

Resistance exercise as a countermeasure to disuse-induced bone loss

L. C. Shackelford, A. D. LeBlanc, T. B. Driscoll, H. J. Evans, N. J. Rianon, S. M. Smith, E. Spector, D. L. Feeback and D. Lai

J Appl Physiol 97:119-129, 2004. ;

doi: 10.1152/jappphysiol.00741.2003

You might find this additional info useful...

This article cites 33 articles, 9 of which you can access for free at:

<http://jap.physiology.org/content/97/1/119.full#ref-list-1>

This article has been cited by 28 other HighWire-hosted articles:

<http://jap.physiology.org/content/97/1/119#cited-by>

Updated information and services including high resolution figures, can be found at:

<http://jap.physiology.org/content/97/1/119.full>

Additional material and information about *Journal of Applied Physiology* can be found at:

<http://www.the-aps.org/publications/jappl>

This information is current as of September 27, 2013.

Journal of Applied Physiology publishes original papers that deal with diverse area of research in applied physiology, especially those papers emphasizing adaptive and integrative mechanisms. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2004 the American Physiological Society. ISSN: 8750-7587, ESSN: 1522-1601. Visit our website at <http://www.the-aps.org/>.

Resistance exercise as a countermeasure to disuse-induced bone loss

L. C. Shackelford,¹ A. D. LeBlanc,^{2,3} T. B. Driscoll,² H. J. Evans,⁴
N. J. Rianon,² S. M. Smith,¹ E. Spector,⁴ D. L. Feedback,¹ and D. Lai⁵

¹National Aeronautics and Space Administration Johnson Space Center, Houston 77058; ²Department of Medicine, Baylor College of Medicine, Houston 77030; ³Division of Space Life Sciences, Universities Space Research Association, Houston 77058; ⁴Wyle Laboratories Life Sciences Systems and Services, Houston 77058; and ⁵School of Public Health, University of Texas, Houston, Texas 77030

Submitted 16 July 2003; accepted in final form 16 March 2004

Shackelford, L. C., A. D. LeBlanc, T. B. Driscoll, H. J. Evans, N. J. Rianon, S. M. Smith, E. Spector, D. L. Feedback, and D. Lai. Resistance exercise as a countermeasure to disuse-induced bone loss. *J Appl Physiol* 97: 119–129, 2004; 10.1152/jappphysiol.00741.2003.—During spaceflight, skeletal unloading results in loss of bone mineral density (BMD). This occurs primarily in the spine and lower body regions. This loss of skeletal mass could prove hazardous to astronauts on flights of long duration. In this study, intense resistance exercise was used to test whether a training regimen would prevent the loss of BMD that accompanies disuse. Nine subjects (5 men, 4 women) participated in a supine maximal resistance exercise training program during 17 wk of horizontal bed rest. These subjects were compared with 18 control subjects (13 men, 5 women) who followed the same bed rest protocol without exercise. Determination of treatment effect was based on measures of BMD, bone metabolism markers, and calcium balance obtained before, during, and after bed rest. Exercisers and controls had significantly ($P < 0.05$) different means, represented by the respective following percent changes: lumbar spine BMD, +3% vs. -1%; total hip BMD, +1% vs. -3%; calcaneus BMD, +1% vs. -9%; pelvis BMD, -0.5% vs. -3%; total body BMD, 0% vs. -1%; bone-specific alkaline phosphatase, +64% vs. 0%; alkaline phosphatase, +31% vs. +5%; osteocalcin, +43% vs. +10%; 1,25 dihydroxyvitamin D, +12% vs. -15%; parathyroid hormone intact molecule, +18% vs. -25%; and serum and ionized calcium, -1% vs. +1%. The difference in net calcium balance was also significant (+21 mg/day vs. -199 mg/day, exercise vs. control). The gastrocnemius and soleus muscle volumes decreased significantly in the exercise group, but the loss was significantly less than observed in the control group. The results indicate that resistance exercise had a positive treatment effect and thus might be useful as a countermeasure to prevent the deleterious skeletal changes associated with long-duration spaceflight.

microgravity; bed rest; bone resorption; bone formation; strength training

UNDER CONDITIONS OF REDUCED weight bearing, bone resorption increases and bone mineral density (BMD) decreases. The response of bone to microgravity is an extreme case of the reduced weight-bearing response and has been well documented in US and Russian crew members on long-duration missions aboard the Russian space station Mir and the International Space Station (ISS). To date, we have collected pre- and postflight bone densitometry measurements on 47 individuals from such flights. Although losses show significant heterogeneity among individuals and between bones of a given subject, bone loss is a consistent finding after spaceflight. Among astronauts and cosmonauts who participated in long-duration (average of 170 days) flights aboard Mir and the ISS,

>50% of the crew members had a $\geq 10\%$ loss in at least one skeletal site, and 22% of the Mir cosmonauts had a 15–20% loss in at least one site.

Bone loss has been shown to be a regional phenomenon in which the areas with the greatest decrease in weight bearing lose the most bone; losses average 1–2%/mo in such regions as the lumbar spine and hip (11, 16, 20) compared with no change in the arms or radius (Mir and ISS astronauts, arms: +0.1%/mo; ISS astronauts, radius and ulna: -0.1%/mo).

Several countermeasure strategies have the theoretical potential to protect against spaceflight-induced bone loss. These include use of artificial gravity, prescription drugs [e.g., bisphosphonates and parathyroid hormone (PTH)], and in-flight exercise regimens that have a significant resistance exercise component. Given present limitations, only the pharmaceutical and exercise approaches are practical.

We have reported on the positive results of a study testing alendronate as a countermeasure to bed rest-induced disuse bone loss (13), but a pharmaceutical approach has certain drawbacks: not all crew members will be able or willing to take such a drug, the drug would benefit only bone and not other body systems, and the drug cannot be used to target specific bone regions.

An exercise countermeasure has the advantage of benefiting multiple body systems (musculoskeletal, cardiovascular, immunological) and can be targeted to those body regions needing protection. Maintenance of muscle strength also reduces risk of injury during falls and impact. Increased muscle strength reduces the risk of impact injury by decreasing joint angular velocity, providing damping of impact loads. Muscles protect bone from fracture by resisting bending moments across long bones.

There is a consensus among exercise scientists that both endurance (aerobic) and resistance exercises are needed as countermeasures to maintain overall crew health and performance during and after spaceflight (2, 5, 30). Although aerobic exercise has been a mainstay of in-flight countermeasures since the days of Skylab, it is clear from our pre- and postflight bone-density measurements and in-flight biochemical assessments that such countermeasures have done little to prevent the loss of bone mineral in-flight (11, 20, 25, 26). In normal, ambulatory subjects, resistance exercise seems to provide an osteogenic stimulus superior to that of aerobic exercise (27, 29); therefore, it may provide more protection against disuse bone loss than aerobic exercise provides.

Address for reprint requests and other correspondence: A. D. LeBlanc, Dept. of Medicine, Baylor College of Medicine, 6550 Fannin St., Ste. 1260, Houston, TX 77030.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Ambulatory training studies involving men and women of diverse ages have demonstrated the osteogenic effects of resistance exercise, including increases in BMD of the spine, hip, and legs (4, 19, 33). A study of hindlimb-suspended rats showed that resistance exercise could attenuate bone loss (3). To date, however, no study has been published that shows the effects of a weight-training type of intensive resistance exercise program on bone loss during long-duration disuse in humans.

Bed rest is used as an analog of spaceflight to study the nature and mechanisms of disuse bone loss and to test countermeasures to bone loss. The musculoskeletal changes documented during bed rest [decreased BMD, increased bone resorption, increased calcium (Ca) excretion, negative Ca balance, and decreased muscle mass and strength] have been found to be qualitatively similar to those occurring in spaceflight (11, 17, 26).

The purpose of this study was to determine whether a maximum-type progressive resistance exercise protocol (defined by the frequency, intensity, and duration of exercise and similar to a competitive weight-lifter's training protocol) would protect against bone loss during 17 wk of continuous bed rest. Our primary hypothesis was that bone loss (as measured by BMD and Ca balance) would be significantly reduced in the exercise group compared with the control group. Our secondary hypothesis was that loss of muscle mass would also be reduced in the exercise group compared with the control group. To provide insight into the time course and mechanisms underlying changes in bone during disuse and disuse with exercise, Ca balance and serum and urinary concentrations of bone metabolism markers were measured.

METHODS

Subjects. The institutional review boards at Baylor College of Medicine and National Aeronautics and Space Administration (NASA) Johnson Space Center reviewed and approved the research protocol for this study, and all test subjects provided written, informed consent. Nine subjects (5 men and 4 women) completed the study, which took place over a period of 3 yr. Each subject served as his or her own control (data from different phases of the study were compared), and the exercising subjects were compared with a group of nonexercising control subjects. There were 18 control subjects: 8 concurrent controls, 5 men (13) and 3 women, who participated in the identical protocol but with no exercise countermeasure, and 10 previous controls, 8 men (13) and two women, from similar 17-wk bed-rest studies conducted previously by the same investigators.

All subjects who participated in this study were recruited from the local community by newspaper advertisements. Prospective subjects were required to pass an Air Force Class III flight physical, which included a general physical examination by a physician, blood pressure measurement, electrocardiogram, complete blood count, and blood and urine analyses. In addition, subjects were required to have BMD values within two standard deviations above or below the age- and sex-matched mean for the spine and hip. Potential subjects were also required to pass drug and psychological (Minnesota Multiphasic Personality Inventory) screens. All prospective subjects were screened for musculoskeletal or cardiovascular disorders that could interfere with exercise capacity. To participate in the study, subjects had to be able and willing to participate as an exercise or control subject.

A total of 20 subjects were recruited to participate in the present study. Subjects were assigned to either the control group (6 men and 4 women) or the exercise group (5 men and 5 women). Assignment was based on order of application and medical clearance for partici-

pation. One female and one male control subject did not complete the study: one resigned from the study after 3 days because of dissatisfaction with having been assigned to the control group, and the other dropped out in the second week because of a major illness in the family. One female exercise subject was dismissed in the first month of the study because she did not abide by the dietary constraints. Thus nine exercise subjects (5 men and 4 women) and eight control subjects (5 men and 3 women) completed the study.

Statistical testing of bone-related parameters (BMD and bone/Ca markers) revealed very few significant differences between the present controls and the previous controls or between men and women (see data for specifics, but examples include ionized Ca and total hip BMD). Therefore, for purposes of statistical testing for a treatment effect, data were combined for this report, resulting in a total of 18 controls (13 men and 5 women). The combined control subjects were 22–56 yr of age (32 ± 9 yr), 157–185 cm in height (171 ± 9 cm), and 63–99 kg in weight (74 ± 9 kg). The exercise subjects were 23–44 yr of age (31 ± 8 yr), 163–185 cm in height (172 ± 9 cm), and 57–92 kg in weight (70 ± 12 kg).

Bed rest. Each subject was housed for 22 consecutive weeks at the Methodist Hospital (Houston) bed-rest facility. The 22-wk study period was divided into three phases: a 3-wk ambulatory control period, a 17-wk bed-rest period, and a 2-wk recovery period. All baseline testing was performed during the pre-bed rest ambulatory control period. During this 3-wk phase, subjects were allowed to engage in activity ad libitum but were accompanied by a member of the metabolic research staff when not on the ward.

During the 17-wk bed-rest phase, subjects were required to remain in horizontal bed rest except to use a bedside commode for bowel movements. When using the commode, subjects were required to keep their legs supported in the horizontal position. The bedside commode was used three to seven times per week by the subjects, who had to request permission from the ward personnel to use it. While in bed, subjects were allowed to raise themselves on one elbow during activities such as eating, reading, and using a notebook computer. Subjects were required to keep the armpit of the supporting arm in contact with the bed at all times. Sitting up was not allowed. Subjects were moved to various testing locations in the hospital on wheeled stretchers. The staff of the research ward closely monitored the activity of the subjects to prevent unauthorized activity.

During the post-bed rest recovery phase, subjects were allowed to resume normal activity ad libitum but were cautioned to avoid excessive walking during the first few days of reambulation. When subjects were not on the research ward, they were accompanied by a member of the staff.

Diet. During the entire 22-wk study, subjects were required to eat a metabolic diet prepared in a manner similar to that used for previous bed-rest studies (10, 16). The diet consisted of seven daily menus, with three meals and an evening snack. All of the food portions were individually weighed, and the subjects were required to eat all of the food served to them. The diet contained, on average, 1 g of Ca, 1.7 g of phosphorus, and 15 g of nitrogen per day. The subjects were also required to consume one vitamin tablet daily, containing 5,000 IU of vitamin A, 400 IU of vitamin D, 3 mg of thiamin, 3.4 mg of riboflavin, 20 mg of niacin, and 90 mg of ascorbic acid. Throughout the 3-yr course of the study, the mineral content of the metabolic diet was confirmed on 20 separate occasions (about every other month). Duplicate meals were prepared, homogenized, ashed, and analyzed according to methods published previously (22). The caloric intake of each subject was adjusted with complex carbohydrates to maintain approximately constant body weight during the study. All subjects began the 22-wk study at an arbitrarily selected daily energy intake. Except for two female exercise subjects who started the study at 2,350 kcal/day (9,800 kJ/day), all subjects began the study with an intake of 2,600 kcal/day (10,900 kJ/day). This starting energy intake had to be adjusted for some subjects (3 controls and 5 exercisers) during the study to maintain constant body weight. Energy intake at the end of

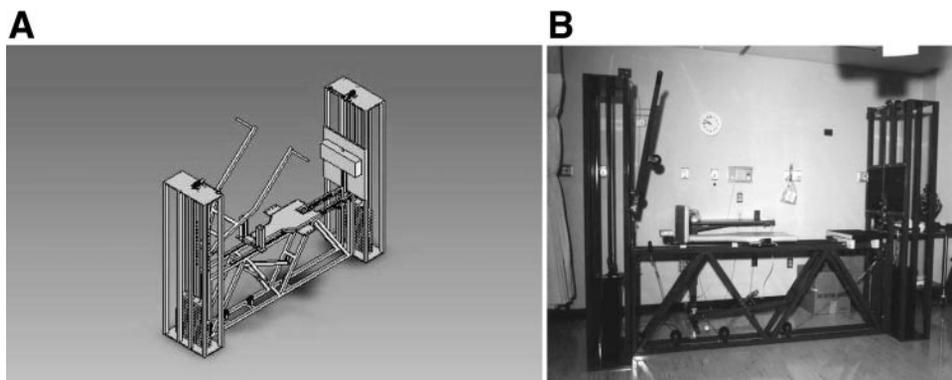


Fig. 1. Diagram (A) and photograph (B) of Horizontal Exercise Machine.

bed rest was 2,100 kcal/day (8,800 kJ/day) for controls and 2,300 kcal/day (9,600 kJ/day) for exercisers. Expressed relative to body mass, energy intake at the beginning of the study was 36 kcal·kg⁻¹·day⁻¹ (150 kJ·kg⁻¹·day⁻¹) for controls and 37 kcal·kg⁻¹·day⁻¹ (155 kJ·kg⁻¹·day⁻¹) for exercisers; at the end of bed rest, both groups averaged 33 kcal·kg⁻¹·day⁻¹ (140 kJ·kg⁻¹·day⁻¹). Although calorie content of the diet was periodically adjusted, the mineral content was not. A diet analysis software package (Nutritionist V, first Databank) was used by the staff dietitian to design and modify the diet. In addition to their food intake, subjects were required to consume a minimum of 1,500 ml of distilled water every day to maintain adequate hydration. Subjects were weighed weekly using an in-bed scale.

Exercise regimen. All exercises were performed on a unique piece of hardware (Fig. 1), the Horizontal Exercise Machine (HEM), developed at the NASA Johnson Space Center specifically for this study. The HEM is a resistance exercise training machine that allows a subject to perform exercises while lying flat, either supine or prone. Resistance is provided by weight plates and cabled pulleys for transferring force. The HEM consists of two weight-stack towers and a central truss; each weight-stack tower holds a 170-kg (375-lb.) stack of weight plates and cabled pulleys. The central truss is the base of support for the bench on which the subject lies while performing the exercises. The bench slides on a pair of case-hardened rails for certain exercises (horizontal leg presses and heel raises) but can be fixed by a stainless steel pin for the remaining exercises.

The exercise subjects were familiarized with the resistance exercise protocol during the pre-bed rest ambulatory period, working with the exercise trainer on 2 different days to learn to perform each of the exercises used in the protocol. During the pre-bed rest training sessions, starting weight loads for each subject were determined. An estimate of the 1-repetition maximum load was made on the basis of the individual subject's size and habitus. The load was adjusted according to the actual number of repetitions performed. A general rule of a 5-lb. adjustment for every two repetitions of deviation from the desired number of repetitions was used.

During the 17-wk bed-rest period, the subjects exercised 6 days/wk. These 6 days consisted of 3 days of lower-body work and 3 days of upper-body work; the upper and lower body were worked on alternate days. Heel raises were performed on all 6 days to maintain ankle plantar flexor conditioning. The exercise trainer monitored all exercise sessions and determined the training progression for each subject. Upper-body exercises were included to provide a total-body workout (upper-body exercise is required in space to prepare for spacewalks) and to keep the subjects motivated to follow the exercise regimen (by allowing subjects to see improvements in upper-body strength and appearance). The following exercises were performed on lower-body exercise days (targeted muscles are shown in parentheses): back extensions (lower back extensors), hip abduction (hip abductors), wide-stance horizontal leg press (hip extensors, hip abductors, knee extensors), narrow-stance leg press (hip extensors, knee

extensors), lying knee-ups (hip flexors, abdominals), single-leg heel raises (plantar flexors, hip abductors), and bilateral heel raises (plantar flexors, paraspinal postural control muscles). The exercises performed on upper-body exercise days were biceps curls (grip, wrist flexors, elbow flexors), triceps press-downs (grip, wrist flexors, elbow extensors), upright rowing (grip, wrist flexors, elbow flexors, shoulder abductors, upper back scapular retractors), bench press (chest, elbow extensors), prone rowing (grip, shoulder extensors, upper back scapular retractors), single-leg heel raises, and bilateral heel raises.

All sets of each exercise were performed in succession before starting another exercise. One to 1.5 min of rest was given between each set. The time between exercises was ~2 min (just long enough to change the configuration of the machine for the next exercise). Neither the three heel-raise exercises nor the two horizontal leg-press exercises were performed consecutively but were alternated with the other exercises for that day. Just before performing a maximum-effort set for each exercise, the subject performed one warm-up set (load set at 2/3 of the weight used for the first maximum set). The number of repetitions for the warm-up sets equaled the number of repetitions to be performed for that day's maximum-effort sets. After performing the warm-up set in each exercise, a subject always performed as many repetitions as possible until failure (when the subject could not complete another full repetition). The goal for all maximum-effort sets was to perform an 11-, 8-, or 5-repetition maximum (RM) (±1 repetition). This approach assured progression in training loads throughout the 17-wk regimen. The training protocol was designed to provide a load-progression regimen similar to that used by resistance-trained athletes (8). If a set was performed outside the desired range of repetitions, the load of subsequent sets was adjusted accordingly. The set and repetition scheme was the same for all exercises, except the heel raises, and is presented in Table 1. Heel raises on lower-body exercise days followed the scheme in Table 1, but heel raises performed on upper-body exercise days used a five-set, 20RM protocol.

When a subject had minor overuse pain, loads were reduced or specific exercises were waived until the symptoms resolved. The subjects performed 90% of the required number of sets and 98% of the required number of repetitions. The average work load for the 5- to 11RM exercises was 74% of 1RM. The average work load for the 20RM heel raises was 58% of 1RM.

Table 1. Set and repetition scheme for exercise training protocol

Bed rest week 1	2 sets	11 RM (range 10–12 reps)
Bed rest weeks 2–3	3 sets	11 RM (range 10–12 reps)
Bed rest weeks 4–6	4 sets	8 RM (range 7–9 reps)
Bed rest weeks 7–9	5 sets	5 RM (range 4–6 reps)
Bed rest weeks 10–11	5 sets	11 RM (range 10–12 reps)
Bed rest weeks 12–14	6 sets	8 RM (range 7–9 reps)
Bed rest weeks 15–17	6 sets	5 RM (range 4–6 reps)

RM, repetition maximum; reps, repetitions.

Bone densitometry. Bone mineral densitometry measurements were obtained with the technique of dual-energy X-ray absorptiometry (DEXA). DEXA scans were performed on the whole body, lumbar spine (L1–L4), hip, forearm, and calcaneus using a Hologic QDR 2000-Plus whole-body densitometer. The measurements were performed three times during the pre-bed rest period, at 6 and 12 wk during bed rest, and three times during *week 17* of bed rest. The pre-bed rest and 17-wk values were used for analysis. Standard scan acquisition and analysis software programs were used to obtain the scans, except for the calcaneus scans. These scans were performed using a special jig and the Hologic forearm scan protocol and were analyzed with subregion software. Subregions of the whole body were those defined by Hologic software and included the pelvis, legs (including the feet), and arms (including the hands). The older published BMD measurements (in the previous controls) were obtained from various DEXA instruments, but each subject's data were obtained on a single instrument by a single operator and analyzed by the same operator. Phantom scans were performed regularly (~20 times/mo and on the morning of each day of testing) to assess the short-term and long-term performance of the densitometer. The precision error for phantom BMD is ~0.5%. The precision of bone and soft-tissue DEXA measurements in our laboratory has been published (14, 15, 28). The following are the most recent *in vivo* laboratory BMD precision data, determined over an interval of 6 mo and expressed as percent coefficient of variance (SD/mean × 100%): lumbar spine, 1.4%; femoral neck, 1.5%; calcaneus, 0.8%; trochanter, 1.8%; ultra distal radius, 2.1%.

Urinary and fecal Ca excretion and Ca balance. Twenty-four-hour urine collections were obtained continuously throughout the study. Each void was collected and stored in a daily aliquot jug at 4°C. At the end of each 24-h period, the total volume was measured, and duplicate aliquots (2% of the total volume) were prepared. One aliquot was acidified with HCl, and both aliquots were then frozen. The aliquots from each day were combined to form two 7-day pools (one acidified and one nonacidified) and stored frozen at -22°C for subsequent analysis. Fecal samples were collected individually and stored in 7-day collections at -22°C for subsequent analysis. Urinary Ca in the weekly acidified pools was analyzed with inductively coupled plasma mass spectrometry. Fecal Ca was analyzed at four time points: during the 3-wk baseline period, and during *weeks 4–6, 10–12, and 15–17* of bed rest. Ca balance was determined for these same periods by using the following equation: Ca balance = Ca intake (in mg/day) – output (urinary Ca + fecal Ca) (in mg/day). Net balance was obtained as the difference between baseline and bed rest.

Markers of bone metabolism and regulatory hormones. Blood and urine samples obtained during the ambulatory period and at *weeks 6, 12, and 17* of bed rest were analyzed to measure changes in markers of bone metabolism and bone regulatory hormones. Urinary markers were measured in samples from the 7-day pooled, nonacidified, frozen aliquots described earlier. Venous blood samples were obtained in the early morning after a 12-h fast on the third day of the weeks listed above. The urine samples were analyzed by methods our laboratory has previously described (12, 24, 25): total Ca (inductively coupled plasma mass spectrometry, Perkin-Elmer), *n*-telopeptide (ELISA, Osteo, Seattle, WA), and pyridinium and deoxypyridinoline cross-links (ELISA, Quidel, Santa Clara, CA). The following analytes were measured in serum: total Ca (spectrophotometry, Beckman Coulter Instruments, Brea, CA), ionized Ca (ion-sensitive electrode, i-STAT, Princeton, NJ), total alkaline phosphatase (spectrophotometry), bone-specific alkaline phosphatase (BSAP; ELISA, Quidel), 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D (DiaSorin, Stillwater, MN), osteocalcin (RIA, Biomedical Technologies, Stoughton, MA), and mid-molecule and intact-molecule PTH (RIA, Nichols Institute Diagnostics, San Juan Capistrano, CA). BSAP, intact-molecule PTH, mid-molecule PTH, and 25-hydroxyvitamin D were obtained from the concurrent controls and exercise subjects only.

Strength testing. Strength testing consisted of isotonic testing (1RM testing on the HEM) and isokinetic testing (using a Biodex dynamometer). The exercise subjects performed 1RM testing as follows: two times before bed rest, and one time at 6, 12, and 17 wk of bed rest. The control subjects performed 1RM testing twice before bed rest and once after 17 wk of bed rest. All subjects performed Biodex testing twice before bed rest and once at 6, 12, and 17 wk of bed rest. Strength testing was performed after MRI testing to avoid affecting the muscle volume measurements. When overuse injuries occurred during the week in which Biodex and 1RM strength testing were scheduled, subjects were tested unless such testing posed a threat of further injury. The 1RM testing replicated several of the exercises used for the strength-training regimen: bench press, wide leg press, back extension, heel raise, knee-up, and right and left abduction. Before beginning 1RM testing of the exercises listed above, subjects performed a warm-up set. Isokinetic strength testing (maximal isokinetic torque) was performed using a Biodex dynamometer located at the Methodist Hospital's Physical Therapy Department. Baseline testing was preceded by one familiarization session. Dynamometer positions for each subject were recorded during the familiarization session and held constant for all tests to ensure the same machine-subject alignment. Peak concentric and eccentric torque were determined for trunk and knee extension/flexion and for ankle plantar/dorsiflexion. The initial maximal effort of each movement was preceded by several submaximal warm-up contractions. All tests were performed at a velocity of 1.05 rad/s (60°/s). The back was tested at 60°/s and 30°/s. Three trials of each movement were performed, with a 2-s pause at full extension and full flexion. For data analysis, peak torque was deemed the best of the three trials. The following standard ranges of motion were used: trunk, 65–125° flexion; knee, 10–95° flexion; ankle, 20° dorsiflexion to 25° plantar flexion.

Lean body mass and muscle volume. Lean body mass (LBM) was determined from whole-body DEXA scans. The reproducibility of these determinations in our laboratory has been published in a precision study (28). Following are the precision values for LBM: arms, 1.8%; legs, 1.3%; whole body, 0.9%.

Muscle volume measurements were obtained from MRI scans of the back and legs. MRI measurements were made with a Siemens 1.5-T Magnetom imager (all present control subjects and 8 of the 9 exercise subjects) or a General Electric imager (1 exercise subject) located at the Methodist Hospital, Department of Radiology. Appropriate phantoms were scanned periodically to assure quality control. Spin echo multi-slice images of the calf and thigh were acquired using the whole-body coil to measure muscle volume changes. Similar images of the lumbar region of the back were obtained with a spine coil (all present control subjects and 8 of the 9 exercisers) or a whole-body coil (1 exercise subject). MRI muscle volume analysis has been described previously (9). Briefly, the muscles were manually outlined to determine their area. The positioning of the images between sessions was determined by the size of the marrow cavity in leg images and from the intervertebral disc (from a sagittal image) in back images. One set of images was selected as the reference set, and the corresponding image from each of the other sessions was outlined, with the reference image on one side of the screen and the image being outlined on the other. After normalization of the images to correct for variations in coil sensitivity, the muscle pixels were separated by windowing, and the number of pixels in the outlined region was counted. To determine the image volume, the areas from all sessions were summed over the maximum length of the overlapping region.

Statistics. Except for the Ca excretion data, all data are expressed as a percent change from pre-bed rest values. The percent changes are reported as means ± SE for the exercise and control groups.

When the primary interest was determining whether there was a difference between the pre-bed rest and post-bed rest values of the exercise and control groups (in BMD, muscle volume, and strength), an analysis of covariance was used to test whether the exercise group

Table 2. Percent change in bone mineral density after 17 wk of bed rest

	Male Controls (n = 13), %Change	Female Controls (n = 5), %Change	All Controls (n = 18), %Change	Male Exercisers (n = 5), %Change	Female Exercisers (n = 4), %Change	All Exercisers (n = 9), %Change
Lumbar spine	-1.7±0.8	-0.3±0.6	-1.3±0.6 ^e	4.4±1.8	2.2±0.8	3.4±1.1 ^{ef}
Femoral neck	-1.9±0.9	-0.6±1.0	-1.5±0.7	0.3±1.1	-0.1±0.7	0.1±0.7
Trochanter	-3.6±0.7	-3.6±1.0	-3.6±0.6 ^e	-0.8±1.5	-4.3±1.6	-2.3±1.2
Total hip	-3.9±0.7 ^e	-2.3±1.0	-3.4±0.6 ^e	0.3±1.2	-2.4±1.4	-0.9±1.0 ^f
Calcaneus	-10.3±2.3	-6.1±3.1	-9.2±1.9 ^e	0.4±0.6	2.1±1.2	1.2±0.7 ^f
Distal radius	-0.5±0.6 ^a	0.8±0.4 ^b	0.0±0.4	-0.7±0.8	-1.3±0.6	-1.0±0.5
Proximal radius	-0.6±0.3 ^a	0.6±0.6 ^b	-0.2±0.3	0.1±0.5	0.3±0.6	0.2±0.4
Total body	-0.7±0.4	-0.7±0.2 ^c	-0.7±0.3 ^e	0.3±0.4	-0.2±1.2	0.1±0.5 ^f
Pelvis	-3.6±0.8	-2.6±1.0 ^c	-3.3±0.7 ^e	-0.4±1.7	1.7±0.7	-0.5±1.0 ^f
Legs	-1.9±0.7	-1.6±0.6 ^c	-1.8±0.6 ^e	-0.3±0.5	-1.4±1.7	-0.8±0.8
Arms	-0.7±0.9	-0.1±0.3 ^c	-0.6±0.7	-0.8±0.5	-0.1±1.1	-0.5±0.5

Values are means ± SE. ^aFive concurrent controls only; ^b3 concurrent controls only; ^c4 controls only; ^d10 controls only; ^ebaseline vs. bed rest ($P < 0.05$); ^fcontrol vs. exercise ($P < 0.05$).

was different from the control group. The software used for the analysis was the method described by Ihaka and Gentleman (6). For each variable to be tested, the pre-bed rest measurements were averaged and used as a covariant. The measurements were coded for study group (exercise or control) and gender. In each case, the slope of the relation of the pre-bed rest value to the bed-rest value was tested by group and sex to ensure that it was valid to use a common slope for the covariant in testing for differences between the groups. In a few cases, this test failed. For several of the variables (BMD, muscle volumes), the measurements were repeated to increase the measurement precision; in these cases, the average of all the measurements at a given time point was used. To test whether there was a significant change between the pre-bed rest values and the post-bed rest values, a paired *t*-test was used (BMD, muscle volume).

When multiple measurements (of urinary markers, serum markers and hormones, and Ca balance) were made during the bed-rest period, ANOVA with repeated measures was used. For these variables, the interest was in determining whether the values during bed rest were different from the pre-bed rest values and whether these changes were different for the two groups (exercise and control). Data are presented both for the entire group and for male and female subjects separately; however, because the number of subjects was small, the male and female data were not subjected to statistical analysis. Significance was assigned to *P* values of ≤ 0.05 .

RESULTS

After 17 wk of bed rest, the control subjects (combined men and women) had statistically significant decreases in BMD in all regions except the femoral neck, radius, and arms (Table 2). BMD in the lumbar spine of the resistance-exercise subjects increased significantly from baseline, but none of the other measured regions changed significantly. Between-group differences were statistically significant for the lumbar spine, total hip, pelvis, calcaneus, and total body. Body mass of subjects did not change significantly at any time during bed rest (Table

3). Exercise subjects gained an average of 1.3 kg during bed rest, whereas control subjects gained an average of 0.6 kg.

During bed rest, urinary excretion of bone resorption markers (*n*-telopeptide, deoxypyridinoline, and pyridinium cross-links) increased significantly from baseline in both the control and exercise groups (Fig. 2, Table 4). The average difference between groups, during bed rest, was not significant for any of these markers. The values reported in the table were not normalized to urinary creatinine excretion. Creatinine correction is a standard normalization procedure to correct for variations in urine output (as when 24-h collections are not possible). However, because urinary creatinine values increased during bed rest in the exercise group ($7 \pm 2.5\%$; $P < 0.05$) but not in the control group, and because our aliquots were based on 24-h rather than spot collections, we thought it was appropriate to report the uncorrected urinary marker results, as our laboratory has done in other studies with similar confounding of creatinine normalization (24). Correction for creatinine did not significantly affect the control subjects' percent change in urinary bone resorption markers (paired *t*-test, $P = 0.44$). Creatinine correction did affect exercisers' values ($P < 0.05$), probably because of changes in creatinine. The bone-formation markers (BSAP, total alkaline phosphatase, and osteocalcin) were unchanged in the control subjects, whereas these same markers were significantly increased in the exercise subjects (BSAP, +64%; total alkaline phosphatase, +31%; osteocalcin, +43%) (Fig. 3). Although 25-hydroxyvitamin D and mid-molecule PTH showed no change in either subject group, 1,25-dihydroxyvitamin D and intact PTH were significantly decreased in the control group. Changes in serum Ca were not significant in either group. Ionized Ca increased a statistically significant 1% in the control group but did not change significantly in the exercise group. Between-group differences were

Table 3. Body mass before and after 17 wk of bed rest

	Male Controls (n = 11)	Female Controls (n = 5)	All Controls (n = 16)	Male Exercisers (n = 5)	Female Exercisers (n = 4)	All Exercisers (n = 9)
Pre-BR body mass, kg	74.0±3.3	67.2±1.6	72.0±2.4	73.0±5.9	64.7±2.8	71.3±4.2
Post-BR body mass, kg	74.6±2.8	67.8±1.3	72.5±2.2	74.2±4.5	66.1±2.6	72.4±3.6
Body mass change, kg	0.5±2.8	0.7±0.4	0.6±0.6	1.2±1.6	1.3±0.3	1.3±1.1
Body mass change, %	1.0±1.1	1.0±0.7	1.0±0.8	2.2±2.0	2.5±1.0	2.3±1.6

Values are means ± SE. BR, bed rest.

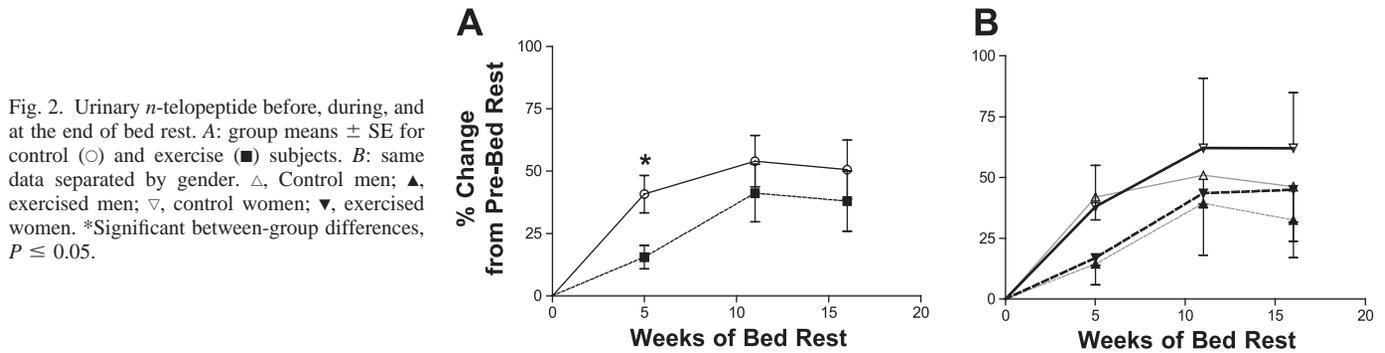


Fig. 2. Urinary *n*-telopeptide before, during, and at the end of bed rest. A: group means \pm SE for control (\circ) and exercise (\blacksquare) subjects. B: same data separated by gender. \triangle , Control men; \blacktriangle , exercised men; ∇ , control women; \blacktriangledown , exercised women. *Significant between-group differences, $P \leq 0.05$.

significant for alkaline phosphatase, BSAP, osteocalcin, 1,25-dihydroxyvitamin D, intact-molecule PTH, and both serum and ionized Ca (controls +1%, exercisers -1%).

In the control subjects, post-bed rest values of urinary and fecal Ca excretion increased (64 and 131 mg/day, respectively) significantly over baseline; Ca balance relative to baseline was -199 mg/day (Table 5). In the exercise subjects, on the other hand, post-bed rest values of urinary Ca excretion (Fig. 4) and net Ca balance were not significantly different from baseline. Between-group differences in total net Ca balance, net urinary Ca balance, and net fecal Ca balance were significant.

After 17 wk of bed rest, exercise subjects showed significant improvements over their pre-bed rest values in 1RM strength for all exercises, ranging from +25% for back extension to over 100% improvement in hip (left and right) abduction (Fig. 5). Control subjects showed decreases in 1RM strength ranging from -6% (knee-ups) to -37% (heel raises). In control subjects, all changes except for the knee-up changes were statistically significant. For all exercises, between-group differences were statistically significant. The 10 control subjects from our laboratory's previous bed rest studies did not perform 1RM testing, so the graph includes data from the present control subjects only. Furthermore, the number of present control subjects was seven, rather than eight, because one of the eight present control subjects did not participate in 1RM strength testing due to a scheduling error. During the 17 wk of resis-

tance training, exercise subjects worked out at loads averaging 74% of their 1RM (range 63–85%, with no difference between the male and female subjects). The heaviest loads lifted during exercise training were for the wide leg press during the last 3 wk of the training period, when the men averaged 181 kg per lift (the maximum individual 3-wk average was 254 kg) and the women averaged 159 kg per lift (maximum 190 kg). The average load for the wide leg press was 72% of the 1RM (calculated by extrapolation between 1RM tests). During the 17-wk bed rest, the wide leg press load averaged 97% of the higher of the two pre-bed rest 1RM tests. The total work during the wide leg press was 6,518 J per person per day (averaged over the full 119 days of bed rest), with an increase of 1,930 J per person per day in the last 8 wk compared with weeks 2 through 9 of bed rest. Alone, neither work performed nor change in work performed during the study correlated with bone density changes. When exercise parameters (total joules and newtons per lift) were normalized to body mass, lumbar spine BMD changes correlated with the wide leg press total joules ($r = 0.87$), narrow leg press total joules ($r = 0.90$), and right heel raise ($r = 0.89$). Change in wide leg press 1RM strength approached significance with changes in lumbar spine BMD ($r = 0.85$). The percent of the initial 1RM at which the subjects trained, averaged over the entire bed-rest period, correlated with BMD changes in the pelvis ($r = 0.88$). Pelvis BMD changes correlated with newtons per lift for knee-ups

Table 4. Change in bone metabolism markers and bone regulatory hormones during 17 wk of bed rest

	All Cont (n = 18), Pre BR	All Cont (n = 18), Avg BR	All Cont (n = 18), %Change	Male Cont (n = 13), %Change	Female Cont (n = 5), %Change	All RE (n = 9), Pre BR	All RE (n = 9), Avg BR	All RE (n = 9), %Change	Male RE (n = 5), %Change	Female RE (n = 4), %Change
NTX	565 \pm 55	811 \pm 74	48.5 \pm 9.6 \ddagger	46.4 \pm 11.6	54.0 \pm 22.3	560 \pm 29	733 \pm 53	31.6 \pm 8.8 \ddagger	28.8 \pm 14.8	35.1 \pm 9.6
PYD	300 \pm 19	431 \pm 24	46.5 \pm 5.6 \ddagger	45.1 \pm 6.4	50.1 \pm 12.4	373 \pm 29	457 \pm 26	25.8 \pm 7.6 \ddagger	34.1 \pm 12.4	15.5 \pm 5.4
DPD	77 \pm 4	104 \pm 6	34.9 \pm 4.5 \ddagger	36.8 \pm 5.4	29.7 \pm 10.8	92 \pm 9	110 \pm 9	23.5 \pm 6.6 \ddagger	23.7 \pm 11.0	23.3 \pm 7.9
Alk Phos	58.4 \pm 3.2	61.0 \pm 4.1	4.9 \pm 3.2	7.9 \pm 3.9	-2.7 \pm 4.9	59.4 \pm 5.8	76.6 \pm 6.1	31.4 \pm 4.6 \ddagger §	35.5 \pm 5.1	26.3 \pm 8.3
BSAP*	15.0 \pm 1.2	14.7 \pm 0.9	-0.1 \pm 4.9	-5.5 \pm 4.9	9.0 \pm 7.5	15.7 \pm 1.9	25.0 \pm 2.6	63.6 \pm 8.3 \ddagger §	63.5 \pm 14.4	63.7 \pm 8.4
Osteocalcin	11.6 \pm 1.0	12.5 \pm 1.0	10.5 \pm 5.8	9.1 \pm 6.9	14.1 \pm 11.3	12.1 \pm 1.1	16.8 \pm 1.4	42.7 \pm 13.8 \ddagger §	33.1 \pm 12.8	54.7 \pm 27.9
1,25(OH) ₂ Vit D										
D	29.8 \pm 2.3	25.7 \pm 2.8	-14.7 \pm 4.8 \ddagger	-15.1 \pm 6.2	-13.8 \pm 6.7	42.1 \pm 4.4	45.9 \pm 3.8	12.1 \pm 7.3 \ddagger	20.9 \pm 12.0	1.0 \pm 25
25 Vit D*	22.4 \pm 1.8	24.3 \pm 2.0	10.9 \pm 8.9	8.4 \pm 14.4	15.0 \pm 6.2	26.5 \pm 2.9	27.5 \pm 2.7	6.0 \pm 6.3	14.3 \pm 8.9	-4.4 \pm 6.5
PTH (MM)*	116 \pm 16.5	111 \pm 18.3	-5.2 \pm 4.1	-12.5 \pm 2.3	7.0 \pm 4.5	124 \pm 14.2	119 \pm 12.5	-2.4 \pm 4.3	-3.3 \pm 6.9	-1.2 \pm 5.6
PTH (IM)*	23.7 \pm 1.7	17.6 \pm 1.2	-25.1 \pm 3.8 \ddagger	-22.8 \pm 6.1	-29.0 \pm 0.7	24.4 \pm 4.2	26.6 \pm 3.5	17.9 \pm 8.9 \ddagger	12.3 \pm 12.8	24.8 \pm 13.1
Serum Ca	9.6 \pm 0.06	9.7 \pm 0.05	0.9 \pm 0.7	1.0 \pm 0.7	0.7 \pm 1.8	9.6 \pm 0.07	9.4 \pm 0.07	-1.3 \pm 0.6 \ddagger	-1.2 \pm 1.0	-1.4 \pm 0.9
Ionized Ca \ddagger	1.2 \pm 0.01	1.3 \pm 0.01	1.1 \pm 0.4 \ddagger	0.8 \pm 0.4	1.6 \pm 1.1	1.2 \pm 0.01	1.2 \pm 0.01	-0.8 \pm 1.1 \ddagger	-1.6 \pm 1.6	0.1 \pm 1.5

Values are means \pm SE. Cont, control; RE, resistance exercise; Avg, average; NTX, urinary *n*-telopeptide in nmol/day; DPD, urinary deoxypyridinoline cross-links in nmol/day; PYD, urinary pyridinium cross-links in nmol/day; Alk Phos, serum alkaline phosphatase in units/l; BSAP, bone-specific alkaline phosphatase in units/l; Osteocalcin, osteocalcin in ng/ml; 1,25 (OH)₂ Vit D, 1,25-dihydroxyvitamin D in pg/ml; 25 Vit D, 25-hydroxyvitamin D in pg/ml; PTH (MM), parathyroid hormone midmolecule in pg/ml; PTH (IM), parathyroid hormone intact molecule in pg/ml; Serum Ca, serum calcium in mg/dl; Ionized Ca, ionized calcium in mM. *Eight control subjects only; \ddagger 16 control subjects only; \ddagger baseline vs. BR ($P < 0.05$); \ddagger control vs. exercise ($P < 0.05$).

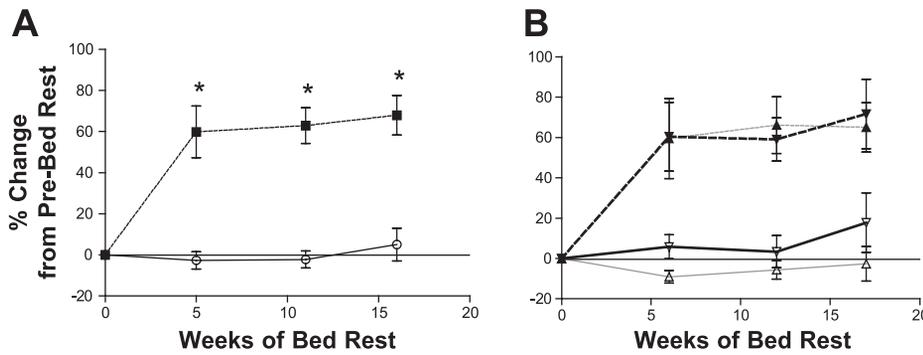


Fig. 3. Serum bone-specific alkaline phosphatase before, during, and at the end of bed rest. A: group means \pm SE for control (○) and exercise (■) subjects. B: same data separated by gender. Δ , Control men; \blacktriangle , exercised men; ∇ , control women; \blacktriangledown , exercised women.

($r = 0.89$) when normalized to body mass. All exercises combined averaged a total work load of 55,276 J per person per day, with an increase of 12,937 J per person per day in the last 8 wk of the bed rest compared with weeks 2 through 9 of bed rest.

The exercise subjects showed essentially no change in isokinetic strength from pre- to post-bed rest (data not shown). Although these subjects' back extensors showed average gains of 21% (30°/s) and 50% (60°/s), these within-group changes were not statistically significant. The previous control subjects' isokinetic data were not combined with the present data because the speed of contractions used during acquisition was different, namely 60°/s instead of 30°/s. However, the present control subjects showed decreases in isokinetic strength very similar to those documented in our laboratory's previous control subjects (17), with the greatest changes occurring in the knee extensors (-35%).

Tables 6 and 7 list the results of MRI and DEXA testing to determine post-bed rest changes in muscle volume and LBM, respectively. The MRI muscle volume results for the eight present control subjects were not combined with previous data because of slight differences in the acquisition protocol; however, the present results were very similar to those published for the eight male control subjects in our laboratory's previous bed-rest studies (17). In the control subjects, the total lower leg lost 23% of pre-bed rest volume, whereas the total thigh lost an average of 13% of pre-bed rest volume. In the exercise subjects after bed rest, lower-body muscle volume, as determined by MRI, had decreased in the total lower leg (-7%) and hamstrings (-8%) significantly less than the losses measured in the control subjects (-23% total lower leg, -13% hamstrings). In the exercise group, muscle volumes of the other (nonhamstring) thigh muscles were essentially unchanged from base-

line. Muscle volume of the psoas back muscles was greater after bed rest in both the exercise and control subjects, but the change was twice as great in the exercisers. DEXA soft tissue measurements indicated that, after bed rest, the control subjects had a statistically significant 11% loss in total leg lean mass (Table 7). Exercisers had a nonsignificant 1% decrease in leg lean mass. Exercisers had significantly increased arm and trunk LBM (+13 and +5%, respectively); control subjects showed a significant increase in arm lean mass of 3% but no change in trunk lean mass. Total lean mass changed significantly in both the control subjects ($-4.0 \pm 0.9\%$) and the exercise subjects ($+3.9 \pm 0.9\%$). Between-group differences were significant for all regions. Total body mass (Table 3) did not change significantly from pre-bed rest values.

Exercise-related complaints. No subject missed an exercise session because of pain or injury, but minor overuse injuries caused the exercise subjects to miss an average of 10% of the required exercise sets. All of the subjects had minor overuse complaints on at least 1 of the 102 days of exercise, averaging 32 days with complaints of pain and ranging from one subject who complained of pain on 2 days to another subject who complained of pain on 70 days. All exercise subjects had at least one complaint in the upper extremity and one complaint in the lower extremity. Eight of the nine subjects complained of low back pain at some time during the study, seven had complaints about the ankle and foot, five complained about the shoulder, four had pain about the forearm, and three had patello-femoral pain complaints. One subject had wrist pain, and another reported pain in the sacroiliac joint. Most complaints were minor, with symptoms of tendonitis or joint pain, and recovery required 1 or 2 days of reduced load or rest from a specific exercise. A few subjects complained of pain specific to the bone, indicating a stress reaction (4 in the forearm, 1 in

Table 5. Calcium excretion and balance during 17 wk of bed rest

	All Controls (n = 18), Pre-BR	All Controls (n = 18), Avg BR	Male Controls (n = 13), Pre-BR	Male Controls (n = 13), Avg BR	Female Controls (n = 5), Pre-BR	Female Controls (n = 5), Avg BR	All RE (n = 9), Pre-BR	All RE (n = 9), Avg BR	Male RE (n = 5), Pre-BR	Male RE (n = 5), Avg BR	Female RE (n = 4), Pre-BR	Female RE (n = 4), Avg BR
Ca Intake	1,023 ± 14	1,019 ± 19	1,002 ± 12	1,007 ± 13	1,077 ± 31	1,048 ± 40	1,008 ± 8	997 ± 9	1,003 ± 6	995 ± 8	1,014 ± 16	999 ± 19
Urine Ca	166 ± 16	230 ± 21*	176 ± 20	248 ± 27	140 ± 23	184 ± 20	189 ± 22	175 ± 24	151 ± 13	144 ± 27	236 ± 36	214 ± 38
Fecal Ca	671 ± 56	802 ± 55*	726 ± 44	868 ± 30	528 ± 161	629 ± 170	743 ± 56	749 ± 20	820 ± 49	723 ± 20	647 ± 97	727 ± 24
Net Ca												
Bal		-199 ± 32*		-208 ± 37		-174 ± 70		21 ± 54†		96 ± 53		-73 ± 87
Net Urine												
Ca Bal		-64 ± 14*		-72 ± 19		-44 ± 12		13 ± 11†		7 ± 19		22 ± 4
Net Fecal												
Ca Bal		-131 ± 31*		-142 ± 36		-102 ± 67		19 ± 51†		98 ± 35		-80 ± 88

Values are means \pm SE. Bal, balance. *Baseline vs. bed rest ($P < 0.05$), †control vs. exercise ($P < 0.05$).

Fig. 4. Urinary calcium excretion before, during, and after bed rest. A: group means \pm SE for control (\circ) and exercise (\blacksquare) subjects. B: same data separated by gender. \triangle , Control men; \blacktriangle , exercised men; ∇ , control women; \blacktriangledown , exercised women.

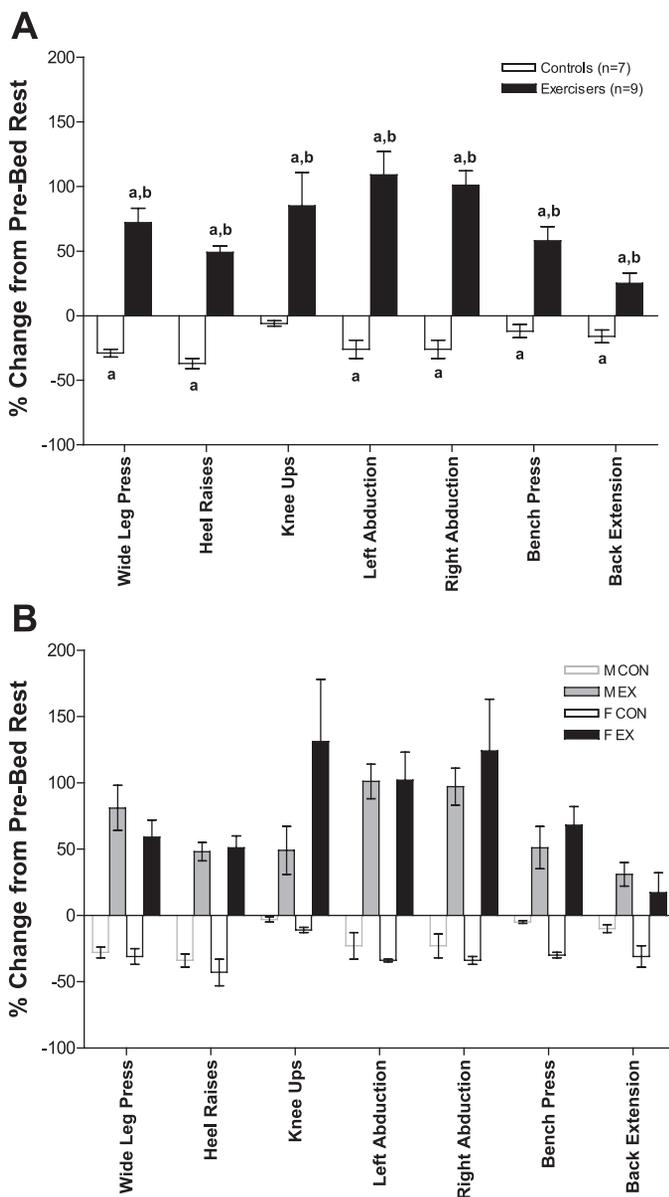
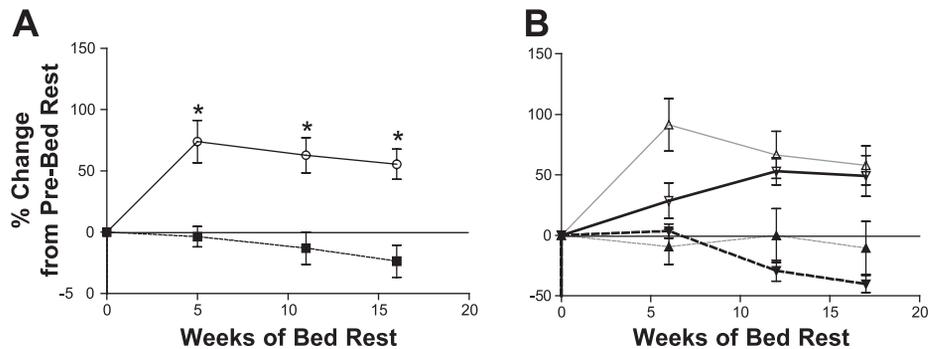


Fig. 5. Change in isotonic strength (1-repetition maximum; kg lifted) after 17 wk of bed rest. Data are means \pm SE. A: group means for control (open bars) and exercise (filled bars) subjects. B: same data separated by gender. M CON, male control subjects; M EX, male exercise subjects; F CON, female control subjects; F EX, female exercise subjects. ^a $P < 0.05$, baseline vs. bed rest; ^b $P < 0.05$, control vs. exercise.

the clavicle, and 1 in the tibia) and requiring several days of rest from a specific exercise associated with the pain. Decreased 1RM performance was sometimes noticed in the subjects who had significant musculoskeletal complaints at the time of testing. Nonsteroidal anti-inflammatory medications were avoided as much as possible because of the potential interference with bone mineralization. Ibuprofen or aspirin were used only when required to hasten recovery from overuse injuries. Five subjects did not use any anti-inflammatory drugs during the 17 wk of bed rest. Four exercise subjects used ibuprofen 200 to 1,600 mg/day or aspirin 1–6 tablets/day during the bed-rest period. The usage consisted of a total of 600 mg of ibuprofen given a subject over 2 days a month apart, 5,200 mg of ibuprofen given to one subject over a period of 5 days during 1 mo, 10,200 mg given to a subject over 14 days, 14 aspirin tablets given over 7 days during a 3-mo period, and 15,800 mg of ibuprofen given to a subject over 16 days during a 3-mo period.

DISCUSSION

DEXA BMD measurements for this study indicated that resistance exercise had a positive treatment effect, most notably in the lumbar spine, total hip, heel, total body, and pelvis; between-group differences for these regions were statistically significant. In the exercise subjects, post-bed rest regional BMD values did not differ significantly from pre-bed rest values, except that lumbar spine BMD increased 3.4%. Although the decrease in trochanter BMD in the exercise subjects was not statistically significant, there was great variability in the trochanter bone density changes, from a gain of 5% to a loss of 9%, indicating that further refinement of the exercise protocol may be warranted. This variability may have been caused by slight differences in the degree of dynamic loading during single-leg heel raises among exercise subjects.

Bone resorption increases during bed rest as measured by histomorphometry (32) or biochemical markers, such as hydroxyproline (12, 31) and collagen cross-links (7, 12, 13, 24, 34). Although the magnitude of change in bed-rest studies ($\sim 50\%$ increase) is less than that of the change seen during spaceflight ($\sim 100\text{--}150\%$ increase) of similar duration, the qualitative similarities are obvious.

In the present study, as expected, control subjects had an increase in resorption markers and essentially no change in bone-formation markers. These results support the idea that disuse conditions cause an uncoupling of the bone-remodeling cycle, with an increase in bone resorption but little or no change in bone formation (12, 24–26). The exercise group had

Table 6. Percent change in muscle volume after 17 wk of bed rest

	Male Controls (n = 5), %Change	Female Controls (n = 3), %Change	All Controls (n = 8), %Change	Male RE (n = 5), %Change	Female RE (n = 4), %Change	All RE (n = 9), %Change
Posterior back	-2.9±1.6	-10±2.9	-5.6±1.9	1.5±1.3	1.8±5.8	1.6±2.5
Psoas back	6.2±3.0	8.8±3.2	7.2±2.1*	14.1±2.6	19.3±1.6	16.4±1.8*
Soleus	-24.8±3.4	-36.7±2.3	-29.3±3.1*	-6.8±3.2	-13.3±3.1	-9.7±2.4*†
Gastrocnemius	-24.3±2.7	-34.3±4.1	-28.1±2.8*	-9.2±1.6	-3.2±3.5	-6.6±2.0*†
Total lower leg	-19.1±2.3	-29.2±2.7	-22.9±2.5*	-6.2±1.7	-8.6±1.9	-7.3±1.3*†
Sartorius	-6.6±0.9	-8.9±4.2	-7.5±1.6*	4.8±2.9	0.9±5.0	3.1±2.6†
Quadriceps	-12.7±1.7	-20.9±3.3	-15.8±2.1*	5.2±2.8	-1.9±2.4	2.0±2.2†
Hamstrings	-10.7±1.4	-17.3±4.7	-13.2±2.1*	-8.1±2.6	-8.5±1.2	-8.3±1.5†
Adductor thigh	-7.3±2.2	-10.7±4.2	-8.6±2.0*	-3.4±2.8	-2.8±2.4	-3.1±1.7
Total thigh	-10.7±1.6	-17.3±3.7	-13.2±2.0*	-0.1±2.1	-4.6±2.8	-2.1±1.8†

Values are means ± SE. *Baseline vs. bed rest ($P < 0.05$); †control vs. exercise ($P < 0.05$).

an increase in both resorption and formation markers, suggesting that resistance exercise caused an increased rate of bone remodeling with less uncoupling. In contrast, treatment with bisphosphonate during bed rest has been shown to decrease both resorption and formation markers (13). Serum and urinary markers of bone metabolism (such as *n*-telopeptide and BSAP) offer the theoretical possibility of assessing countermeasure effectiveness in-flight, providing a "real-time" picture of bone remodeling. However, use of bone markers in this way may be limited by their lack of regional specificity and by individual subject variability. Although bone formation may be occurring in some regions, losses may still be occurring in others, with bone markers reflecting only cumulative effects over the entire skeleton.

Bone biopsy and histomorphometry have shown that, during bed rest, bone formation decreases (1, 32). However, measurement of biochemical markers has indicated that bone formation either remains unchanged (12, 24, 31, 34) or decreases (18) during bed rest. These results likely reflect a difference between site-specific (biopsy) and systemic (biochemical markers) indexes of bone formation. After bed rest, markers of bone formation are generally increased (12, 24, 34).

Control subjects had a small but significant increase in serum ionized Ca along with reduced PTH and 1,25-dihydroxyvitamin D, consistent with previous findings (12, 24, 34). These changes are also consistent with disuse-related release of Ca from bone, decreased PTH secretion by the parathyroid gland in response to ionized Ca, and consequently decreased conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D in the kidney under the influence of PTH. A decrease in 1,25-dihydroxyvitamin D results in decreased gut absorption of Ca, evidenced by increased fecal Ca excretion by these subjects and by subjects measured previously in bed rest (12) and spaceflight (26). Exercise subjects did not show these changes;

their ionized Ca, PTH, and 1,25-dihydroxyvitamin D concentrations were unchanged. This suggests that intestinal Ca absorption was maintained in the exercise group; moreover, the findings may provide insight into the mechanism of the effect of exercise. Increased Ca absorption secondary to exercise has not been studied extensively; however, limited data from humans (35, 36) and animals (23) support this theory.

The ability of resistance exercise to maintain bone (at least overall) was also reflected in Ca balance, which was preserved in the exercise subjects but was significantly negative in the control subjects. Ca balance, like the biochemical markers, is limited as an indicator of bone formation or loss by its lack of regional specificity; it is, however, very sensitive to changes in bone metabolism. The exercisers' lack of hypercalciuria, which is a hallmark of disuse conditions, indicates that the exercise countermeasure could reduce space crew members' risk of developing renal stones.

For the exercisers at the end of bed rest, lower-body muscle mass and volume were essentially unchanged to slightly decreased, whereas 1RM strength was significantly increased. One explanation for this is that the 1RM strength gains from exercise training reflect the exercise subjects' improved technique as well as improved neural control (such as improved motor-neuron recruitment and pattern of neuron firing). Lower-body isokinetic strength testing of the exercise subjects did not improve at the end of bed rest. These results support the idea of specificity of training: exercise subjects trained isotonicly and showed improvement in lower-body isotonic (1RM) strength testing but not in isokinetic testing. It is also worth noting that the only lower-body muscle group not specifically targeted by the training regimen was the hamstrings, and in the exercise group the hamstrings were the only thigh muscle to show loss of volume by MRI. The volume loss in the soleus was not totally unexpected, because the original plan to exer-

Table 7. Lean body mass before and after 17 wk of bed rest

	All Cont (n = 15), Pre BR	All Cont (n = 15), Avg BR	All Cont (n = 15), %Change	Male Cont (n = 11), %Change	Female Cont (n = 4), %Change	All RE (n = 9), Pre BR	All RE (n = 9), Avg BR	All RE (n = 9), %Change	Male RE (n = 5), %Change	Female RE (n = 4), %Change
Total body	55.3±2.8	53.2±2.7	-4.0±0.9†	-3.1±1.1	-6.5±1.2	47.9±3.7	49.8±3.8	3.9±0.9†‡	3.8±0.7	0.7±4.1
Legs	17.3±1.0	15.6±1.0	-10.6±1.5†	-8.4±1.2	-16.4±3.2	14.8±1.4	14.7±1.5	-1.4±1.5‡	-0.5±1.7	-2.6±2.8
Trunk*	27.6±1.5	26.9±1.4	-2.4±1.1	-1.7±1.5	-4.0±0.9	24.0±1.5	25.2±1.5	5.0±1.2†‡	4.1±0.8	6.3±2.5
Arms	6.6±0.5	6.8±0.6	2.3±0.9†	3.4±1.0	-0.8±0.6	5.5±0.7	6.2±0.8	13.3±1.3†‡	12.0±1.3	14.8±2.3

Values are means ± SE. *Thirteen control subjects only; †baseline vs. bed rest ($P < 0.05$); ‡control vs. exercise ($P < 0.05$).

cise the soleus in isolation (with bent knee heel raises) was abandoned because of safety concerns. Specifically, during definition of the exercise protocol, the arm curl bar was balanced across the lower thighs with the knees bent 90°. This presented a hazard of slipping during heavily loaded exercise. The loss in the gastrocnemius was not as pronounced but could indicate a need for gastrocnemius exercise of its knee flexor function or in the flexed knee position. Furthermore, the finding that calf-muscle volume decreased in the exercisers could indicate that even daily resistance exercise targeting the calf may not be enough to completely overcome the lack of gravity on these all-important postural muscles. An increase in psoas back-muscle volume in the control subjects may have occurred because the subjects used these muscles during bed rest in ways not normally used while subjects are ambulatory.

Subject complaints of pain associated with the exercise protocol were minor but pervasive, reported at one time or another by all subjects. Development of local pain occurs frequently in any exercise program, and subjects needed only to rest before returning to exercise. Similarly, astronauts have experienced mild overuse pain when performing exercises on orbit and have modified exercises accordingly. Further modification of the exercises performed in this study may make them more effective for regions that may not have been adequately loaded during this exercise protocol and may reduce the risk of overuse in other regions. For example, the research subjects' frequent complaints of low back pain, coupled with the increase in lumbar spine BMD, indicate that the spine received more than adequate exercise with this protocol.

Crew members on Mir and the ISS have lost bone density despite the time-consuming (up to 3 h/day) exercise regimens they followed (26). For resistance exercise on ISS flights, crew members have used an in-flight resistance exercise device (the Interim Resistance Exercise Device or iRED), which can provide loads of up to 135 kg (300 lb) (21), but hardware problems have limited the use of this equipment.

It is likely that a resistance-exercise countermeasure would be used as but one tool in the effort to prevent musculoskeletal disuse on long-duration spaceflights. The ISS exercise protocol currently includes treadmill running and cycle ergometry. Although this bed-rest study clearly showed that impact is not necessary to maintain bone density in the calcaneus, it is possible that, when performed with sufficient loading, treadmill running and cycle ergometry contribute to maintenance of bone.

Ideally, more than one countermeasure option would be available to crew members for a given physiological concern. In the case of bone, countermeasures that are alternatives to resistance exercise could include a pharmaceutical countermeasure such as an oral bisphosphonate. Having more than one countermeasure available would provide a backup in case one countermeasure could not be used for a particular crew member. For example, a crew member might have to discontinue a drug countermeasure if it had untoward side effects during flight. Similarly, injury or equipment failure could prevent continued participation in an effective exercise countermeasure. In the near future, tests should be performed to determine whether alendronate, combined with the standard in-flight exercise protocols and equipment, can protect against bone loss on long-duration ISS flights.

In summary, this study showed that a progressive isotonic strength-training regimen had a significant protective effect on several changes associated with bed-rest disuse, including loss of BMD, unfavorable changes in serum and urinary bone markers, and hypercalciuria. The training regimen also seemed to offer a significant treatment effect for reducing the effects of disuse on muscle mass and strength. The training regimen may not fully protect all bone regions and muscle groups in every individual; protection of the trochanter and calf muscles may require further refinement of the exercise protocol. Furthermore, the BMD results do not directly address the most important question: How is bone strength, not just bone mass, affected by use of resistance exercise in bed rest or spaceflight? Despite these limitations, it seems prudent to increase the resistance-exercise component of present spaceflight exercise protocols and to determine how well bone and muscle loss during spaceflight can be prevented by resistance exercise, either alone or in combination with other countermeasure strategies.

ACKNOWLEDGMENTS

We thank Dr. Edward Powers for providing medical care to the subjects; Thomas C. Woltz for serving as the exercise trainer for this study; Chris Miller for overseeing the development, manufacturing, setup, and testing of the HEM; and Mary Jane Maddocks and Becky Shaughnessy for assisting with study coordination and support. We also thank Jane Krauhs for reviewing the manuscript.

GRANTS

This work was supported by NASA Grant NCC9-61; the General Clinic Resource Center at Baylor College of Medicine, Houston, TX; National Center for Research Resources Grant M01 RR-00188; and the National Space Biomedical Research Institute, Baylor College of Medicine.

REFERENCES

1. **Arnaud SB, Sherrard DJ, Maloney N, Whalen RT, and Fung P.** Effects of 1-week head-down tilt bed rest on bone formation and the calcium endocrine system. *Aviat Space Environ Med* 63: 14–20, 1992.
2. **Convertino VA.** Physiological adaptations to weightlessness: effects on exercise and work performance. *Exerc Sport Sci Rev* 18: 119–166, 1990.
3. **Fluckey JD, Dupont-Versteegden EE, Montague DC, Knox M, Tesch P, Peterson CA, and Gaddy-Kurten D.** A rat resistance exercise regimen attenuates losses of musculoskeletal mass during hindlimb suspension. *Acta Physiol Scand* 176: 293–300, 2002.
4. **Gleeson PB, Protas EJ, LeBlanc AD, Schneider VS, and Evans HJ.** Effects of weight lifting on bone mineral density in premenopausal women. *J Bone Miner Res* 5: 153–158, 1990.
5. **Greenleaf JE, Bulbulian R, Bernauer EM, Haskell WL, and Moore T.** Exercise-training protocols for astronauts in microgravity. *J Appl Physiol* 67: 2191–2204, 1989.
6. **Ihaka R and Gentleman R.** A language for data analysis and graphics. *J Comp Graph Stat* 5: 299–314, 1996.
7. **Inoue M, Tanaka H, Moriwake T, Oka M, Sekiguchi C, and Seino Y.** Altered biochemical markers of bone turnover in humans during 120 days of bed rest. *Bone* 26: 281–286, 2000.
8. **Kramer WJ and Fleck SJ.** Resistance training: basic principles (Part 4 of 4). *Phys Sports Med* 16: 69–81, 1988.
9. **LeBlanc A, Lin C, Shackelford L, Sinityn V, Evans H, Belichenko O, Schenkman B, Kozlovskaya I, Oganov V, Bakulin A, Hedrick T, and Feedback D.** Muscle volume, MRI relaxation times (T2), and body composition after spaceflight. *J Appl Physiol* 89: 2158–2164, 2000.
10. **LeBlanc A, Schneider V, Krebs J, Evans H, Jhingran S, and Johnson P.** Spinal bone mineral after 5 weeks of bed rest. *Calcif Tissue Int* 41: 259–261, 1987.
11. **LeBlanc A, Schneider V, and Shackelford L.** Bone mineral and lean tissue loss after long duration space flight. *J Musculo Neur Interact* 1: 157–160, 2000.

12. **LeBlanc A, Schneider V, Spector E, Evans H, Rowe R, Lane H, Demers L, and Lipton A.** Calcium absorption, endogenous excretion, and endocrine changes during and after long-term bed rest. *Bone* 16, Suppl 4: 301S–304S, 1995.
13. **LeBlanc AD, Driscoll TB, Shackelford LC, Evans HJ, Rianon NJ, Smith SM, Feedback DL, and Lai D.** Alendronate as an effective countermeasure to disuse induced bone loss. *J Musculo Neuron Interact* 2: 335–343, 2002.
14. **LeBlanc AD, Evans HJ, Marsch C, Schneider V, Johnson PC, and Jhingran SG.** Precision of dual photon absorptiometry measurements. *J Nucl Med* 27: 1362–1365, 1986.
15. **LeBlanc AD, Schneider VS, Engelbretson DA, and Evans HJ.** Precision of regional bone mineral measurements obtained from total-body scans. *J Nucl Med* 31: 43–45, 1990.
16. **LeBlanc AD, Schneider VS, Evans HJ, Engelbretson DA, and Krebs JM.** Bone mineral loss and recovery after 17 weeks of bed rest. *J Bone Miner Res* 5: 843–850, 1990.
17. **LeBlanc AD, Schneider VS, Evans HJ, Pientok C, Rowe R, and Spector E.** Regional changes in muscle mass following 17 weeks of bed rest. *J Appl Physiol* 73: 2172–2178, 1992.
18. **Lueken SA, Arnaud SB, Taylor AK, and Baylink DJ.** Changes in markers of bone formation and resorption in a bed rest model of weightlessness. *J Bone Miner Res* 8: 1433–1438, 1993.
19. **Menkes A, Mazel S, Redmond RA, Koffler K, Libanati CR, Gundberg CM, Zizic TM, Hagberg JM, Pratley RE, and Hurley BF.** Strength training increases regional bone mineral density and bone remodeling in middle-aged and older men. *J Appl Physiol* 74: 2478–2484, 1993.
20. **Oganov VS, Grigoriev AI, Voronin LI, Rakhmanov AS, Bakulin AV, Schneider VS, and LeBlanc AD.** Bone mineral density in cosmonauts after flights lasting 4.5–6 months on the Mir orbital station. *Aviakosm Ekolog Med* 26: 20–24, 1992.
21. **Schneider SM, Amonette WE, Blazine K, Bentley J, Lee SM, Loehr JA, Moore AD Jr, Rapley M, Mulder ER, and Smith SM.** Training with the International Space Station interim resistive exercise device. *Med Sci Sports Exerc* 35: 1935–1945, 2003.
22. **Schneider VS.** *Modification of Negative Calcium Balance and Bone Loss During Prolonged Bed Rest.* Washington, DC: NASA, 1981. (NASA Tech. Rep. T-660-1981)
23. **Shiga K, Hara H, Okano G, Ito M, Minami A, and Tomita F.** Ingestion of difructose anhydride III and voluntary running exercise independently increase femoral and tibial bone mineral density and bone strength with increasing calcium absorption in rats. *J Nutr* 133: 4207–4211, 2003.
24. **Smith SM, Davis-Street JE, Feserman JV, Calkins DS, Bawa M, Macias BR, Meyer RS, and Hargens AR.** 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. *J Bone Miner Res* 18: 2223–2230, 2003.
25. **Smith SM, Nillen JL, Leblanc A, Lipton A, Demers LM, Lane HW, and Leach CS.** Collagen cross-link excretion during space flight and bed rest. *J Clin Endocrinol Metab* 83: 3584–3591, 1998.
26. **Smith SM, Wastney ME, Morukov BV, Larina IM, Nyquist LE, Abrams SA, Taran EN, Shih CY, Nillen JL, Davis-Street JE, Rice BL, and Lane HW.** Calcium metabolism before, during, and after a 3-mo spaceflight: kinetic and biochemical changes. *Am J Physiol Regul Integr Comp Physiol* 277: R1–R10, 1999.
27. **Snow-Harter C and Marcus R.** Exercise, bone mineral density, and osteoporosis. *Exerc Sport Sci Rev* 19: 351–388, 1991.
28. **Spector E, LeBlanc A, and Shackelford L.** Hologic QDR 2000 whole-body scans: a comparison of three combinations of scan modes and analysis software. *Osteoporos Int* 5: 440–445, 1995.
29. **Stone MH.** Connective tissue and bone response to strength training. In: *Strength and Power in Sport*, edited by Komi PV. Oxford, UK: Blackwell Scientific, 1991, p. 279–290.
30. **Tesch PA, Buchanan P, and Dudley GA.** An approach to counteracting long-term microgravity-induced muscle atrophy. *Physiologist* 33, Suppl 1: S77–S79, 1990.
31. **Van der Wiel HE, Lips P, Nauta J, Kwakkel G, Hazenberg G, Netelenbos JC, and Van der Vijgh WJ.** Intranasal calcitonin suppresses increased bone resorption during short-term immobilization: a double-blind study of the effects of intranasal calcitonin on biochemical parameters of bone turnover. *J Bone Miner Res* 8: 1459–1465, 1993.
32. **Vico L, Chappard D, Alexandre C, Palle S, Minaire P, Riffat G, Morukov B, and Rakhmanov S.** Effects of a 120 day period of bed-rest on bone mass and bone cell activities in man: attempts at countermeasure. *Bone Miner* 2: 383–394, 1987.
33. **Vuori I, Heinonen A, Sievanen H, Kannus P, Pasanen M, and Oja P.** Effects of unilateral strength training and detraining on bone mineral density and content in young women: a study of mechanical loading and unloading on human bones. *Calcif Tissue Int* 55: 59–67, 1994.
34. **Zerwekh JE, Ruml LA, Gottschalk F, and Pak CY.** The effects of twelve weeks of bed rest on bone histology, biochemical markers of bone turnover, and calcium homeostasis in eleven normal subjects. *J Bone Miner Res* 13: 1594–1601, 1998.
35. **Zittermann A, Sabatschus O, Jantzen S, Platen P, Danz A, Dimitriou T, Scheld K, Klein K, and Stehle P.** Exercise-trained young men have higher calcium absorption rates and plasma calcitriol levels compared with age-matched sedentary controls. *Calcif Tissue Int* 67: 215–219, 2000.
36. **Zittermann A, Sabatschus O, Jantzen S, Platen P, Danz A, and Stehle P.** Evidence for an acute rise of intestinal calcium absorption in response to aerobic exercise. *Eur J Nutr* 41: 189–196, 2002.