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Evaluation of Treadmill Exercise in a Lower Body Negative Pressure Chamber as a Countermeasure for Weightlessness-Induced Bone Loss: A Bed Rest Study With Identical Twins

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ABSTRACT

Counteracting bone loss is required for future space exploration. We evaluated the ability of treadmill exercise in a LBNP chamber to counteract bone loss in a 30-day bed rest study. Eight pairs of identical twins were randomly assigned to sedentary control or exercise groups. Exercise within LBNP decreased the bone resorption caused by bed rest and may provide a countermeasure for spaceflight.

Introduction: Bone loss is one of the greatest physiological challenges for extended-duration space missions. The ability of exercise to counteract weightlessness-induced bone loss has been studied extensively, but to date, it has proven ineffective. We evaluated the effectiveness of a combination of two countermeasures—treadmill exercise while inside a lower body negative pressure (LBNP) chamber—on bone loss during a 30-day bed rest study.
Materials and Methods: Eight pairs of identical twins were randomized into sedentary (SED) or exercise/LBNP (EX/LBNP) groups. Blood and urine samples were collected before, several times during, and after the 30-day bed rest period. These samples were analyzed for markers of bone and calcium metabolism. Repeated measures ANOVA was used to determine statistical significance. Because identical twins were used, both time and group were treated as repeated variables.
Results: Markers of bone resorption were increased during bed rest in samples from sedentary subjects, including the collagen cross-links and serum and urinary calcium concentrations. For N-telopeptide and deoxypyridinoline, there were significant ($p < 0.05$) interactions between group (SED versus EX/LBNP) and phase of the study (sample collection point). Pyridinium cross-links were increased above pre-bed rest levels in both groups, but the EX/LBNP group had a smaller increase than the SED group. Markers of bone formation were unchanged by bed rest in both groups.
Conclusions: These data show that this weight-bearing exercise combined with LBNP ameliorates some of the negative effects of simulated weightlessness on bone metabolism. This protocol may pave the way to counteracting bone loss during spaceflight and may provide valuable information about normal and abnormal bone physiology here on Earth.
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Key words: spaceflight, bed rest, simulated weightlessness, bone resorption, bone markers

INTRODUCTION

SPACEFLIGHT EXERCISE PROTOCOLS implemented to date have failed to protect against weightlessness-induced bone loss.[1–4] While frequency (number of cycles) and load are key parameters in assessment of exercise, the failure to

protect bone during weightlessness is generally hypothesized to result from using insufficient loads to maintain bone mass.[5–8] The bungee-cord loading apparatus used by crew members onboard the Mir space station was too uncomfortable to generate loads over 60–70% of their body weight,[9] thus strengthening this hypothesis. Mir crewmembers with varying degrees of exercise compliance (very high to very low) but similar mechanical loads had virtually the same

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amount of bone calcium loss,[10] again suggesting that inadequate load may be the main reason bone loss was not affected. Bone remodeling studies[5,11] suggest that bone tissue maintenance is relatively insensitive to the frequency of loading per day. Evidence of this from spaceflight exists—running on a treadmill for 2–3 h/day did not prevent bone loss in Mir cosmonauts.

Ground-based studies have also examined the effectiveness of varied exercise protocols on maintaining or increasing bone mass. In a recent 8-month randomized controlled study of ambulatory women,[12] gains in bone density produced by high-impact exercise were maintained by twice-per-week aerobic and step exercises. High-impact exercise was also found to promote increased bone mass in female athletes.[13]

Ground reaction forces during running are about three times higher than during walking, and studies with human subjects suggest that bone is more responsive to load magnitude than to load frequency. Thus, we hypothesized that high-impact treadmill exercise, such as that obtained with treadmill exercise during lower body negative pressure (LBNP) exposure,[14,15] would be efficacious for mitigating the bone loss associated with microgravity.

In addition to exercise-related loading of bone, circulatory factors may also play a role in weightlessness-induced bone loss. During spaceflight, blood pressure stimuli at the feet are low because gravitational blood pressures are absent.[16] Reduced blood flow to the lower extremities has been postulated as a mechanism related to, or responsible for, bone loss during weightlessness.[17] Thus, reduced loads on the musculoskeletal system and the loss of gravitational blood pressures together may explain the 1–2% bone loss per month experienced during spaceflight.[17] In addition to producing a higher musculoskeletal load, the proposed exercise within LBNP system could also create Earth-like vascular loads during exercise in microgravity. This would allow a combination of countermeasures (loading and circulatory factors) aimed at mitigating bone loss.

The concept of treadmill exercise during LBNP has developed over the past decade.[14,18,19] We have recently tested supine treadmill exercise within an LBNP chamber, in which a vertical treadmill is mounted inside an LBNP chamber. These tests demonstrated that one can comfortably run on the treadmill for well over 40 minutes daily at up to 1.2 times body weight (~60 mm Hg). The system includes partial compression shorts over the lower abdomen, as a prophylaxis for preventing excessive blood pooling or herniation in the lower abdomen. Previous bed rest data suggest that this system improves cardiovascular and muscular responses to bed rest, and the responses are equivalent to upright treadmill exercise.[15,20–22]

In the current study, we tested the hypothesis that treadmill exercise during LBNP will ameliorate weightlessness-induced bone loss. Head-down tilt bed rest, a ground-based analog of weightlessness, was used as the model system. While subjects undergoing bed rest for less than 5 weeks do not show significant decreases in skeletal mineral content (as determined by DXA), clear evidence from biochemical markers shows that the adult human skeleton is affected by unloading within hours to days.[23,24] We measured bio-

TABLE 1. SUBJECT CHARACTERISTICS (MEAN ± SD)

Characteristic	Sedentary group	Exercise/LBNP group
n	8	8
Age (years)	27.1 ± 5.0	27.1 ± 5.0
Height (cm)	176 ± 14	174 ± 13
Weight (kg)*		
Beginning of study	69.4 ± 10.9	66.5 ± 9.5
End of bed rest	69.5 ± 10.9	66.9 ± 9.6

* No significant difference between groups or before and after bed rest.

chemical markers of bone and mineral metabolism in blood and urine samples collected before, during, and after 30 days of head-down tilt bed rest.

The effects of both real and simulated weightlessness include loss of bone mass,[25–27] decreased calcium absorption,[28] increased calcium excretion,[23,25,27–30] increased renal stone risk,[29,31] and decreased serum concentrations of parathyroid hormone[23,27] and 1,25-dihydroxyvitamin D.[23,27,28] While the qualitative effects of bed rest on bone and calcium homeostasis are similar to those of spaceflight, the magnitude of these effects is generally reduced in the ground analog.

MATERIALS AND METHODS

Subjects

Eight pairs of male identical twins were selected for inclusion in the study after a thorough medical examination. Subject characteristics are shown in Table 1. Within twin pairs, one twin was randomly assigned to the sedentary group (SED) and the other to the exercise/LBNP (EX/LBNP) group, as determined by coin toss.

These studies were reviewed and approved by the Institutional Review Boards of the University of California at San Diego, the NASA Ames Research Center, and the NASA Johnson Space Center. All subjects provided informed written consent before participating.

Study design and bed rest protocol

The study was conducted at the University of California at San Diego General Clinical Research Center (GCRC), where subjects resided throughout the ambulatory, bed rest, and post-bed rest phases. A 6-day ambulatory session was conducted immediately before bed rest to familiarize subjects with the facility and testing procedures, and to conduct initial data collection.

The subjects were restricted to food and fluids provided by the GCRC. Daily dietary sodium intake was controlled at approximately 170 mEq (about 3.5 g/day), and energy intake was also controlled (about 2500–3000 kcal/day, depending on exercise level). Caloric intake was adjusted to maintain body weight. Calcium intake was 989 ± 116 and 1011 ± 125 mg/day pre-bed rest for the SED and EX/LBNP groups, respectively, and during bed rest was 979 ± 104 and 988 ± 145 mg Ca/day.

Body weight, fluid intake, bowel movement frequency, and urine output were monitored daily. During the ambu-
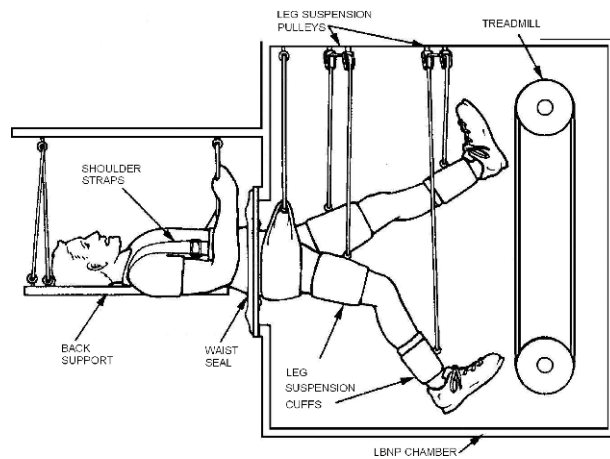
FIG. 1. Schematic diagram of the treadmill exercise within an LBNP chamber. A back support and leg suspension system is used to reduce the effects of gravity while exercising in the supine position.

latory phase, an activity log was maintained for each subject. The goal was for the subjects to maintain a neutral or positive fluid balance and to maintain body weight during the course of the study.

During the entire 30-day bed rest phase, all subjects remained in a 6° head-down tilt except during periods for bathing and exercise (totaling 0.5–1.5 h/day), during which time they were horizontal (0° tilt).

Exercise protocol

The exercising twin ran while supine in the LBNP chamber (see diagram in Fig. 1) 6 days/week, for 40 minutes each day, with 5 minutes of static LBNP at the conclusion of the run. Pressure in the LBNP chamber was adjusted to increase load and thereby increase exercise intensity. During supine treadmill exercise, LBNP (52–63 mm Hg) was applied to produce footward forces equivalent to those for upright running on Earth initially at 1.0 times body weight, increasing to 1.2 times body weight according to subject tolerance. The following interval exercise protocol was used: 7 minutes to warm up at 40% peak oxygen uptake, followed by 3 minutes at 60%, 2 minutes at 40%, 3 minutes at 70%, 2 minutes at 50%, 3 minutes at 80%, 2 minutes at 60%, 3 minutes at 80%, 2 minutes at 50%, 3 minutes at 70%, 2 minutes at 40%, 3 minutes at 60%, and 5 minutes to cool down at 40% peak oxygen uptake (40 minutes total) with an additional 5 minutes of supine, stationary exposure to 50 mm Hg LBNP.

Bone biochemical markers

Blood and urine samples were collected for subsequent analysis of biochemical markers of bone and calcium metabolism. Blood samples were collected by standard phlebotomy techniques into evacuated blood collection tubes. Fasting (8 h) blood samples were collected in the morning as follows: each of the 3 days immediately before bed rest, bed rest days 4 ($n = 2$ for each group) or 5 ($n = 6$ for each group), 12, 19, 26, and 31 (just before reambulation), and on the first 2 days after reambulation.

Urine samples (24-h pools) were collected on the 3 days before bed rest, on bed rest days 5 and 6, 12 and 13, 19 and 20, and 26 and 27, and on the first 2 days after reambulation. Urine voids were refrigerated immediately after collection, and aliquots were prepared for individual analytes. Blood and urine samples were stored frozen at $-80°C$ until analysis.

Total calcium in serum was measured by ion-sensitive electrode techniques (Beckman CX7; Beckman Instruments). Urinary total calcium was measured by inductively coupled plasma emission mass spectrophotometry techniques.[32]

Serum osteocalcin (intact molecule) was measured by commercial radioimmunoassay (Biomedical Technologies), with binding recognition site at the carboxy terminal end of the molecule. Serum parathyroid hormone was determined using a commercially available immunoradiometric assay to the intact peptide (Nichols Institute). Vitamin D metabolites in serum were determined using commercially available kits (Diasorin, Inc.). Serum bone-specific alkaline phosphatase was measured by enzyme linked immunosorbent assay (Quidel Corp., Santa Clara, CA, USA). Total alkaline phosphatase was determined using a colorimetric kinetic rate assay on an automated instrument (Beckman CX7).

Urine samples were analyzed for collagen cross-links using commercially available kits (Pyrilinks and Pyrilinks-D; Quidel Corp. and Osteomark ELISA kit; Ostex International, Inc., Seattle, WA, USA), as previously reported.[10,33] The PYD and DPD assays used did not involve a hydrolysis step, and thus, results reflect free cross-link excretion (as opposed to total).

Statistical analyses

Two-way repeated measures ANOVA was performed to assess the effect of exercise on adaptation to bed rest. Interpretation of the ANOVA results was facilitated by the assumption that pre-bed rest treatment groups were equivalent for the urinary and serum variables assessed, because the subjects were identical twins assigned to different treatment arms. As a consequence, differences between the means of the sedentary and exercised groups at the various points in time indicate treatment effects. The Bonferroni *t*-test was used post hoc to determine main effect differences between bed rest or reambulation data and pre-bed rest data and to assess specific differences between sedentary and exercised subjects over time. When significant interaction effects were identified, only these were reported (i.e., not main effects).

Two subjects were missing samples for one data collection session (urine data for bed rest day 5/6). Statistical analyses were performed with this reduced data set (i.e., six subjects) for this time point, because of the inability to perform the statistical analyses with an unbalanced data set.

The difference in body weight before and after bed rest was evaluated using two-way repeated measures ANOVA (with one repeated factor). Significance was assigned to $p < 0.05$. Statistical analyses were performed using SigmaStat (SPSS Inc.).

All data presented are mean $\pm$ SD. Data from multiple pre-bed rest sample collections were averaged for each

TABLE 2. SERUM MARKERS OF BONE AND CALCIUM METABOLISM BEFORE, DURING, AND AFTER 30 DAYS OF BED REST

	Pre-BR	BR 5*	BR 12	BR 19	BR 26	BR 31	Post-BR 1	Post-BR 2
Calcium (mmol/liter)								
SED	2.34 ± 0.07	2.37 ± 0.08	$2.40 \pm 0.09^{\ddagger}$	$2.39 \pm 0.08^{\dagger}$	$2.39 \pm 0.06^{\dagger}$	$2.38 \pm 0.08^{\dagger}$	2.36 ± 0.07	2.35 ± 0.12
EX/LBNP	2.32 ± 0.05	2.35 ± 0.08	$2.36 \pm 0.07^{\ddagger}$	$2.36 \pm 0.06^{\dagger}$	$2.35 \pm 0.06^{\dagger}$	$2.35 \pm 0.06^{\dagger}$	2.35 ± 0.07	2.37 ± 0.07
25(OH)-vitamin D (nmol/liter)								
SED	63 ± 28	69 ± 34	67 ± 24	70 ± 33	59 ± 17	62 ± 26	68 ± 24	59 ± 6
EX/LBNP	65 ± 22	66 ± 26	67 ± 25	73 ± 33	62 ± 16	63 ± 21	61 ± 19	66 ± 13
1,25(OH)$_2$-vitamin D (pmol/liter)								
SED	117 ± 39	$87 \pm 28^{\dagger}$	90 ± 26	$91 \pm 28^{\dagger}$	$84 \pm 17^{\dagger}$	$92 \pm 15^{\ddagger}$	99 ± 25	100 ± 14
EX/LBNP	131 ± 34	$106 \pm 21^{\dagger}$	105 ± 21	$97 \pm 16^{\dagger}$	$102 \pm 26^{\dagger}$	$90 \pm 24^{\ddagger}$	114 ± 43	104 ± 27
Parathyroid hormone (pg/ml)								
SED	21.9 ± 8.2	20.0 ± 8.2	$17.0 \pm 6.0^{\dagger}$	$16.5 \pm 6.5^{\ddagger}$	$16.9 \pm 6.5^{\dagger}$	$13.9 \pm 5.7^{\S}$	18.3 ± 5.9	22.6 ± 7.6
EX/LBNP	23.4 ± 11.8	20.0 ± 9.6	$19.1 \pm 10.4^{\dagger}$	$17.7 \pm 7.2^{\ddagger}$	$19.0 \pm 9.0^{\dagger}$	$15.7 \pm 10.8^{\S}$	22.0 ± 13.4	24.8 ± 9.1
BSAP (μg/liter)								
SED	22.9 ± 6.3	23.3 ± 5.0	24.1 ± 7.9	25.0 ± 10.2	24.3 ± 6.1	22.8 ± 5.3	25.9 ± 11.3	19.3 ± 4.7
EX/LBNP	23.3 ± 5.9	25.0 ± 11.3	23.3 ± 8.3	24.7 ± 8.4	23.8 ± 6.0	26.4 ± 12.8	26.1 ± 10.6	21.1 ± 5.9
Alkaline phosphatase (U/liter)								
SED	58 ± 12	60 ± 14	61 ± 11	61 ± 11	62 ± 11	61 ± 10	$63 \pm 10^{\dagger}$	56 ± 10
EX/LBNP	56 ± 10	55 ± 9	57 ± 8	56 ± 9	59 ± 9	59 ± 9	$60 \pm 10^{\dagger}$	60 ± 15
Osteocalcin (ng/ml)								
SED	17.7 ± 2.9	18.2 ± 1.9	17.7 ± 3.2	19.1 ± 3.5	18.6 ± 3.7	18.1 ± 3.6	16.7 ± 4.5	18.4 ± 2.8
EX/LBNP	16.8 ± 2.3	17.9 ± 2.7	17.2 ± 4.1	17.8 ± 2.6	17.3 ± 2.1	15.9 ± 3.5	16.3 ± 3.0	18.5 ± 2.4

Subjects were identical twins randomized into sedentary (SED) or engaged in treadmill exercise in an LBNP chamber (EX/LBNP).

BR, bed rest.

Data are mean $\pm$ SD for eight subjects per group except "Post-BR 2" for which $n = 4$ (note: "Post-BR 2" data were not included in statistical analyses).

* For six subjects in each group, data were collected on BR 5, and for two subjects, samples were collected on BR 4.

Significant difference from pre-bed rest for main effect (session) are denoted as $^{\dagger} p < 0.05$; $^{\ddagger} p < 0.01$; $^{\S} p < 0.001$.

subject before statistical analyses. Because of missing data from four of the eight subjects in each group (samples not collected), the post-bed rest day 2 serum data were not included in the statistical analysis.

RESULTS

All subjects completed the entire 39-day protocol in the GCRC without adverse events. As intended by the design, subjects did not lose body weight during the course of the study, regardless of treatment group (Table 1). There were no differences in pre-bed rest data between SED and EX/LBNP groups for any of the parameters (Tables 2 and 3). Urine volumes tended to be higher in the SED group compared with the EX/LBNP, but this was statistically significant only on bed rest day 12 (Table 3).

Serum calcium was increased during bed rest but tended to increase less in the exercise group than in controls (Table 2). Urine calcium excretion followed the same general pattern as serum (Table 3). Both serum and urinary calcium levels quickly returned to pre-bed rest levels after bed rest ended. Serum parathyroid hormone concentrations declined significantly beginning at BR12, and returned to pre-bed rest levels on the day after reambulation (Table 2). While there was no significant difference between the experimental and control means, the decrease during bed rest was greater in the control subjects. At the first measurement during bed rest (bed rest day 4 or 5) and throughout the bed rest period, serum concentrations of the active form of vitamin D, 1,25(OH)$_2$-vitamin D, were about 20% below pre-bed rest levels (Table 2), and they returned to pre-bed

rest levels on reambulation. Vitamin D stores [represented by serum 25(OH)-vitamin D concentrations] did not change in either group during bed rest. Exercise had no effect on serum vitamin D metabolite or parathyroid hormone concentrations.

Bone formation markers did not change during bed rest. Serum concentrations of bone-specific alkaline phosphatase (BSAP) and osteocalcin were unchanged during bed rest, in both SED and EX/LBNP groups (Table 2). Serum total alkaline phosphatase was slightly elevated toward the end of the bed rest period, becoming significantly elevated 24 h after reambulation (Table 2). There was no difference between sedentary and exercised subjects in the response of bone formation markers to bed rest.

Bone resorption markers were increased during bed rest, with the exercise/LBNP mitigating the effect of bed rest. NTX excretion in SED subjects was elevated from pre-bed rest levels beginning on BR12/13, returning towards pre-bed rest levels on reambulation (Table 3). NTX excretion in the EX/LBNP subjects became elevated compared with pre-bed rest levels on BR27 and remained elevated 1 day after reambulation. PYD levels in SED subjects were similarly elevated beginning on BR12/13 and remained elevated on reambulation. Likewise, DPD levels in the EX/LBNP subjects were elevated from pre-bed rest levels on BR26 and remained elevated for 1 day after reambulation. DPD and NTX excretion were significantly higher in SED subjects than EX/LBNP subjects beginning on BR12/13. There were no differences between the groups in these markers after reambulation. PYD excretion followed similar

TABLE 3. URINE MARKERS OF BONE AND CALCIUM METABOLISM BEFORE, DURING, AND AFTER 30 DAYS OF BED REST

	Pre-BR	BR 5/6	BR 12/13	BR 19/20	BR 26/27	Post-BR
Calcium (mmol/day)						
SED	5.67 ± 3.19	5.34 ± 3.31	6.86 ± 3.43	6.59 ± 3.05	7.48 ± 3.35*	5.66 ± 2.76
EX/LBNP	5.73 ± 3.15	5.03 ± 2.91	5.70 ± 3.31[†]	5.92 ± 3.25	6.03 ± 3.19[†]	5.71 ± 3.71
N-telopeptide (nmol/day)						
SED	580 ± 331 (40.2 ± 22.0)[‡]	631 ± 257	850 ± 393*	943 ± 412*	924 ± 359*	717 ± 285
EX/LBNP	566 ± 277 (42.1 ± 22.4)[‡]	574 ± 150	659 ± 314[†]	693 ± 250[†]	745 ± 303*[†]	753 ± 327*
Pyridinium crosslinks (nmol/day)						
SED	291 ± 143 (19.8 ± 8.7)[‡]	327 ± 172	414 ± 193**	456 ± 166**	498 ± 181**	436 ± 173[§]
EX/LBNP	308 ± 136 (22.1 ± 9.0)[‡]	321 ± 134	350 ± 133**	386 ± 139**	413 ± 153**	423 ± 174[§]
Deoxypyridinoline crosslinks (nmol/day)						
SED	73 ± 43 (5.1 ± 3.2)[‡]	71 ± 30	103 ± 50*	113 ± 53*	120 ± 54*	102 ± 38*
EX/LBNP	65 ± 23 (4.7 ± 2.1)[‡]	59 ± 20	79 ± 22[†]	81 ± 24[†]	89 ± 28*[†]	91 ± 30*
Creatinine (mmol/day)						
SED	14.6 ± 3.9	16.5 ± 5.3	16.2 ± 3.8	16.7 ± 4.7[§]	16.6 ± 3.8[¶]	14.8 ± 3.1
EX/LBNP	14.4 ± 3.2	16.2 ± 4.0	14.5 ± 4.6	15.5 ± 3.3[§]	15.7 ± 3.5[¶]	15.8 ± 4.3
Urine volume (ml/day)						
SED	2321 ± 357	2460 ± 699	2405 ± 517[§]	2465 ± 561	2454 ± 764	1857 ± 704
EX/LBNP	2466 ± 600	2026 ± 638	1905 ± 494[§]	2311 ± 886	2312 ± 607	2140 ± 817

Subjects were identical twins randomized into sedentary (SED) or treadmill exercise within LBNP (EX/LBNP) groups.

BR, bed rest.

Data are mean ± SD for eight subjects per group, except BR 5/6 data where $n = 6$ (see Statistical analyses for details).

* Denotes differences ($p < 0.05$) from the pre–bed rest means for individual group (determined from post hoc analysis when group × study day interaction was significant).

[†] Denotes significant ($p < 0.05$) differences between SED and EX/LBNP subjects.

[‡] Pre–bed rest crosslink data in parentheses are expressed as nanomoles crosslink per millimole creatinine. A t-test was performed to compare this pre–bed rest data, although none of these were statistically significant ($p > 0.05$ for all three crosslinks expressed per creatinine).

Significant differences from pre–bed rest for main effect (session) are denoted as [§] $p < 0.05$; [¶] $p < 0.01$; ** $p < 0.001$.

trends as seen with the other cross-links; however, this trend was not statistically significant. In SED subjects, collagen cross-link excretion (NTX, PYD, or DPD) was elevated 50–75% above pre-bed rest levels, whereas the corresponding increases in the EX/LBNP subjects were only 20–30% above pre-bed rest levels (Fig. 2).

Urine creatinine excretion was higher toward the end of bed rest in both groups, returning to pre-bed rest levels on reambulation (Table 3). Pre-bed rest collagen cross-link data, when expressed per creatinine (Table 3), were not significantly different between SED and EX/LBNP groups.

DISCUSSION

Exercise has been evaluated extensively as a countermeasure against the bone loss associated with aging and disease processes. Many studies have evaluated the effects of exercise, whether as single bouts[34–36] or training regimens,[37–40] on markers of bone remodeling. Most of these studies have either failed to show an effect of the exercise protocol[39] or shown increases in resorption but little or no change in formation.[35,37] A few have given indications of increases in bone turnover and remodeling (that is, increased resorption and formation).[34,36,38,40]

In this study, the exercise protocol was coupled with the added stimulus of LBNP. One interesting aspect of this combination is that it allows greater foot strike force (up to 1.2 times body weight) than previous spaceflight exercise protocols (0.6–0.7 times body weight), while at the same time, being comfortable for the subject during a 40-minute bout of treadmill running. Another interesting aspect is the cardiovascular involvement of the LBNP protocol in combination with the exercise.

It is striking that the exercise/LBNP protocol mitigated the increased bone resorption, when most ground-based ambulatory exercise studies have shown that exercise training increases bone resorption. One potential explanation for this finding is that the exercise combined with the LBNP-induced increase in lower-extremity blood flow may stimulate the maintenance of bone integrity. Related studies in rats have hypothesized a link between blood flow and the bone loss of weightlessness,[17] but this is the first finding in humans, to our knowledge, showing that a postulated increase of blood flow to the lower extremity can actually mitigate bone loss of simulated weightlessness. One obvious question remains: what are the relative contributions of the LBNP protocol and the exercise protocol to this phenomenon?

Studies of the effects of exercise on bone have provided varied results (see discussion above), some of which are likely related to differences between studies. Many factors
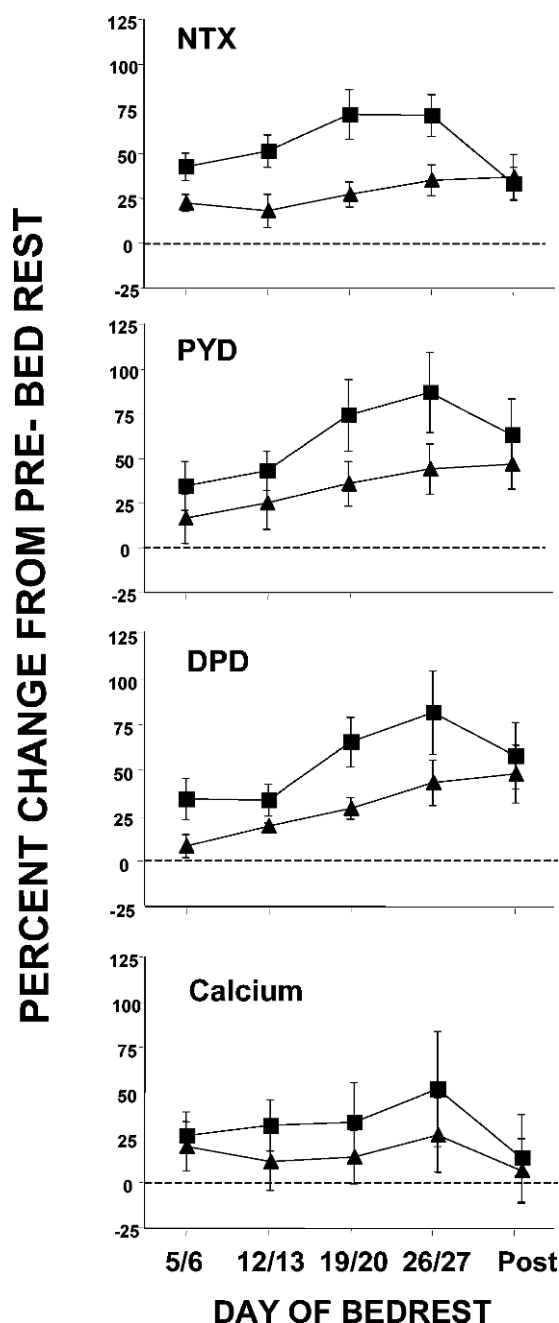
FIG. 2. Urinary excretion of bone resorption markers during 30 days of head-down tilt bed rest in sedentary (■) or exercise within LBNP (▲) subjects. Data are expressed as percent change from pre-bed rest, calculated for each subject, with mean and SEM calculated for each group. Statistical analyses were performed on raw data (see Table 3), and notations are NOT included here.

need to be evaluated, including subject types (gender, age, menopausal status, exercise training history), exercise protocol (acute/chronic, aerobic/resistive), and even which bone markers are evaluated (marker type, serum or urine, temporal relationship between sampling and training). The amelioration of the bone resorption during bed rest was confirmed with three biochemical markers of bone resorp-

tion (NTX, PYD, and DPD), and even with the less specific urinary calcium. The trend for all cross-links (and even urinary calcium) to have a lesser increase in EX/LBNP compared with SED treatments is convincing. Furthermore, we report here data on 24-h excretion of the collagen cross-links without normalization to creatinine (as is very commonly done). This eliminates the potential for changes in creatinine excretion (as seen in this study), caused by either bed rest or exercise, to affect the results.

The bone loss of spaceflight has been associated with increased resorption, in both flight[10,33,41–43] and ground-based analog studies.[24,27,28,33,44] Although the magnitude of change in bed rest studies (such as the ~50% increase in collagen cross-link excretion seen here) is lower than the change seen during space flight (~100–150% increase),[10,33] the qualitative similarities are obvious. The study reported here (SED subjects) confirmed these earlier findings,[33] with an approximately 50–75% increase in resorption markers during bed rest.

The impact of spaceflight on bone formation is not as clear as its effect on resorption, with studies showing either unchanged or decreased osteoblast activity.[10,41,42,45–47] Some of this lack of clarity seems to be methodological, with the site-specific assessments (such as biopsy and histomorphometry) showing a decrease in bone formation during bed rest,[23,28,44] and the systemic markers typically showing no change.[27,28] Whereas one study has documented a decrease in bone formation markers,[24] the data presented here support the idea that bone formation markers do not change during simulated spaceflight.

The bone loss of spaceflight affects primarily the lower extremities[1,3] in relation to their weight-bearing role. The biochemical markers measured in blood or urine samples provide a reflection of systemic changes in bone metabolism. Nonetheless, it is likely that the changes reflected in the biochemical markers are indicative of the metabolic changes in the weight-bearing bones (e.g., spine, femur, calcaneus). With regard to the effects of the exercise regimen studied here, these effects are also most likely localized to the lower extremities. Whereas a longer bed rest period would have allowed us to document changes in bone using densitometry techniques, the difficulty and cost of such a study would have been much greater. The consistent and rapid changes in biochemical markers provide valuable information and allow for interpretation of effects from much shorter studies.

Trends for increased serum calcium and decreased circulating parathyroid hormone and $1,25(OH)_2$-vitamin D are expected findings of bed rest and spaceflight studies. This may initially seem to suggest that parathyroid hormone is not involved with the increased bone resorption, but in our opinion, this reflects the feedback control of the calcium regulation system. These findings support the theory that weightlessness-induced bone loss is an adaptive phenomenon, associated with an intact calcium regulating system.

Two technical points add to the significance of this study. First, although it is commonly understood that bed rest studies need to last a minimum of 5–6 weeks before changes in bone may be observed, biochemical markers provide strong evidence that these changes begin very

quickly after initiation of bed rest. In fact, resorption markers tended to be elevated the first time they were measured in this study (although not statistically significantly), and it is likely that earlier sample collection may have revealed increased resorption activity even earlier. Second, although use of collagen cross-links is often criticized because of their high subject-subject variability, this paper shows their clear utility in defining the effects of simulated weightlessness and assessing efficacy of countermeasures to the same.

Counteracting the negative effects of spaceflight on human physiology is a challenging endeavor, and bone loss is one of the effects that present the greatest challenges. The amount of time required to recover the lost bone is estimated to be two to three times the length of the mission.[10] This will become especially critical when spaceflight missions increase in duration, as humans venture outside of low-Earth orbit. While various exercise protocols have been evaluated during spaceflights over the past 30 years, none have proven successful to date. The data presented here show that treadmill exercise in an LBNP chamber ameliorates the increased bone resorption typically seen during spaceflight and bed rest. Combining this protocol with resistive exercise may also improve the countermeasure efficacy. In summary, LBNP in combination with exercise may provide a viable countermeasure against the bone loss of spaceflight, along with benefits for other physiologic systems. The potential for this, or a similar system, to improve bone health in individuals on Earth is evident, but needs to be tested further.

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