr>
<tr>
<td>PV (ml)</td>
<td>2685.8 ± 169.2</td>
<td>2438.4 ± 103.4</td>
<td>2607.4 ± 150.8</td>
<td>2390.7 ± 114.4</td>
<td>2987.6 ± 289.2</td>
</tr>
<tr>
<td>Urinary Ca (mg/24 hr)</td>
<td>135.4 ± 22.8</td>
<td>201.0 ± 23.5</td>
<td>177.6 ± 28.2</td>
<td>212.5 ± 17.7</td>
<td>199.3 ± 21.3</td>
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**Experimental Conditions**

Admission of subjects was staggered so that three subjects were admitted daily to the HRF and released six days later. Subjects were supervised 24 hr/day while in the HRF to assure that they adhered to the bed rest regimen. Transportation to and from VO2peak and tilt test rooms was in horizontal or −6° head-down position as appropriate; showering was done in horizontal position and excretory functions in −6° head-down position. During −6° HDBR, subjects were allowed one pillow. To facilitate eating, a subject was allowed to raise his head and rest it in his hand as long as the upper part of his arm was resting flat against the mattress. Smoking, caffeine containing drinks, and medications (including vitamins) were not allowed during the study. Lights were on from 0700 hr to 2300 hr for all subjects except those standing at 2315 hr and walking at 2330 hr.

All subjects were maintained throughout the study on a standard diet of 2500-2800 Kcal/day including 190 mEq Na (±20) and 90 mEq potassium (K) (±10). Subjects were required to drink a minimum of one liter/day of fluids which included fluids from their food trays; there was no upper limit on the amount of fluids allowed. Subjects were required to keep accurate food and fluid intake
records during the study including records of the amount of sodium consumed.

**Experimental Procedures**

In the two week period preceding each HDBR exposure, subjects came on an outsubject basis to establish peak oxygen consumption. Upon admission to the facility for each HDBR exposure, an admission physical was done which included measurement of height, weight, and vital signs (oral temperature, heart rate, respiration, and blood pressure). Vital signs were taken each day during the HDBR exposure at 0700 and 1900 hr; weight and vital signs were again determined following the VO₂ peak test on the recovery day prior to release.

On the day after admission, there was one ambulatory control day when early-morning fasting PV and tilt tolerance were determined. On the following day, after awakening, getting up, having a standardized light breakfast and generally being ambulatory for 2 hr, subjects began the HDBR period. Treatments started 1 hr after beginning HDBR. Plasma volume and tilt tolerance were again determined on day 4 of HDBR. At the end of this tilt test, subjects, without ever ambulating, were placed back in the -6° head-down position and returned to their beds. Subjects following walking or standing treatments resumed treatment the hour following the tilt test, thus missing only the treatment replaced by the 30-min tilt test. Subjects remained in the -6° head-down position until the next day (the recovery day) when a VO₂ peak test was again performed using a supine bicycle ergometer. Following completion of this last test, subjects were allowed to ambulate and were then released.

**Blood Samples**

Blood samples (supine) were taken on the ambulatory control day and on HDBR day 4 for PV determination and for analysis of electrolytes and fluid regulating hormones. In addition, a blood sample was taken 4 hr after beginning HDBR and also analyzed for various fluid regulating hormone changes as compared to a supine control blood sample taken after lying quietly supine for 45 min on the ambulatory control day. Blood samples were collected using the Becton Dickinson Vacutainer system, as appropriate, into test tubes containing heparin, EDTA, or no anticoagulant. The use of anticoagulants had no effect on subsequent analyses. The tubes were kept in crushed ice and then centrifuged at 4°C for 20 min at 2500 rpm. Plasma or serum were removed, allocated into individual tubes for each assay, and immediately frozen for later analysis.

**Urine Samples**

Volumes and times of void-by-void specimens were recorded daily. Urine pools (24-hr) were collected throughout each study for the determination of fluid and electrolyte balance. Aliquots from each pool were frozen and stored for later analysis.

**Tilt Tolerance Test**

Orthostatic tolerance testing was performed pre-bed rest on the ambulatory control day, after subjects had been lying quietly in the supine position (horizontal) for 45 min prior to the test. Testing was performed again on HDBR day 4. Testing both times was performed on a NASA developed tilt table. On the ambulatory control day, subjects were transported by gurney to the tilt room in horizontal position; on HDBR day 4, transportation was by gurney with subjects in -6° head-down tilt position. In the tilt room, subjects slid over onto the tilt table (horizontal on ambulatory control day; -6° head-down on HDBR day 4) and positioned themselves in the center of the table with their feet just touching the table foot rest. A folded towel was placed under the heels for better positioning of the feet during tilt. A wide band, secured on both sides of the table, was placed loosely across the waist to make the subject feel more secure. After a 5-min pre-tilt control period, subjects were tilted within 10-15 sec to 60° head-up for 30 min or until signs and symptoms of pre-syncope (e.g., nausea, dizziness, sweating, lightheadedness, and tunnel vision) occurred. Subjects were then immediately returned to a horizontal (ambulatory control day) or -6° head-down (HDBR day 4) position for a recovery period of at least 10 min. An Ohmeda 2300 (Ohmeda, BOC Group Inc., Englewood, Colo.) Finapres™ blood pressure (BP) monitor was used to monitor BP and heart rate (HR) continuously during the test (5-min pre-tilt control period, tilt, post-tilt recovery period). An arm rest, attached to the tilt table, was used to keep the subject's hand at heart level at all times to ensure the collection of consistent and reliable data. Pre- and post-tilt manual blood pressure readings were also taken and recorded. Upon completion of the test, subjects were transported back to their beds by gurney (in horizontal position on ambulatory control day; in -6° head-down tilt position on HDBR day 4). A medical monitor was in attendance at all times during the tests.

The Finapres fingercuff BP monitoring device used to provide beat-by-beat measurement of peripheral BP measures BP using a small finger cuff that contains a photoplethysmographic volume transducer and an inflatable air bladder. The cuff is connected to a fast-response servo control system that instantaneously regulates the pressure applied to the finger through the bladder and, thus, the pressure applied to the walls of the arteries. As BP
increases, the arterial wall expands, increasing the volume of the finger. This volume differential is measured by the plethysmographic transducer. The Finapres monitor responds to the increasing volume by increasing cuff pressure until the original arterial size and blood volume (BV) are again reached. The external pressure continuously adjusted by the cuff closely follows the intra-arterial pressure within the finger, allowing measurement of the external pressure itself as a function of the arterial BP.

**Peak Oxygen Consumption Test**

Prior to and following 4 days of -6° HDBR, VO2_peak was determined using indirect calorimetry during supine leg cycling ergometry. During an initial visit, subjects were familiarized with the exercise testing equipment and completed an incremental submaximal test. In the two week period preceding the first HDBR exposure, a VO2_peak test was administered. Several days later, and before beginning HDBR, subjects completed a second VO2_peak test to verify results of the first test. In the two week period preceding the four subsequent HDBR exposures, all subjects came again on an outpatient basis to establish peak oxygen consumption. Post-HDBR VO2_peak tests were administered on the recovery day of all HDBR exposures. For the post-HDBR tests on the recovery day, subjects were transported by gurney between the HRF and the test room (before and after completion of the tests) in -6° head-down tilt position. Subjects were horizontal during the administration of all VO2_peak tests.

The term VO2_peak is used rather than maximal oxygen consumption (VO2_max) since a plateau or decrease in oxygen consumption with an increase in load was not a criterion for measurement. VO2_peak is defined as the highest minute oxygen consumption measured during incremental loads on the ergometer.

The protocol for VO2_peak consisted of a 5-min warmup at 400 kg-m-min⁻¹ which was followed by three 2-min incremental loads of 200 kg-m-min⁻¹ estimated to elicit VO2_peak. If the subject was able to complete the third incremental load, an additional increase of 200 kg-m-min⁻¹ was given. The subjects exercised to volitional fatigue, or the test was terminated if the subject was unable to maintain a pedaling frequency above 50 rpm. After completion of the test, resistance was reduced so that the subject could recover comfortably. Hand grips and shoulder braces were used for stabilization during the test.

The three loads used to reach the peak load were estimated from the results of the submaximal test. In the first VO2_peak test, the three peak loads were set to achieve exhaustion with the third load (within 5–6 min). If the subjects were not able to exercise for at least 1 min at the peak load, or required a fourth load to reach exhaustion, the peak loads were adjusted.

Reported results are submaximal HR response to the initial 5-min warmup prior to the peak protocol (400 kg-m-min⁻¹), the average VO2 of the highest four 15-sec values obtained during the peak power output, and the maximal HR achieved during the test.

Exercise testing was performed on a Quinton model 846T Imaging/Ergometer Table (Quinton Instruments, Seattle, Wash.). The metabolic gas collection system utilized an on-line data acquisition system. Subjects breathed through a low-resistance, high flow Rudolph valve. Inspired gas volumes were measured using a Pneumoscan S-301 spirometer (Vacumed, Ventura, Calif.). Oxygen and carbon dioxide concentrations were measured on Ametek Applied Electrochemistry (Pittsburg, PA) S-3A1 and CD-3A analyzer respectively. Analog data was converted to digital by Vista model 17002 system and software (Vacumed, Ventura, Calif.) and fed to an IBM PC AT for calculation. Heart rate data was collected on a Hewlett Packard cardiotachometer model 78905A and ECG module model 78203C (Hewlett Packard, Medical Products Group, Waltham, Mass.).

**Plasma Volume**

Plasma volume was measured using a modified Evans blue dye dilution method (Greenleaf et al., 1979; Greenleaf and Hinghofer-Szalkay, 1985). The procedure was performed on each subject on the ambulatory control day and again on HDBR day 4 after subjects were awakened at 0700 hr and before they were served breakfast. A Becton Dickinson Vacutainer Blood Collection Set with a 21-gauge needle, attached to a 3-way stopcock, was used to obtain blood samples. A pre-injection blood sample was drawn. The pre-weighed Evans blue dye solution (2.5 ml/subject) in the syringe was injected and a second blood sample was drawn 10 min later. The empty syringe used to inject the Evans blue was saved for weighing in order to calculate the amount of Evans blue dye actually used for each PV test. The IV line was kept patent with a slow drip of sterile 5% dextrose solution. No more than 50 ml of dextrose was used over the 10-min period.

The blood samples were centrifuged and plasma stored at -70° C. At the completion of the study, analysis was done on all samples by a modified column extraction procedure of Greenleaf and Hinghofer-Szalkay (1985) using Sephadex columns (Pharmacia LKB Biotechnology, Uppsala, Sweden) in place of Solka Floc columns.
Blood volume was calculated using the subject's PV and hematocrit (Hct) values with BV (in ml) = PV (in ml) × \[
\frac{100}{100 - (0.91 \times \text{Hct})}.
\]

Blood Analyses

Hematocrit measurements were made using a microhematocrit centrifuge (CT-2900, Adams Micro-Hematocrit Centrifuge, Clay-Adams, Inc., N.Y.). Vasopressin and catecholamine measurements for a subject were done in a single assay for each hormone. Vasopressin (AVP) was measured by radioimmunoassay (RIA) using the method of Keil and Severs (1977); the within-assay coefficient of variability was 9% and assay sensitivity was 0.3 pg/ml. Plasma norepinephrine (NE) and epinephrine (E) were separated by high performance liquid chromatography (HPLC) and then measured by electrochemical detection (Bioanalytical Systems, West Lafayette, Ind.); within-assay variabilities were 5% (NE) and 3% (E) and sensitivities for both were 5 pg/ml (Wade et al., 1991). Plasma renin activity (PRA) was measured by RIA for angiotensin I (kit from New England Nuclear, Boston, Mass.) with a within-assay coefficient of variability of 7%, an inter-assay coefficient of variability of 8%, and sensitivity of 30 ng/ml/hr. Aldosterone and cortisol were measured using RIA kits from Diagnostic Products Corporation (Los Angeles, Calif.); within-assay coefficients of variability, inter-assay coefficients of variability, and sensitivities were 4%, 8%, and 2.5 ng/dl for aldosterone and 3%, 4.4%, and 1 μg/100 ml for cortisol, respectively. Atrial natriuretic peptide (ANP) was measured by Waters column extraction (Waters Chromatography Division, Millipore Corporation, Milford, Mass.) followed by RIA (kit from Peninsula Laboratories, Inc., Belmont, Calif.), with a within-assay coefficient of variability of 5%, an inter-assay coefficient of variability of 15%, and sensitivity of 1.5 pg/tube. Plasma Na and K were measured by ion specific electrode (ISE) (Cobas Mira, Roche Diagnostic Systems, Nutley, N.J.). Plasma osmolality was measured using an Advanced Digimatic Osmometer (Model 3D11, Advanced Instruments, Inc., Needham Heights, Mass.). Serum total protein was measured by hand refractometer (National, No. 15064, Japan) and serum creatinine by an alkaline picric acid (APA) method (Cobas Mira, Roche Diagnostic Systems, Nutley, N.J.). Total cholesterol, high density lipoprotein (HDL) cholesterol, and triglyceride concentrations were measured by an enzymatic chemistry procedure (Cobas Mira, Roche Diagnostic Systems, Nutley, N.J.) and low density lipoprotein (LDL) cholesterol was calculated using the formula LDL cholesterol = Total cholesterol - [HDL cholesterol + (Triglycerides/5)].

Urine Analyses

Urinary creatinine was measured by APA (Cobas Mira, Roche Diagnostic Systems, Nutley, N.J.) and the glomerular filtration rate (GFR) calculated using the formula GFR = (Urine creatinine excretion rate/Serum creatinine)/1440. Urinary osmolality was measured using an Advanced Digimatic Osmometer (Model 3D11, Advanced Instruments, Inc., Needham Heights, Mass.). Urinary cortisol was measured using a RIA kit from Diagnostic Products Corporation (Los Angeles, Calif.); the within-assay coefficient of variability was 3%, the inter-assay coefficient of variability was 4.4%, and sensitivity was 1 μg/100 ml. Urinary Ca concentration was measured by Arsenazo III colored complex (Cobas Mira, Roche Diagnostic Systems, Nutley, N.J.) and urinary Na and K were measured by ISE (Cobas Mira, Roche Diagnostic Systems, Nutley, N.J.).

Statistics

The general experimental design consisted of nine subjects who received five experimental treatments (including no intervention). Each subject received each of the five treatments (in random or near random order) and were measured before and after each treatment. Some of the dependent variables were measured over time (daily) during HDBR. The general statistical model is therefore a five treatment randomized block (subjects are the blocking factor) with two or five repeated measures. When only two repeated measures were taken (pre- and post-treatment), the repeated measures were differenced and the differences were analyzed. This is equivalent to the test of interaction in a standard repeated measures ANOVA. The test of the difference scores evaluates differences in the rate of change for the given dependent variable across treatments after subject-to-subject variation has been removed (subjects are crossed with treatments). When more than two repeated measures were taken, the rate of change was quantified by the slope of the response across the repeated measures. The slopes were either first order (linear) or second order (quadratic) depending on the shape of the response function. These slopes were then analyzed using the same model as the difference scores. To correct for differences due to starting points, slopes and differences were adjusted for pre-HDBR values by analysis of covariance (pre-HDBR values were used as a covariate). The statistical tests for both slopes and differences evaluate the differences in rate of change across the five treatments after correcting for initial values. A more detailed description of this approach is given by Laird (1983).

Line or bar charts were used for graphical analysis. Mean variation is presented on the graphs as error bars (standard
Errors). Estimates of variation and mean values are based on the raw data (data specific to that mean) when no statistical test was performed. When a statistical test was performed, pooled estimates of variation and adjusted means from the analysis of covariance were presented. Unadjusted means for all variables are given in the appendix. Specific comparisons (least significant differences) between treatment means were calculated from the pooled error obtained from the ANCOVA. In the event of missing observations (due to subject withdrawal or equipment failure), statistical estimates were adjusted by least squares. Since the general linear model was the basis for all the statistical analyses, adjustment due to missing data was inherent to the statistical methodology.

Exact statistical probabilities (Type I error rates) are given along with the size of the test statistic and the degrees of freedom. The statistical probabilities can be interpreted as the chances of observing a difference as large or larger than the one observed if in fact the treatments had no effect. Thus, a low probability (P value) would indicate an unusual situation under the hypothesis of no treatment differences. It might then be concluded that the observed experimental difference is a result of factors other than random experimental variation. In order to help control the experimentwise Type I error rate, statistical tests were only applied to dependent variables of primary interest (orthostatic tolerance, VO_2peak, PV, urinary Ca).

Raw data for all dependent variables are in the appendix.

Statistics: orthostatic tolerance—The statistical methods applied to HR and BP data from the tilt test are different from those described above due to the large amounts of data (beat to beat) collected during the testing. Similarly, analysis of fainting rates (proportion fainting during the tilt test) required more specialized statistical procedures appropriate for the analysis of proportions.

One discrete and four continuous measures were used as dependent variables in quantifying orthostatic intolerance. The discrete variable was the proportion of the nine sub-jects who successfully completed a tilt test without experiencing syncope or pre-syncopal symptoms ("survival rate"). The four continuous variables were HR, mean arterial pressure (MAP), and the two components of MAP, systolic (SBP) and diastolic (DBP) pressures. The statistical analysis used the final 3 min prior to the 60° head-up tilt as the control period. The tilt test lasted for 30 min. Heart rate and BP (Finapres at heart level at all times) were measured continuously before and during the tilt test and digitally recorded every 3 sec on magnetic tape. This equates to approximately 700 data points per subject per variable. Fifteen sec averages were taken to reduce the data to 140 data points per subject per variable. This time series of 140 points occurring during the pre-tilt control and the tilt test was used in the statistical analysis. Ten “survival rates" (proportion completing a tilt test without experiencing syncope or pre-syncopal symptoms) were calculated from the five pre-HDBR (control) and the five post-HDBR (treatment) tilt tests.

Statistical comparison of the pre-HDBR control measures to the post-HDBR treatment conditions was accomplished with the use of control charts (i.e., Shewhart charts) and multiple regression techniques (time series analysis). A control chart was constructed for each of the five dependent variables from the five pre-HDBR measurements.

This was possible since multiple control measures were taken on the same subject prior to each HDBR exposure and subsequent treatment. From this replicated pre-HDBR information, variation in the physiological system when it is functioning normally could be estimated. This inherent variation (which also includes experimental variation and measurement error) is graphically depicted in the form of approximate 95% control limits on the control chart. Experimental inference can then be made by comparing the response under stress or during different treatments to these limits. This statistical approach is similar to methods described by Box et al. (1978) in that data internal to the experiment is used as the reference distribution rather than reference distributions based on normal theory (i.e., z, t, F, etc.). A more detailed account of these statistical procedures is given by Montgomery (1985).

Control charts for survival rate were constructed in the same manner as those for HR and MAP. For comparative purposes, a survival rate analysis based on asymptotic normal theory was performed. The overall survival rate from the five multiple control periods was tested against each of the five treatment conditions using the Mantel-Haenszel procedure for matched case control studies with multiple controls per case (Kleinbaum et al., 1982).

Results

Orthostatic Tolerance: Heart Rate And Blood Pressure

The control charts for HR, MAP, SBP, and DBP are presented in figures 1–4 respectively. Panel A of all four figures presents the average time series from the five pre-HDBR measurements, the overall linear model, and the approximate 95% control limits during the 30-min tilt test (plus 3 min of supine pre-tilt). Panel B in each figure compares the no intervention treatment (0 Gz) to the control limits while panels C and D compare the 2 and 4 hr treatments to the control limits for both standing and walking.
Figure 1. Heart rate response to 60° head-up tilt by treatment conditions (Panel A, average-time series from the five pre-HDBR measurements, the overall linear model, and the approximate 95% control limits during the 30-min tilt test (plus 3 min of supine pre-test)); Panel B, no intervention (0 Gz) compared to the control limits for both standing and walking; Panel C, 2 hr treatments compared to the control limits for both standing and walking).
Figure 2. Mean arterial pressure response to 60° head-up tilt by treatment conditions [Panel A, average-time series from the five pre-HDBR measurements, the overall linear model, and the approximate 95% control limits during the 30-min tilt test (plus 3 min of supine pre-tilt); Panel B, no intervention (0 Gz) compared to the control limits; Panel C, 2 hr treatments compared to the control limits for both standing and walking; Panel D, 4 hr treatments compared to the control limits for both standing and walking].
Figure 3. Systolic blood pressure response to 60° head-up tilt by treatment conditions [Panel A, average-time series from the five pre-HDBR measurements, the overall linear model, and the approximate 95% control limits during the 30-min tilt test (plus 3 min of supine pre-tilt); Panel B, no intervention (0 Gz) compared to the control limits; Panel C, 2 hr treatments compared to the control limits for both standing and walking; Panel D, 4 hr treatments compared to the control limits for both standing and walking].
Heart rate responses—The average pre-HDBR (ambulatory control) HR response to tilt demonstrated an increase in HR from 60 beats per minute (bpm) during supine pre-tilt to 76 bpm at approximately 1 min post-tilt (+16 beats) (fig. 1, Panel A). Beats per minute continued to increase at a rate of 0.56 beats/5min during tilt, reaching an average maximum of 79 bpm by the end of the test. The 95% control limits were calculated at approximately ±7 beats around the least squares fit of the average time series (fig. 1, Panel A).

The no intervention comparison (control HDBR with no +1 G z exposure) was well outside the control limits indicating a much different HR response to tilt after HDBR (fig. 1, Panel B). The pre-tilt HR was elevated by approximately 6 bpm (66 bpm) and rose to 102 bpm following tilt (+36 bpm). At 1390 sec into tilt, three of the nine subjects had failed the test. A slight survivor effect must be considered when evaluating the no intervention series past this point (i.e., only survivors are being evaluated during the latter part of the tilt test).

The 2 hr stand and walk series (fig. 1, Panel C) were also outside the control limits. As with the no intervention treatment, the supine HR was elevated by approximately 5 bpm rising to around 91 bpm following tilt (+26 beats). Heart rate for the 2 hr stand tended to increase during tilt in comparison to a somewhat constant HR for the 2 hr walk treatment.

The 4 hr stand and walk series were also outside the control limits during tilt but showed a slight improvement during pre-tilt (fig. 1, Panel D). Both series jumped to approximately 89 bpm following tilt (+25 beats) and remained outside the control limits for the remainder of the test. Heart rate for both 4 hr conditions remained relatively constant during tilt. Although the 4 hr stand series and 4 hr walk series were both outside the pre-HDBR control limits, they showed a marked improvement over the no intervention (0 G z) treatment.

Although all the intervention treatments seemed to be an improvement over no intervention, none of the treatments completely restored HR response to pre-HDBR levels (all fell outside the control limits). All of the 2 and 4 hr walk and stand curves seemed comparable, except for a slightly elevated HR (both pre- and post-tilt) for the 2 hr conditions. Some possible interaction between exposure time and activity level can be seen in Panels C and D of figure 1. The HR for standing tends to be five to six beats higher than that for walking given the 2 hr condition, whereas little difference between standing and walking is seen in the 4 hr condition. Although not statistically discernible, perhaps activity is beneficial only when +G z exposure is low (i.e., low in duration and/or intensity).

Mean arterial pressure responses—Average supine MAP during pre-HDBR was approximately 81 mmHg rising to 99 mmHg (+18 mmHg) post-tilt (fig. 2, Panel A). Mean arterial pressure continued to rise at a rate of approximately 0.52 mmHg/5min during tilt reaching an average maximum of approximately 103 mmHg by the end of the test. The 95% control limits were calculated at ±22 mmHg around the least squares fit of the average time series (fig. 2, Panel A).

For the 0 G z treatment, MAP remained within the 95% control limits until the end of the tilt test (fig. 2, Panel B). The overall series tended to remain in the lower quarter of the control band through the majority of the test while dropping to near pre-tilt values by the end of the test. Supine pre-tilt values were slightly lower following HDBR (approximately 3 mm/Hg).

Although pressure for both 2 hr conditions tended to drop off at 1500 seconds, all of the intervention treatments maintained pressure within the 95% control limits (fig. 2, Panels C and D). As with HR, a slight interaction can be seen. While the 4 hr curves are basically indistinguishable and have slight positive slopes, the 2 hr curves begin to separate and decline at 800 sec. Changes in MAP for the 2 hr conditions confirm changes observed in HR. While the 2 hr standing condition produced higher HR than the 2 hr walk, the 2 hr walk produced greater pressure increases than the 2 hr stand. This reflects the attempt to compensate for lower pressure with higher HR.

Systolic blood pressure and diastolic blood pressure responses—Similar results can be seen for SBP (fig. 3) and DBP (fig. 4). Since MAP is a linear composite formed from SBP and DBP, the responses across the tilt test, by definition, would be similar.

Orthostatic Tolerance: Syncope Prevalence

The control chart and Mantel-Haenszel statistics for "survival rates" (proportion of subjects completing a tilt test without experiencing syncope or pre-syncopal symptoms) are presented in figure 5. Panel A of figure 5 gives the survival rates for the five pre-HDBR tests and the five post-HDBR treatments. Survival rates are based on nine subjects except for both 2 hr conditions, where one subject dropped out of the study, and the 0 G z condition, where one subject became ill. Survival rates during pre-HDBR ranged from 1.00 to 0.75 with an average survival rate of 0.86. The lower bound of the approximate 95% control limit calculated from the range and standard deviation of the five pre-HDBR rates was 0.62, [0.86 – 1.96(0.12)]. The upper limit is bounded by 1.00. This indicates that under normal ambulatory conditions it would not be that unlikely that this sample of nine
Figure 5. Tilt test survival rates (proportion completing the test) by treatment conditions for pre- and post-HDBR measurements (Panel A, survival rates and lower limit of the approximate 95% confidence interval; Panel B, Mantel-Haenszel comparison of post-HDBR rates to the pre-HDBR average). The traditional chi squared statistic was converted to $z$ for ease of interpretation (critical value of $z = 1.96$ for a Type I error rate of 0.05). Value of $z$ statistics and associated Type I probability levels are included in the figure. OG, S4, W4, S2, and W2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions.
subjects would vary in their response to the tilt test (survival rate) by as much as ±24%. Therefore, until a treatment demonstrated a decrease in average survival rate of at least 24%, the difference would not be large enough to conclude an effect when compared to normal variation in survival rates when the subjects were ambulatory. Panel B presents the results of the Mantel-Haenszel comparisons of each post-HDBR survival rate to the average pre-HDBR rate. The traditional chi squared statistic was converted to z for ease of interpretation (critical value of z = 1.96 for a Type I error rate of 0.05).

The results from both analyses show a decrease in survival rates (orthostatic tolerance) from pre-HDBR conditions for the 0 G, 4 hr walk, and 2 hr walk, although the 2 hr walk condition was borderline. Both standing conditions seemed to maintain pre-HDBR levels with the 4 hr condition showing slightly better results. Walking did not improve the survival rate as both walking conditions were lower than both standing conditions. The results seem to indicate that passive Gz provides more protection than active Gz at least when compared to low level activity (walking). Perhaps the active conditions were less effective due to a lower orthostatic challenge resulting from augmented venous return during walking.

It was expected that HR and BP data would to some degree coincide with the survival rates. Only in the no intervention condition (0 Gz) did this seem to be true. Heart rate for the no intervention condition showed the highest elevation during tilt and was associated with the lowest survival rate. However, no relationship was seen between HR or BP and survival rates within the four intervention conditions.

**Peak Oxygen Consumption**

The results for the pre- to post-HDBR differences in VO₂peak are given in figure 6. The 95% confidence intervals around the treatment mean differences indicated that a non zero decline in VO₂peak occurred across all treatments (none of the confidence intervals included zero). All of the intervention treatments (Ps ≤ 0.0124) demonstrated significantly smaller decreases in VO₂peak than the 0 Gz condition. However, the 2 hr standing treatment, although lower, was not significantly different from the untreated condition (P = 0.1702). As expected, the walking conditions showed less of a decrease than the standing conditions. However, the 4 hr standing condition was an improvement over the 0 Gz condition. The results tended to favor the activity conditions with less of the effect due to exposure time.

**Plasma Volume**

The results for the pre- to post-HDBR difference in PV are given in figure 7. Both 4 hr conditions showed less PV loss by the end of HDBR as compared to the untreated, 0 Gz condition (S4: P = 0.0623; W4: P = 0.0383). No difference in PV change from pre- to post-HDBR was detected between the 0 Gz and the 2 hr conditions (P ≥ 0.7375). The results indicate that treatment conditions with a longer exposure time (4 hr) were most effective, with little difference attributable to activity (walking versus standing). However, the 4 hr walking condition produced the least amount of pre- to post-HDBR change (see adjusted means and confidence intervals).

**Urinary Calcium**

Since urinary Ca was measured daily during HDBR, a more complete analysis over days based on statistical modeling was possible. Figure 8 (Upper Panels) presents the actual urinary Ca output and actual % change in urinary Ca by treatment condition and day. Figure 8 (Lower Panel) gives the predicted urinary Ca based on a quadratic polynomial model. Predicted urinary Ca was based on a linear statistical model containing a constant, a linear, and a quadratic term. The coefficients from the linear and quadratic components for each treatment condition were compared to assess differences in response across HDBR. This was done by estimating the linear and quadratic terms for each subject and then testing the difference in these terms across treatment conditions. As described in the methods section, after the linear and quadratic coefficients were estimated for each subject, these coefficients were analyzed with analysis of covariance holding the initial Ca output (value at ambulatory control day) constant.

Figure 9 presents the analysis of the linear component. Although all of the treatment conditions showed a positive linear increase in Ca excretion over HDBR days, the rate of change was, for the most part, constant for all treatment groups. On the average, the linear rate of change per day was approximately 10 mg (see adjusted means and confidence levels). The analysis of the quadratic component is given in figure 10. This component represents the amount of change (bend in the trend) over time. The results indicate that the quadratic trend over time for the two standing conditions (both 2 and 4 hr) was not different from the trend for the 0 G condition (untreated). In contrast, the two walking conditions were much flatter with only slight positive quadratic trends (W2: P = 0.0109; W4: P = 0.0262). (See fig. 8, Predicted Calcium.)
### Peak VO2

**Adjusted* Mean Differences**  
*(Pre- Minus Post-Bedrest)*

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**Pairwise Comparisons (OG to Other Treatments)**

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* Adjusted for Pre-Bedrest Values. Overall F test for treatment differences (ANCOVA) = 4.96; df = 4, 29; P = .0036.

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*Figure 6. Pre- to post-HDBR differences in $\dot{V}O_2^{\text{peak}}$. OG, S2, W2, S4, and W4 denote respectively the no treatment and the 2 hr stand, 2 hr walk, 4 hr stand, and 4 hr walk treatment conditions. Values are adjusted mean differences ± SE (pooled).*
Plasma Volume

Adjusted* Mean Differences
(Pre- Minus Post-Bedrest)

\[ \text{SE(pooled)} = 55.84 \]

* Adjusted for Pre-Bedrest Values. Overall F test for treatment differences (ANCOVA) = 2.12; df = 4, 29; \( P = .1035 \).

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Pairwise Comparisons (OG to Other Treatments)

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Figure 7. Pre-to post-HDBR differences in plasma volume. OG, S2, W2, S4, and W4 denote respectively the no treatment and the 2 hr stand, 2 hr walk, 4 hr stand, and 4 hr walk treatment conditions. Values are adjusted mean differences ± SE (pooled).
Figure 8. Actual (upper panels) and predicted (lower panel) urinary calcium by treatment condition and HDBR days. Values for actual and predicted urinary Ca are means; values for actual urinary Ca (% change) are means ± SE. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days. O0G, S2, S4, and W4 denote respectively the no treatment and the 2 hr stand, 2 hr walk, 4 hr stand, and 4 hr walk treatment conditions.
Urinary Calcium
Adjusted* Linear Coefficients

* Adjusted for Pre-Bedrest Values. Overall F test for treatment differences (ANCOVA) = 0.545; df = 4, 29; P = .7038.

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Pairwise Comparisons (OG to Other Treatments)

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Figure 9. Linear change in urinary calcium by treatment conditions during HDBR. OG, S2, W2, S2, and W4 denote respectively the no treatment and the 2 hr stand, 2 hr walk, 4 hr stand, and 4 hr walk treatment conditions. Values are means ± SE (pooled).
* Adjusted for Pre-Bedrest Values. Overall F test for treatment differences (ANCOVA) = 3.82; df = 1, 29; P = .0130.

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Figure 10. Quadratic change in urinary calcium by treatment conditions during HDBR. OG, S2, W2, S4, and W4 denote respectively the no treatment and the 2 hr stand, 2 hr walk, 4 hr stand, and 4 hr walk treatment conditions. Values are means ± SE (pooled).
In summary, the two walking conditions attenuated the increase in Ca output seen in the 0 G and standing conditions. The 0 G and standing conditions showed a steady increase in Ca excretion across HDBR which levelled off by HDBR day 4. The walking conditions showed very little trend as Ca output did not increase, and remained relatively stable and comparable to values obtained prior to HDBR (ambulatory control day).

Conclusions

Can cardiovascular deconditioning be attenuated with periodic exposure to +1 Gz? The answer seems to depend on which physiological variable is being evaluated and how cardiovascular adaptation is defined. The results of this study indicate that cardiovascular changes attributable to HDBR can be attenuated to some degree by periodic +1 Gz gravitational field (standing). Mild activity within this field did seem to augment the effects of this countermeasure although orthostatic intolerance was attenuated to a greater extent by standing than walking. Although this may at first seem surprising, the specificity of the treatment in the standing conditions may have allowed the subjects to maintain their orthostatic tolerance in response to the tilt test, since standing imposed a greater orthostatic challenge than walking because walking contributes to venous return via the skeletal muscle pump.

Although HR and BP were not restored completely to pre-HDBR levels by any of the four treatments during HDBR, the response (survival rate) to the tilt test in the subjects when they stood 2 or 4 hr/day did return to what was typically observed during their ambulatory control, pre-HDBR tilt tests. The control of BP during orthostatic challenge involves complex physiological mechanisms. The results here suggest that orthostatic intolerance can be controlled even when peripheral cardiovascular measures are altered. Indeed it is controlled because of appropriate cardiovascular responses (Convertino, 1993). Compensatory mechanisms clearly allow for some alterations in such cardiovascular indexes without complete loss of the ability to maintain adequate head level pressures. The data also indicate that the orthostatic response in these subjects following HDBR is mostly compensated for by an increase in HR. Changes in BP were far less than those seen for HR and in most cases fell within the control limits.

A summary of the results is given in table 3. No single treatment was most effective across all dependent variables. The results suggest that the preventive effects of different countermeasures are system specific. As expected, Ca balance responded favorably to activity in +1 Gz whereas orthostatic tolerance and PV were less dependent on activity and more dependent on the orthostatic stimulus provided by the +Gz field. It is not known whether muscle and bone require additional G force intensity over and above that provided by standing or whether they respond specifically to activity whatever the G field. The momentary high G stress and muscle activity provided by walking seem to be important components for maintaining bone density and fitness (Whalen et al., 1988).

In most cases, differences between the 2 and 4 hr exposures were small. This suggests that 2 hr/day of standing or walking would be sufficient to counteract the negative effects of microgravity. In the case of Ca excretion, less than 2 hr of walking may suffice. The most efficient time schedule for this 2 hr of activity needs further investigation. Should the countermeasures be given in one 2-hr dosage or should dosages of shorter duration (as we did here) be given throughout the day? There is ample evidence throughout physiology that systems respond to signal and intensity change rather than to the duration of the stimulus. Factors such as fatigue, crew work

Table 3. Summary of results. S2, S4, W2, and W4 denote the 2 hr stand, 4 hr stand, 2 hr walk, and 4 hr walk treatment conditions

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+++ Most effective.
++ Effective.
+ Partially effective.
o Not effective.
schedules, and crew size in the real environment of spaceflight will also need to be considered. Using centrifugation to increase the intensity of the G stimulus further introduces the possibility of undesirable Coriolis side effects. Ideally the shorter exposures would work best from a flight logistics, psychological, and compliance perspective. Future studies in this field must assess comprehensive physiological changes if they are to contribute to our understanding of G stimulus requirements.

The most effective and physiologically comprehensive G replacement treatment is one that is most like what we experience in our everyday life on Earth, including both passive Gz and Gz with activity. What is surprising is how little of that exposure the human body seems to need to maintain normal health. Whatever the eventual optimal protocol, the present findings clearly emphasize that periodic Gz exposure can adequately prevent deconditioning associated with simulating the effects of weightlessness.

References


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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment condition and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions.
### Daily Weight, kg

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OG, 1GS4, and 1GW4 denote respectively the no treatment condition and the 4 hr stand and 4 hr walk treatment conditions. C1, BR1, BR2, BR3, BR4, and R+1 denote ambulatory control, HDBR, and recovery days.
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1GS2 and 1GW2 denote respectively the 2 hr stand and 2 hr walk treatment conditions. C1, BR1, BR2, BR3, BR4, and R+1 denote ambulatory control, HDBR, and recovery days.
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| MEAN | 106 | 169 | 111 | 177 |
| SE  | 4   | 5   | 5   | 5   |

OG and 1GS4 denote respectively the no treatment and the 4 hr stand treatment conditions. Pre-BR and Post-BR denote pre- and post-HDBR.
### Peak VO2 submaximal and maximal heart rate (HR) in beats per min (bpm), where "submaximal" is HR observed after 5 min at 400 kg-m/min

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### Peak VO2 submaximal and maximal heart rate (HR) in beats per min (bpm), where "submaximal" is HR observed after 5 min at 400 kg-m/min

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1GW4 and 1GS2 denote respectively the 4 hr walk and 2 hr stand treatment conditions. Pre-BR and Post-BR denote pre- and post-HDBR.
Peak VO2 submaximal and maximal heart rate (HR) in beats per min (bpm), where "submaximal" is HR observed after 5 min at 400 kg-m/min

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Peak VO2, L/min

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0G, 1GS4, 1GW4, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, and 2 hr walk treatment conditions. Pre-BR and Post-BR denote pre- and post-HDBR.
### Peak VO2, L/min

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### Peak VO2, ml/kg/min

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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Pre-BR and Post-BR denote pre- and post-HDBR.
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### Orthostatic Tolerance, no faint (NF) or faint (F plus time into tilt at which syncope occurred, rounded to nearest 15 seconds)

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### Orthostatic Tolerance, no faint (NF) or faint (F plus time into tilt at which syncope occurred, rounded to nearest 15 seconds)

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OG, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Pre-BR and Post-BR denote pre- and post-HDBR.
Tilt Test: Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg), Mean Arterial Pressure (mmHg), and Heart Rate (bpm) before and after a 60° head-up tilt test

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<th>Diastolic Blood Pressure, mmHg</th>
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<table>
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<th>Heart Rate, bpm</th>
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Pre-Tilt values are averages of measurements taken in the 15-second period preceding one minute before start of tilt. Test End Point values are single point measurements taken at completion of the test (30 min or syncope). Parenthetic end point values are an average over the last 15 seconds prior to end of test. Pre-Tilt and Test End Point values for Subjects #249, #394, #405 for Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), and Heart Rate (HR) are single point hand recorded values; Mean Arterial Pressure (MAP) for these subjects was calculated using the formula: MAP = (SBP - DBP)/3 + DBP. Measurements for Subject #439 were not used: data was deleted because subject had fever and fainted at 2:30 min.
Tilt Test: Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg), Mean Arterial Pressure (mmHg), and Heart Rate (bpm) before and after a 60° head-up tilt test

**0G Condition (Post-HDBR)**

<table>
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<th>Systolic Blood Pressure, mmHg</th>
<th>Diastolic Blood Pressure, mmHg</th>
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Pre-Tilt values are averages of measurements taken in the 15-second period preceding one minute before start of tilt. Test End Point values are single point measurements taken at completion of the test (30 min or syncope). Parenthetic end point values are an average over the last 15 seconds prior to end of test. Test End Point values for Subjects #439, #435, #434 are averages of measurements taken in the final 15-sec period of the test before completion of the test.
Tilt Test: Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg), Mean Arterial Pressure (mmHg), and Heart Rate (bpm) before and after a 60° head-up tilt test

**S4 Condition (Pre-HDBR)**

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Pre-Tilt values are averages of measurements taken in the 15-second period preceding one minute before start of tilt. Test End Point values are single point measurements taken at completion of the test (30 min or syncope). Parenthetic end point values are an average over the last 15 seconds prior to end of test. Test End Point values for Subject #394 are averages of measurements taken in the final 15-sec period of the test before completion of the test.
### Tilt Test: Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg), Mean Arterial Pressure (mmHg), and Heart Rate (bpm) before and after a 60° head-up tilt test

#### S4 Condition (Post-HDBR)

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Pre-Tilt values are averages of measurements taken in the 15-second period preceding one minute before start of tilt.
Test End Point values are single point measurements taken at completion of the test (30 min or syncope). Parenthetic end point values are an average over the last 15 seconds prior to end of test.
## Tilt Test: Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg), Mean Arterial Pressure (mmHg), and Heart Rate (bpm) before and after a 60° head-up tilt test

### W4 Condition (Pre-HDBR)

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Pre-Tilt values are averages of measurements taken in the 15-second period preceding one minute before start of tilt. Test End Point values are single point measurements taken at completion of the test (30 min or syncope). Parenthetic end point values are an average over the last 15 seconds prior to end of test. Test End Point values for Subjects #434, #405 are averages of last 15 second measurements. Pre-Tilt and Test End Point values for Subject #243 and Test End Point values for Subject #279 for Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), and Heart Rate (HR) are single point hand recorded values; Mean Arterial Pressure (MAP) for these subjects was calculated according to the formula: MAP = (SBP - DBP)/3 + DBP.
### Tilt Test: Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg), Mean Arterial Pressure (mmHg), and Heart Rate (bpm) before and after a 60° head-up tilt test

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Pre-Tilt values are averages of measurements taken in the 15-second period preceding one minute before start of tilt. Test End Point values are single point measurements taken at completion of the test (30 min or syncope). Parenthetic end point values are an average over the last 15 seconds prior to end of test.
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Pre-Tilt values are averages of measurements taken in the 15-second period preceding one minute before start of tilt. Test End Point values are single point measurements taken at completion of the test (30 min or syncope). Parenthetical end point values are an average over the last 15 seconds prior to end of test.
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Pre-Tilt values are averages of measurements taken in the 15-second period preceding one minute before start of tilt. Test End Point values are single point measurements taken at completion of the test (30 min or syncope). Parenthetical end point values are an average over the last 15 seconds prior to end of test.
### Tilt Test: Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg), Mean Arterial Pressure (mmHg), and Heart Rate (bpm) before and after a 60° head-up tilt test

#### W2 Condition (Post-HDBR)

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Pre-Tilt values are averages of measurements taken in the 15-second period preceding one minute before start of tilt. Test End Point values are single point measurements taken at completion of the test 30 min or syncope). Parenthetic end point values are an average over the last 15 seconds prior to end of test. Pre-Tilt and Test End Point values for Subject #434 and Test End Point values for Subject #439 for Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), and Heart Rate (HR) are single point hand recorded values; Mean Arterial Pressure (MAP) for these subjects was calculated according to the formula: MAP = (SBP - DBP)/3 + DBP.
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<th>1GS4 Control</th>
<th>1GS4 BR4</th>
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OG, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4.
## Plasma Volume, ml/kg

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| MEAN | 32.8 | 29.6 | 31.4 | 30.2 | 35.9 | 34.3 |
| SE   | 2.5  | 2.0  | 1.9  | 1.9  | 3.6  | 3.4  |

## Plasma Volume, ml/kg

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| MEAN | 29.2 | 26.4 | 28.2 | 26.2 |
| SE   | 1.3  | 0.9  | 0.8  | 1.0  |

OG, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4.
### Blood Hematocrit, Hct (%)

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| MEAN        | 43.3                  | 46.6  | 44.1                    | 44.5    | 43.3                    | 44.1    |
| SE          | 1.0                   | 1.2   | 1.2                     | 1.4     | 1.1                     | 1.2     |

### Blood Hematocrit, Hct (%)

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| SE          | 1.3                    | 1.3      | 1.1                     | 1.1     |

OG, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4.
### Blood Volume, ml

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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4.
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OG, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4.
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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4.
0G, 1GS4, and 1GW4 denote respectively the no treatment and the 4 hr stand and 4 hr walk treatment conditions. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1. Plasma AVP (Supine Control and BR1 4-HR) data were not analyzed for the 2 hr stand and 2 hr walk conditions.
**Plasma Norepinephrine (NE), pg/ml**

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**Plasma Norepinephrine (NE), pg/ml**

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0G, 1GS4, and 1GW4 denote respectively the no treatment and the 4 hr stand and 4 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from samples drawn on HDBR day 4. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1. Plasma E data were not analyzed for the 2 hr stand and 2 walk conditions.
### PRA, ng/ml/hr

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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4.
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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1.
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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4.
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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1.
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OG, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1.
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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4.
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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR 1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1.
### Plasma Osmolality, mOsm/kg

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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4.
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### Plasma Osmolality, mOsm/kg

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OG, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1.
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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4. Serum Total Protein data were not analyzed for the first HDBR exposure.
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OG, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1. Serum Total Protein data were not analyzed for the first HDBR exposure.
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OG and 1GW4 denote respectively the no treatment and the 4 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1.
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0G and 1GW4 denote respectively the no treatment and the 4 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1.
### Plasma LDL Cholesterol, mg/dl

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0G and 1GW4 denote respectively the no treatment and the 4 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1. Plasma LDL cholesterol values were calculated using the formula: Plasma LDL cholesterol = Plasma total cholesterol - [Plasma HDL cholesterol + (Plasma triglycerides/5)].
## Plasma Triglycerides, mg/dl

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0G and 1GW4 denote respectively the no treatment and the 4 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1.
## Serum Creatinine, mg/dl

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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Ambulatory Control values are from blood samples drawn on the ambulatory control day (first samples drawn that day); BR4 values are from blood samples drawn on HDBR day 4.
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0G, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. Supine Control values are from blood samples drawn on the ambulatory control day after subjects had been lying quietly for 45 min (second samples drawn that day); BR1 4-HR values are from blood samples drawn 4 hr after beginning HDBR on HDBR day 1.
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| SE          | 76   | 108  | 252 | 143  | 109 |

### Urinary Creatinine, mg/24 hr

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| MEAN        | 2043 | 2146 | 2223 | 2458 | 2265 |
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### Urinary Creatinine, mg/24 hr

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| MEAN        | 2037 | 2348 | 2158 | 2296 | 2147 |
| SE          | 138  | 54   | 147  | 159  | 150  |

0G, 1GS4, and 1GW4 denote respectively the no treatment condition and the 4 hr stand and 4 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
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1GS2 and 1GW2 denote respectively the 2 hr stand and 2 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
### Glomerular Filtration Rate (GFR), dl/min

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### Glomerular Filtration Rate (GFR), dl/min

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OG, 1GS4, 1GW4, 1GS2, and 1GW2 denote respectively the no treatment and the 4 hr stand, 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. GFR values were calculated using the formula: GFR = (Urinary creatinine excretion rate/Serum creatinine)/1440. Ambulatory Control values were calculated using urinary creatinine data from the ambulatory control day and serum creatinine data from blood samples drawn on the ambulatory control day (first samples drawn that day). BR4 values were calculated using urinary creatinine data from HDBR day 4 and serum creatinine data from blood samples drawn on HDBR day 4.
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1GS4, 1GW4, and 1GS2 denote respectively the 4 hr stand, 4 hr walk, and 2 hr stand treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
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0G, 1GS4, and 1GW2 denote respectively the no treatment condition and the 4 hr stand and 2 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.

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1GW4, 1GS2, and 1GW2 denote respectively the 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
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0G, 1GS4, and 1GW4 denote respectively the no treatment condition and the 4 hr stand and 4 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory and HDBR days.
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**MEAN** | **158.7** | **179.5** | **187.7** | **170.7** | **160.5**

**SE** | **8.9** | **7.1** | **7.7** | **4.4** | **4.3**

### Sodium (Na) Intake, mEq/24 hr

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**SE** | **16.9** | **13.1** | **9.5** | **6.7** | **7.7**

0G, 1GS2, and 1GW2 denote respectively the no treatment condition and the 2 hr stand and 2 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
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1GS4, 1GW4, and 1GS2 denote respectively the 4 hr stand, 4 hr walk, and 2 hr stand treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
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0G and 1GW2 denote respectively the no treatment condition and the 2 hr walk treatment condition. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
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1GS4 and 1GW4 denote respectively the 4 hr stand and 4 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.

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Sodium (Na) Balance, mEq/24 hr, determined from Urinary Sodium (Na) minus Sodium (Na) Intake

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1GS2 and 1GW2 denote respectively the 2 hr stand and 2 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.

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1GS2 and 1GW2 denote respectively the 2 hr stand and 2 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
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1GS4, 1GW4, and 1GS2 denote respectively the 4 hr stand, 4 hr walk, and 2 hr stand treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
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| SE         | 6.4  | 5.3  | 3.4  | 5.0  | 2.4  |

OG and 1GW2 denote respectively the no treatment condition and the 2 hr walk treatment condition. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
Potassium (K) Balance, mEq/24 hr, determined from Urinary Potassium (K) minus Potassium (K) Intake

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1GS4 and 1GW4 denote respectively the 4 hr stand and 4 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
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1GS2 and 1GW2 denote respectively the 2 hr stand and 2 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.

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0G, 1GS4, and 1GW4 denote respectively the no treatment condition and the 4 hr stand and 4 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
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0G, 1GS2, and 1GW2 denote respectively the no treatment condition and the 2 hr stand and 2 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
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1GS4, 1GW4, and 1GS2 denote respectively the 4 hr stand, 4 hr walk, and 2 hr stand treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
### Daily Fluid (Urinary) Output, ml/24 hr

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| MEAN        | 2022 | 1656 | 1421 | 1410 | 1213 |
| SE          | 198  | 209  | 114  | 150  | 142  |

### Daily Fluid Balance, ml/24 hr, determined from Fluid Out minus Fluid In

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| MEAN        | 9    | 109  | 440  | 322  | 342  |
| SE          | 220  | 92   | 180  | 131  | 151  |

### Daily Fluid Balance, ml/24 hr, determined from Fluid Out minus Fluid In

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| MEAN        | 61   | 242  | 153  | 288  | 256  |
| SE          | 89   | 76   | 65   | 70   | 60   |

OG, 1GS4, and 1GW2 denote respectively the no treatment condition and the 4 hr stand and 2 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
### Daily Fluid Balance, ml/24 hr, determined from Fluid Out minus Fluid In

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1GW4, 1GS2 and 1GW2 denote respectively the 4 hr walk, 2 hr stand, and 2 hr walk treatment conditions. C1, BR1, BR2, BR3, and BR4 denote ambulatory control and HDBR days.
Intermittent Gravity: How Much, How Often, How Long?

Joan Vernikos and David A. Ludwig*

Ames Research Center
Moffett Field, CA 94035-1000

National Aeronautics and Space Administration
Washington, DC 20546-0001

Point of Contact: Joan Vernikos, Ames Research Center, MS 239-11, Moffett Field, CA 94035-1000; (415) 604-3736

*University of North Carolina, Greensboro, North Carolina

Continuous exposure to gravity may not be necessary to prevent the deconditioning effects of microgravity. It is not known, however, what the minimum gravity (G) exposure requirements are, whether they vary for different physiological systems, or whether passive Gz (gravity in the head-to-toe vector) or activity in a G field is more effective in preventing deconditioning. It is also not known what the optimal characteristics of the G stimulus should be in terms of amplitude, duration, and frequency. To begin to address these questions, we conducted a 4-day -6° head-down bed rest (HDBR) study. Nine males (aged 30–50 yr) were subjected, over a period of seven months, to four different +1 Gz exposure protocols (periodic standing or controlled walking each for a total of 2 or 4 hr/day in individual 15-min doses), plus a control (0 Gz) of continuous HDBR. The study consisted of one ambulatory control day, 4 full days of -6° HDBR, and a recovery day when subjects were released at the end of HDBR after completion of tests. A battery of tests was selected and standardized in order to evaluate the known early responses to HDBR. Dependent variables of interest included orthostatic tolerance (30 min at 60° head-up tilt) and hemodynamics during head-up tilt, peak oxygen consumption (VO2peak) plasma volume (PV), and urinary calcium (Ca).

The results were as follows: (1) 4 hr standing completely prevented and 2 hr walking partially prevented post-HDBR orthostatic intolerance. Walking at 3 mi/hr for 4 hr/day provided no additional benefit. (2) Intermittent walking attenuated, but did not prevent, the decrease in VO2peak. (3) Both 4 hr conditions showed less PV loss by the end of HDBR; both 2 hr conditions were without effect. (4) Both 2 and 4 hr walking essentially prevented urinary Ca excretion and were more effective than standing. It is concluded that different physiological systems benefit differentially from passive +1 Gz or activity in +1 Gz, and the intensity of the stimulus may be an important contributing factor.

Key Words: Head-down bed rest, Intermittent gravity, Weightlessness

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Point of Contact: Joan Vernikos, Ames Research Center, MS 239-11, Moffett Field, CA 94035-1000; (415) 604-3736
*University of North Carolina, Greensboro, North Carolina

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