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Resistive exercises, with or without whole body vibration, prevent vertebral marrow fat accumulation during 60 days of head-down tilt bed rest in men

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Trudel G, Coletta E, Cameron I, Belavý DL, Lecompte M, Armbrecht G, Felsenberg D, Uhthoff HK. Resistive exercises, with or without whole body vibration, prevent vertebral marrow fat accumulation during 60 days of head-down tilt bed rest in men. *J Appl Physiol* 112: 1824–1831, 2012. First published March 22, 2012; doi:10.1152/japplphysiol.00029.2012.—Fat accumulates in the bone marrow of lumbar vertebrae with bed rest. Exercise with or without whole body vibration may counter this effect. Our objectives were to measure *1*) the vertebral fat fraction (VFF) of men subjected to bed rest who performed resistive exercises with (RVE, $n = 7$) or without whole body vibration(RE, $n = 8$) or no exercise (CTR, $n = 9$) using three MRI techniques; and *2*) changes in peripheral blood counts. Twenty-four healthy men (age: 20–45 yr) underwent −6° head-down tilt (HDT) bed rest for 60 days. MRI was performed using three techniques (fat saturation, proton spectroscopy, and in and out of phase) to measure the fat fraction of L_3, L_4, and/or L_5 at baseline, mid-HDT, and end-HDT. Erythrocytes and leukocytes were counted at HDT *days 19*, *33*, *47*, *54*, and *60*. The mean absolute VFF was increased in the CTR group at mid-HDT and end-HDT (+3.9 ± 1.3 and +3.6 ± 1.2%, respectively, both $P < 0.05$). The RE group had a smaller VFF change than the CTR group at mid-HDT (−0.9 ± 1.2 vs. +3.9 ± 1.3%, $P < 0.05$). The RVE group had a smaller VFF change than the CTR group at end-HDT (−2.6 ± 1.9 vs. +3.5 ± 1.2%, $P < 0.05$). Erythrocyte counts were increased in all groups at HDT *day 19* and HDT *day 33* and in the RE group at HDT *day 54* (all $P < 0.05$). Bed rest for 60 days at −6° HDT increased lumbar VFF in men beyond natural involution. RVE and RE regimens effectively prevented VFF accumulation. Higher erythrocyte counts were not altered by RVE or RE. Whole body vibration, along with RE administered to people with prolonged immobility, may prevent fat accumulation in their bone marrow.

bone marrow; magnetic resonance imaging; erythrocytes

WEIGHTLESSNESS AND IMMOBILIZATION lead to musculoskeletal alterations affecting the spine. These include altered bone density, stiffness, and architecture; increased calcium excretion; and reduced paravertebral muscle mass, strength, and resistance to insulin (47). Bed rest has been used as a model for microgravity (47). One recently recognized additional effect of bed rest on the spine is vertebral marrow fatty accumulation (48).

Vertebral marrow fat content has been measured since 1965 (26). Longitudinal fatty marrow involution proceeds throughout life at an average annual rate of 0.6–0.7%, which translates into an average monthly increase of 0.05% (30, 33, 36). Using histology and, more recently, MRI, investigators have identified several conditions that acutely alter vertebral marrow fat content: glucocorticoid administration, paralysis, ovariectomy, alcohol abuse, and osteoporosis (15, 23, 27, 35). We measured increases in vertebral marrow fat in 24 women who underwent −6° head-down tilt (HDT) bed rest for 60 days as a model for space flight [Women International Space Simulation for Exploration (WISE) study] (48). The mean absolute vertebral marrow fat fraction increased by 2.5% over the 60 days, ~25 times faster than physiological fat accumulation. Neither a regimen combining aerobic and resistive exercises, nor a leucin-rich diet prevented the vertebral marrow fatty accumulation.

Whole body platforms vibrating at various frequencies, amplitudes, and directions have been used for fitness, therapy, and performance (39). They were observed to *1*) increase muscle oxygen consumption, temperature, and performance; *2*) increase mineral density at the hip (25, 50); *3*) improve balance and decrease falls in the elderly (9); and *4*) decrease muscle atrophy secondary to immobilization (6). This generated interest in the potential for whole body vibration to decrease systemic fat, alone or in combination with exercise or diet (1, 21, 34, 44, 51). However, marrow fat control appears to be independent of systemic fat (3, 17, 19, 48), and the effect of whole body vibration on marrow fat has, to our knowledge, never been studied. Based on a potential inverse relationship between bone formation and marrow fat, the direct and indirect stimulation of bones through muscle contractions from exercise, with or without whole body vibration, may decrease marrow fat (56).

The effect of bed rest as a surrogate for microgravity on vertebral fat fraction (VFF) has, to our knowledge, never been studied in men, who outnumber women among astronauts and cosmonauts and have higher baseline fat marrow content than women (30, 33, 45). Similarly, the ability of exercise, with or without whole body vibration during bed rest, to prevent VFF increases, and hemopoietic alterations are unknown. Increases in VFF have important physiological implications on bone metabolism (24, 54, 56, 57), hemopoietic metabolism, and immunity/inflammation (7, 13, 43), as well as on energy metabolism and thermogenesis (22, 29, 32), and potential clinical applications in real microgravity environments, and on Earth for bedridden patients or people with limited mobility. In women, bed rest and vertebral marrow fat accumulation in the

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hemopoietically active bone marrow led to increased leukocyte counts (48).

The 2nd Berlin BedRest Study (BBR2–2) tested the effects of resistive exercises, with and without whole body vibration, during 60 days of −6° HDT bed rest (5). In that study, 24 men were randomly assigned to perform resistive exercises with whole body vibration (RVE, $n = 7$), resistive exercises only (RE, $n = 8$), or no exercise (CTR, $n = 9$). Our objectives in the present study were *1*) to measure the VFF of the participants using three MRI techniques; and *2*) to determine the effects on peripheral blood counts. Our hypotheses were that *1*) VFF increases in men during HDT bed rest; *2*) both RVE and RE limit VFF accumulation; *3*) all three MRI techniques can monitor VFF change; and *4*) changes in VFF are associated with changes in peripheral blood counts.

METHODS

The BBR2–2 was carried out at the Benjamin Franklin Campus of the Charité Universitätsmedizin in Berlin, Germany. The methods have been described elsewhere (5). We are reporting data collected during 9 days of baseline data collection followed by 60 days of −6° HDT bed rest.

The inclusion and exclusion criteria were extensive (5). Relevant inclusion criteria for the current study included psychological and medical health, male sex, height 155–195 cm, and age 20–45 yr. Relevant exclusion criteria included addiction to alcohol; chronic disease; metabolic or hormonal disturbance; cardiovascular disease; coagulation disorder; any muscle, bone, or joint disease; metal implant; low back pain; spinal surgery; severe scoliosis; results of dual-energy X-ray absorptiometry of the lumbar spine and hip less than −1.5 SD; trabecular density of the lumbar spine as determined by quantitative computed tomography <120 mg/ml; and any regular medication intake. The study was approved by the Ethics Committee of the Charité Universitätsmedizin. All subjects gave their informed, written consent before participating in the study, and all received 24-h nursing and medical supervision.

Exercise and Vibration Protocols

The countermeasure exercise protocol is discussed in detail elsewhere (5). In brief, exercises targeted the load-bearing regions where most bone and muscle are lost during bed rest (i.e., lower quadrant and lumbar region). The training program consisted of high-load resistive exercise training aimed at achieving muscle hypertrophy (55). A single-set regimen was chosen to minimize exercise time (5, 55). Training was performed 3 days/wk. The subject lay in the HDT posture on a sliding back rest with padded shoulder restraints. The feet were positioned on the footplate, and force was also transmitted via the shoulder restraints to the shoulders. A pneumatic system generated the required pressure. The force levels were monitored via sensors in the footplate. In the vibration group, additional force was generated via a side-alternating movement of the footplate. After a short warm-up, the following exercises were performed on the Galileo Space exercise device (Novotec Medical , Pforzheim, Germany): *1*) bilateral leg press (~75–80% of pre-bed-rest maximum voluntary contraction); *2*) single-leg heel raises (~1.3 times body weight); *3*) double-leg heel raises (~1.8 times body weight); and *4*) back and forefoot raise (performing hip and lumbar spine extension against gravity with ankle dorsiflexion, but with ~1.5 times body weight applied at the shoulders).

The RVE group performed the same exercises as the RE group, except that whole body vibration was applied. The corresponding vibration parameters were as follows: *1*) frequency 24 Hz, amplitude 3.5–4 mm, and peak acceleration ~8.7 *g*, where $g = 9.81\ ms^{-2}$; *2*) frequency 26 Hz, amplitude 3.5–4 mm, and peak acceleration ~10.2 *g*; *3*) frequency 26 Hz, amplitude 3.5–4 mm, and peak acceleration ~10.2 *g*; and 4) frequency 16 Hz, amplitude 3.5–4 mm, and acceleration ~3.9 *g*. Acceleration parameters refer to the platform; effective acceleration values on the subject depend on subject position and muscle stiffness and are generally lower. The maximum resulting ground reaction forces transmitted to the feet produce effective acceleration at the feet in the order of 0.7 *g* (unpublished observations).

MRI

All subjects underwent MRI 8 or 9 days before the start of bed rest (baseline), after 27 or 28 days of bed rest (mid-HDT), and after 55 or 56 days of bed rest (end-HDT). To allow time for equalization of body fluid, subjects rested in bed in the horizontal position for 2 h before scanning. After the rest period, subjects were positioned in the supine position on the scanning bed of a 1.5-T Siemens Magnetom Symphony syngo MR 2004A (Siemens, Erlangen, Germany). The subject's legs were in neutral rotation (kneecaps oriented to the ceiling and first metatarsal oriented vertically) with the knee in 20° of flexion. The arms were placed at 20° of abduction with the elbows relaxed and the forearms placed parallel to the trunk. Sandbags were used to ensure and maintain this position. A standardized pillow was placed under the subject's head. The lower lumbar vertebrae were imaged using three techniques: proton density (PD) turbo spin echo (TSE), with and without fat saturation (fatsat), magnetic resonance spectroscopy (MRS), and T_1 FLASH in-phase and out-of-phase imaging (IOP).

fatsat. The vertebrae were imaged using sagittal PD-TSE, with and without fatsat, with a field of view of 300 mm, echo time (TE) of 13 ms, repetition time (TR) of 2,000 ms, slice thickness of 6 mm, and flip angle of 180° for the refocusing of pulses. Images from midvertebra sections were imported into Image J (National Institutes of Health, Bethesda, MD) and transformed from 16 to 32 bits, then adjusted to a 512 × 512 pixel matrix. A standardized rectangular region of interest (ROI) was drawn exclusive of superior and inferior endplates and of anterior and posterior cortices for L_3, L_4, and L_5. The mean pixel density for each ROI was measured on the images, where black was 0 and white was 1,486. The ROI mean pixel densities were used in the following formula to calculate the fat fraction for each vertebra: fat fraction = 100 × (PD − PD Fatsat)/PD, where PD = PD without fatsat, and PD Fatsat = PD with fatsat (40). The mean value of L_3, L_4, and L_5 constituted the VFF value used for each patient at each time point with this technique.

MRS. Since magnetic resonance (MR) proton spectra take longer to acquire, we imaged the L_5 vertebral body only, using a TE = 30 ms, TR = 4,000 ms, flip angle of 90°, and water suppression bandwidth of 35 Hz in a volume of 15 ×15 × 15 mm^3 with 128 averages. Images were postprocessed on a Siemens workstation, where three fat peaks were added to obtain the total fat peak height and peak integral: $(\text{-}CH_2\text{-})_n$, -CH=CH-CH_2-, and CH_3- (16). The total fat peak integral was then compared with the water peak integral. The VFF was calculated using the formula: fat fraction = fat integral/(water integral + fat integral) × 100.

IOP. The vertebrae were imaged using sagittal spoiled gradient echo (i.e., FLASH) sequences in phase and out of phase with a field of view of 300 mm, TE = 2.44 ms out of phase, and 4.76 ms in phase, a TR = 160 ms, a flip angle of 8°, a slice thickness of 6 mm, and a voxel size of 1.3 × 0.7 × 6.0 mm^3. The midvertebra images for L_3, L_4, and L_5 were processed in Image J using the same methods as for the fatsat data. The ROI mean pixel densities were used in the following formula to calculate the fat fraction for each vertebra: fat fraction = (in phase − out phase)/(2 × in phase) × 100. As this formula will calculate VFF values <50% only (11, 40), we used spectroscopy images to correct when the fat fraction was ≥50% (11, 40). If the total fat peak integral of the spectroscopy image exceeded the water peak integral, the fat fraction was calculated using the following formula: fat fraction = 1 − (in phase − out phase)/(2 × in

phase) × 100 (11). The mean VFF of L_3, L_4, and L_5 constituted the VFF value used for each patient at each time point with this technique.

Erythrocyte, Leukocyte, and Platelet Counts

Fasting (>10 h) blood samples were collected immediately after the subjects awoke, at the same time each morning, to minimize diurnal changes. Samples were drawn at baseline, HDT *day 19* (HDT19), *day 33* (HDT33), *day 47* (HDT47), *day 54* (HDT54), and *day 60* (HDT60). Counts were processed on a Sysmex System XE 2100 hemocytometer (Norderstedt, Germany).

Data Analysis

All data were analyzed using SPSS 18.0 (SPSS-IBM, Armonk, NY). The sample size in the BBR2–2 was based on bone parameters, not VFF (4); however, statistically significant VFF changes were detected in a related study with 24 patients undergoing a similar course of 60-day bed rest (48). With these data, we estimated a correlation of 0.77 between repeated measures of VFF and a between-subject SD of 16.1% (relative to the mean VFF of all subjects). Given these data, and assuming a power of 0.8 and an α-level of 0.05, the present study design, with three repeated measures, should detect a difference of 3.8% in VFF between the RE and RVE groups (G*Power version 3.1.2; http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3). Since there was no statistically significant difference in fat content between the three vertebrae (L_3, L_4, and L_5), we report the average of L_3, L_4, and L_5 for fatsat and IOP. Absolute VFF is reported as percent ± 1 SE. To compare the three participant groups, each with different baseline VFF values, and the three MRI techniques, each with different baseline VFF values, we report absolute VFF changes from baseline. This allowed us to combine the data from all three techniques and to define our primary outcome measure.

We used repeated-measures ANOVA to compare within-group changes in VFF and blood count during bed rest. We used one-way ANOVA and post hoc Gabriel *t*-tests to assess differences in VFF between the three groups at each time point. To further test between-group differences, we conducted secondary analyses of three-group ANOVAs using the data for all three MRI techniques at both mid-HDT and end-HDT; if the results were significant, we examined two-group ANOVAs between all group pairs (CTR vs. RE, CTR vs. RVE, and RE vs. RVE) to determine which of the groups differed.

RESULTS

One participant in the RE group left the study after mid-HDT, and in one participant in the CTR group no MR spectrum was obtained at end-HDT. The participants' baseline demographic characteristics and VFF are shown in Table 1. The RVE group had higher baseline VFF than the RE group, both with the fatsat technique and with all techniques combined ($P < 0.05$), and their mean age was 3 yr older [nonsignificant (NS)]; no difference remained after correction for age. There were no statistically significant differences between the three groups on other VFF measures or blood counts (data not shown).

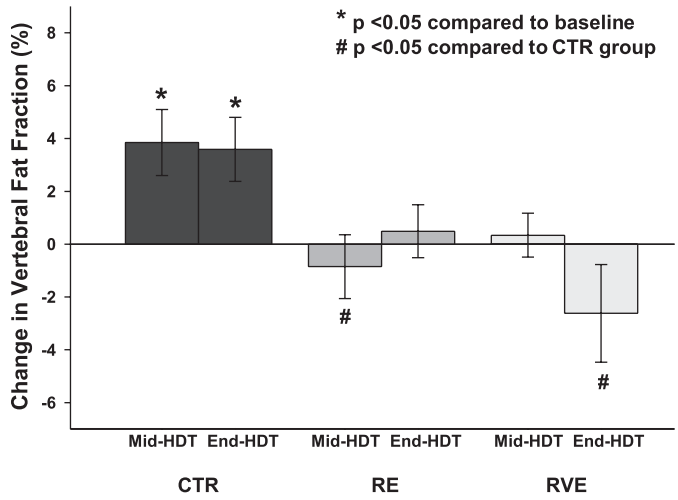

Fig. 1. Absolute change in vertebral fat fraction (VFF) during 60 days of −6° head-down tilt (HDT) bed rest in men: all MRI techniques. The CTR (control) group accumulated fat, while the RE (resistive exercises) and RVE (resistive exercises with whole body vibration) protocols prevented fat accumulation. Values are means ± SE. *$P < 0.05$ from baseline. #$P < 0.05$ from CTR group.

Absolute VFF Values

The three MRI techniques provided different estimates of VFF (Table 1). The fatsat technique consistently gave higher VFF values than MRS and IOP, but not significantly so, in all groups at baseline and at both mid-HDT and end-HDT.

Changes in VFF

All MRI techniques. The mean absolute VFF in the CTR group increased from baseline to both mid-HDT and end-HDT (+3.9 ± 1.3 and +3.6 ± 1.2%, respectively, both $P < 0.05$; Fig. 1). The RE group had a lower VFF than the CTR group at mid-HDT (−0.9 ± 1.2 vs. +3.9 ± 1.3%, $P < 0.05$; Fig. 1). The RVE group had a lower VFF than the CTR group at end-HDT (−2.6 ± 1.9 vs. +3.6 ± 1.2%, $P < 0.05$; Fig. 1). The three-group ANOVA showed a group interaction for VFF changes (*F*-statistic 8.68, $P < 0.05$). The two-group ANOVA confirmed significant group interactions between CTR vs. RVE and between CTR vs. RE (*F*-statistic 13.38 and 10.64, respectively, both $P < 0.05$), but not between RVE vs. RE.

Table 1. *Baseline demographic characteristics and vertebral fat fraction of subjects who performed resistive exercises with or without whole body vibration or no exercise during 60 days of −6° head-down tilt bed rest*

					Vertebral Fat Fraction (MRI Technique), %			
Group	*n*	Age, yr	Weight, kg	BMI, kg/m^2	Fatsat	MRS	IOP	All (n = 72)
All	24	33 ± 2	78.9 ± 1.8	24.3 ± 0.4	47.3 ± 1.5	41.8 ± 1.7	42.6 ± 1.7	43.9 ± 1.0
RVE	7	34 ± 4	81.1 ± 2.5	24.9 ± 0.5	52.1 ± 1.9*	46.6 ± 2.5	47.3 ± 3.7	48.7 ± 1.6*
RE	8	31 ± 2	75.0 ± 4.5	23.4 ± 0.8	42.5 ± 2.5	38.5 ± 2.7	38.2 ± 1.8	39.7 ± 1.4
CTR	9	33 ± 3	80.6 ± 1.7	24.6 ± 0.7	48.0 ± 2.1	40.9 ± 2.9	42.8 ± 2.5	43.9 ± 1.5

Values are means ± SE; *n*, no. of subjects. RVE, resistive exercises with whole body vibration; RE, resistive exercises without whole body vibration; CTR, no exercise; BMI, body mass index; fatsat, proton density turbo spin echo with and without fat saturation; MRS, magnetic resonance spectroscopy; IOP, T_1 FLASH in-phase and out-of-phase imaging. *$P < 0.05$ compared with RE.

Individual MRI techniques. With the fatsat technique, the mean change in VFF from baseline in the CTR group was +2.6 ± 1.6% (NS) at mid-HDT and +5.4 ± 2.2% ($P < 0.05$) at end-HDT (Fig. 2*A*). With MRS, the corresponding changes were +2.7 ± 2.5 and +2.4 ± 1.9% (NS; Fig. 2*B*), and with the IOP technique, +5.6 ± 2.6 and +0.7 ± 1.2% (NS; Fig. 2*C*).

In the RE group, the mean changes in VFF at mid-HDT and end-HDT with the fatsat technique were 0.0 ± 2.6 and +3.6 ± 1.9%, respectively (NS; Fig. 2*A*). With MRS, the corresponding changes were −0.8 ± 1.3 and −1.8 ± 1.4% (NS; Fig. 2*B*), and with the IOP technique, +1.8 ± 2.1 and −0.4 ± 1.4% (NS; Fig. 2*C*).

Finally, in the RVE group, the mean VFF changes at mid-HDT and end-HDT with the fatsat technique were +0.2 ± 0.9 and −2.4 ± 2.7%, respectively (NS; Fig. 2*A*). The corresponding changes with MRS were −0.1 ± 1.2 and −0.9 ± 1.9% (NS; Fig. 2*B*), and with the IOP technique, +1.2 ± 2.0 and −1.9 ± 4.2% (NS; Fig. 2*C*).

Erythrocyte, Leukocyte, and Platelet Counts

Erythrocyte counts were increased in all three groups at HDT19 and HDT33 and only in the RE group at HDT54 (all $P < 0.05$; Fig. 3*A*). Leukocyte counts were increased at HDT47 only in the CTR group (+540 ± 220 × 10^6/l, $P < 0.05$; Fig. 3*B*). Platelet counts were increased in all three groups, but not significantly so (Fig. 3*C*).

DISCUSSION

Vertebral bone marrow fat accumulated in inactive participants who underwent 60 days of −6° HDT bed rest. Participants who followed protocols of exercise with or without whole body vibration were protected against vertebral fat accumulation. These results confirmed our first and second hypotheses, respectively.

The VFF increases that we observed in the CTR group, 3.9% after 1 mo and 3.6% after 2 mo, are more than 36-fold faster than the normal involution of bone marrow (predicted to be 0.05% at 1 mo and 0.1% at 2 mo) (30, 33, 36). To our knowledge, this study is only the second investigation on changes in fat related to bed rest in hemopoietically active human vertebral bone marrow. This first experiment in men confirms the findings of the WISE study in women (48). Vertebral fat marrow content was higher at baseline in men (42.6%; IOP technique) than in women (29%) (48). The fact that men have higher bone marrow fat content compared with women has already been well documented (30, 33, 45). Besides the difference of sex, only one MRI technique (IOP) was used in the WISE study, in the coronal plane. In the BBR2–2, three MRI techniques were used in the sagittal plane.

In the WISE study, neither the nutritional countermeasure nor the countermeasure of aerobic low-body negative pressure plus RE prevented VFF accumulation (48). In the present study, while the CTR group showed large VFF increases, the RE countermeasure prevented VFF accumulation, and the combination of whole body vibration with RE decreased the VFF, compared with the CTR group.

The accelerated bone marrow involution has important physiological deleterious implications on bone metabolism (24, 54, 56, 57), hemopoietic metabolism, and immunity/inflammation (7, 13, 43), as well as on energy metabolism and thermogenesis

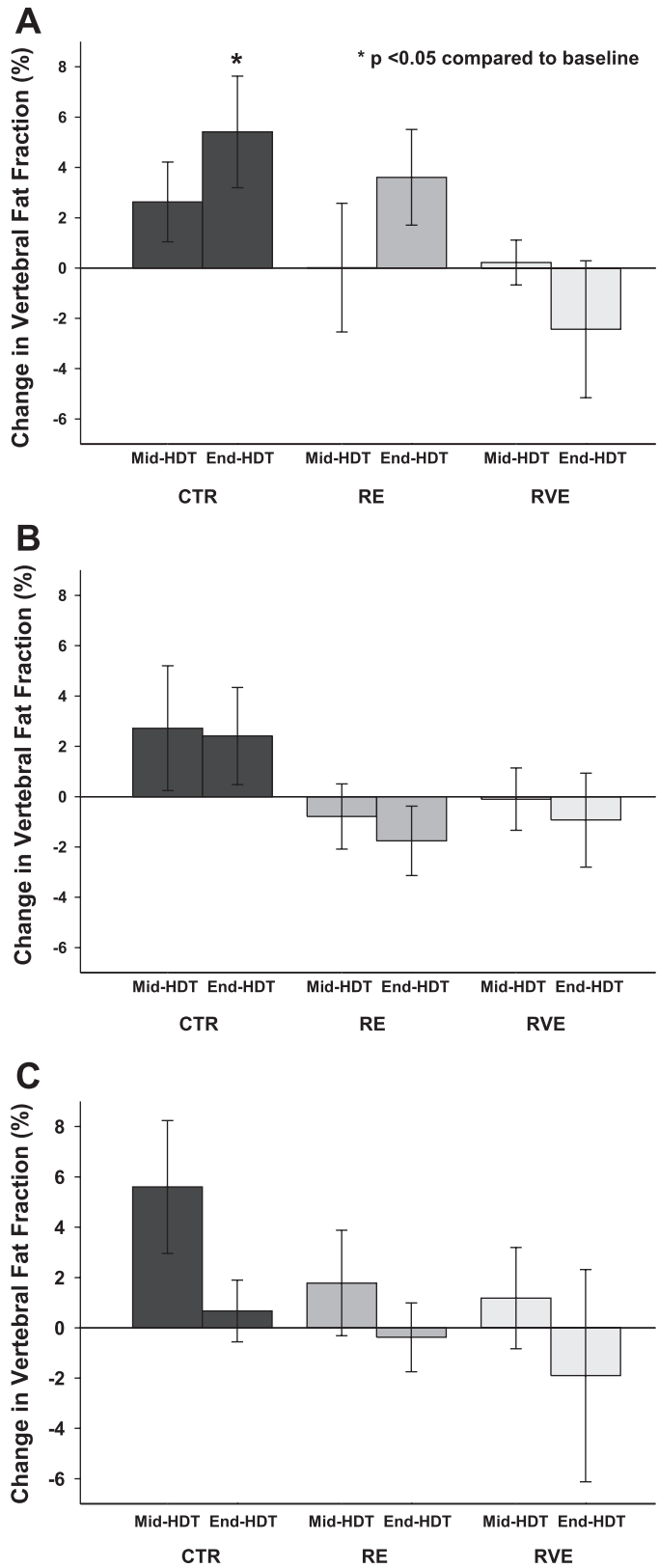

Fig. 2. Absolute change in VFF: individual MRI techniques. *A*: fat saturation. *B*: magnetic resonance spectroscopy. *C*: in-phase and out-of-phase imaging. The three imaging techniques show similar trends in VFF change for each intervention (CTR, RE, and RVE). Values are means ± SE. *$P < 0.05$ from baseline.

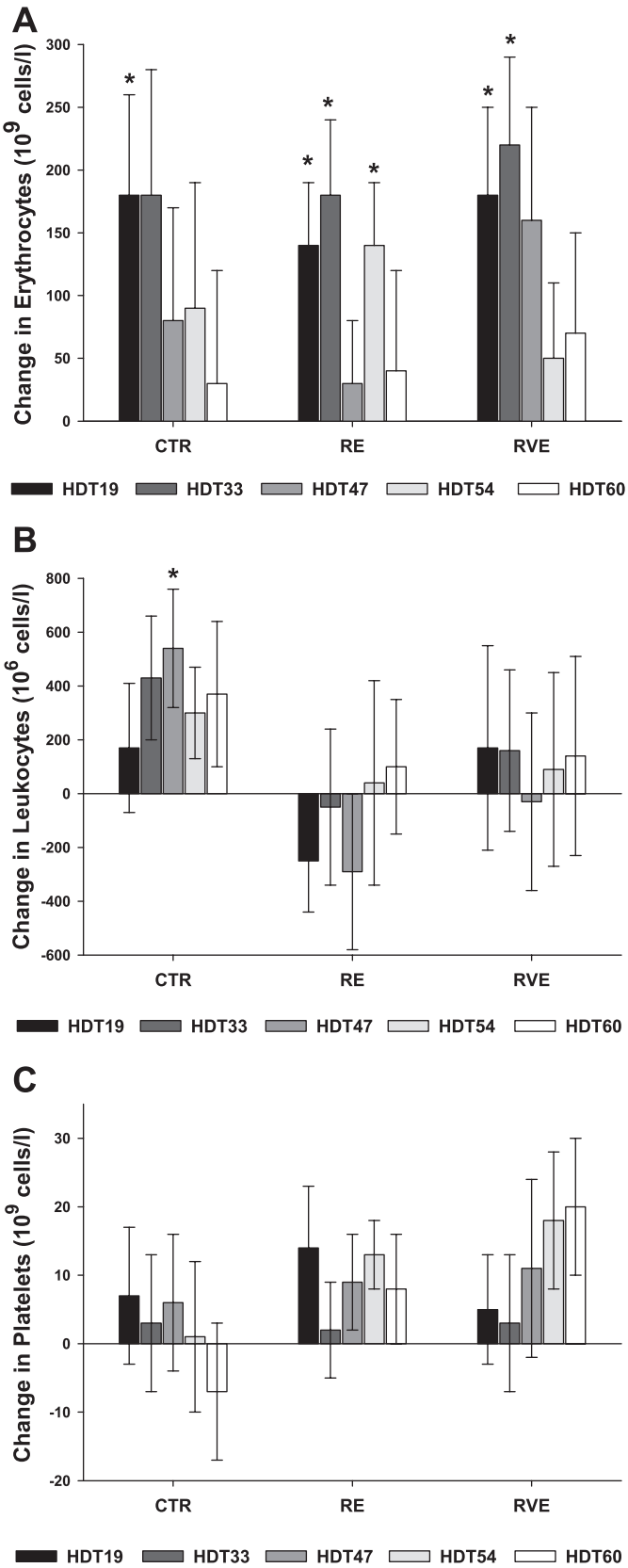

Fig. 3. Absolute change in hematological counts. *A*: erythrocytes. *B*: leukocytes. *C*: platelets. The increase in hematological counts is suggestive of panhemopoietic stimulation. HDT19, HDT33, HDT47, HDT54, and HDT60: HDT *days 19*, *33*, *47*, *54*, and *60*, respectively. Values are means ± SE. $*P < 0.05$ from baseline.

(22, 29, 32). The mechanisms by which bed rest causes vertebral marrow fat accumulation are still speculative. Decreased loading of the spine may trigger differentiation of pluripotential cells located in the bone marrow in favor of adipocytes (adipocyte switch) (10, 18, 43, 52). Proliferation of existing adipocytes is another possible mechanism. Similarly, the mechanisms by which RE and RVE prevented and decreased vertebral fat accumulation, respectively, compared with CTR are unknown. A direct effect of vibration on bone and bone marrow and an indirect effect through vertebral musculature contraction-relaxation are possible. RE, in combination with whole body vibration, may stimulate pluripotential cell differentiation away from adipocytes (10, 18, 52). Diverging evidence and opinions exist on whole body vibration inhibition of existing adipocytes (12, 34, 44). In the present study, we used a vibration amplitude of 3.5–4.0 mm and a frequency of 26 Hz for lower limbs and 16 Hz for back raise. Higher vibration amplitudes produce larger forces. A lower vibration frequency of 16 Hz for the back raise exercise targeted type I (slow twitch) paravertebral muscle, allowing adequate time for contraction and relaxation. A possible reason why the exercise regimen in the WISE study did not prevent VFF accumulation is that the protocol was very different from that of the BBR2–2: it included aerobic exercise and low-body negative pressure, less RE, and no whole body vibration (48).

Our findings have potential clinical applications. They may translate to real microgravity environments such as exist in space. Resistive exercise regimens, with or without whole body vibration, could recreate forces on the axial skeleton. The exercise equipment and the vibration device are portable and can be flown aboard spacecrafts or to orbital space stations or planetary space bases. The protocol studied in the BBR2–2 would require relatively little astronaut time, 1 h three times per week. This protocol may also find applications on Earth for bedridden patients or people with limited mobility. Patients who are bedridden in intensive care units or hospital wards may have limited ability to perform high-load RE or RVE. The interventions we tested are more easily applied to a medically stable person with decreased mobility. In the present study, no subject was tested with vibration only; therefore, it is not known whether vibration alone would provide the same benefits to bedridden patients in hospital.

In the present study, we used three MRI signal fat-fraction techniques for measuring fatty marrow content: fatsat, MRS, and IOP. As expected, and as previously reported (8, 40), they gave slightly different absolute VFF values. However, the three techniques identified similar trends in VFF changes between groups and time points, which confirmed our third hypothesis. The fatsat technique is suitable for estimating the signal fat fraction. It estimates the signal fat fraction with a dynamic range of 0–100%. MRS is the most direct method for separating the water and fat components from the marrow signal. The placement of the ROI occurs at the time of imaging (as opposed to postprocessing) and may be affected by motion. MRS techniques are longer, which also makes them more vulnerable to the effects of motion. In the present study, given the limitation in machine time, a MR spectrum from only one vertebra (L_5) was obtained. Finally, the MRS package is an optional item that is not part of the basic MR equipment at all MR imaging centers. The IOP technique, using magnitude-based chemical shift, is a commonly used MRI approach for fat

assessment but achieves a dynamic range of only 0–50%. Resolving the signal fat fraction to the full 0–100% range requires using both magnitude and phase information, as we did in the WISE study, or using complementary water spectra, as we did in the BBR2–2, which introduces an additional postprocessing step. While VFF over 50% was not frequent in women in the WISE study (mean baseline VFF 29%) (48), it occurred regularly in men in the present study (mean baseline VFF 42.6% with the IOP technique). All three MRI techniques for measuring VFF provided valid data. Importantly, expressing VFF data as change from baseline allows clear appreciation of the direction of change with bed rest.

Alterations in vertebral marrow fat content may impact circulating levels of peripheral blood elements (20, 43, 46, 52). In the present study, we found increased erythrocyte counts up to 54 days after the start of bed rest and increased leukocyte counts up to 47 days, confirming our fourth hypothesis. The −6° HDT bed-rest position leads to a cephalad fluid shift (47). Diuresis adjusts the blood volume to lower levels [−15% after 7 days (38)], which leads to hemoconcentration. Decreases in the number of blood cells are, therefore, needed to return blood cell concentrations to pre-bed-rest normal values. For erythrocytes, the mechanism of neocytolysis reported after descent from altitude or reentry from space predicts decreased erythrocyte production, acquisition of senescent characteristics, and lysis of young erythrocytes to restore erythrocyte concentration within ~7 days (41, 42). A paradoxical increase in erythropoietic production in a state of hemoconcentration was reported in elite cyclists returning from altitude training; it resolved by *day 9* (37). Increased circulating leukocyte and platelet concentrations after enforced HDT bed rest were a novel finding in the WISE study, and the mechanisms are still unclear (31, 48). Adipocytes can be metabolically active in promoting hemopoiesis (7, 14, 20, 31, 46, 52, 53). Conversely, fat taking up bone marrow space may lead to yellow marrow, which can decrease hemopoietic production (18).

The countermeasures of RE or whole body vibration can directly influence hemopoietic status (49). However, contrary to the changes in VFF, neither RVE nor RE inhibited the increase in erythrocyte counts compared with the CTR group. The effect of many factors, the −6° HDT bed rest, the fluid shift, the countermeasures, the change in fat content, and the venipuncture needed for the study, was an increased erythrocyte count in all three groups and an increased leukocyte count in the CTR group. The increased erythrocyte concentration in the RE group after 54 days of bed rest, long after fluid shifts have been completed, may have been due to survival of existing erythrocytes or to continued or heightened erythrocyte production (2, 28). It is important to monitor erythropoietin in this situation. Preliminary results from the BBR2–2 suggest a drop in erythropoietin concentrations during the first days of bed rest, followed by a gradual increase throughout bed rest (personal communication, A. Stahn and H.C. Gunga). RVE and RE may have limited the increase in leukocyte count with bed rest. Therefore, we conclude that bed rest increased hemopoietic counts, possibly related to the increased vertebral marrow fat content, and that RE with and without whole body vibration caused limited blunting of the leukocyte response.

Limitations

The small number of subjects per group may limit the interpretation of some of the statistical testing, since power was calculated for bone density changes (4). Experiments with a larger population may clarify the trends noted in this study. For example, the apparent differences in VFF between the RVE and RE groups were not significant with either statistical method. Our sensitivity analysis based on prior bed-rest data in women and the IOP technique (48) suggested that the study was powered to detect a difference of 3.8% between groups. The averaged VFF estimates from the three MRI techniques predicted a slightly higher sensitivity. Higher baseline VFF and age in the RVE group than in the RE group occurred, despite randomization; the study was run in four campaigns, with equal numbers of participants per group in each campaign, to avoid seasonal effects. After correction for age-related bone marrow involution (0.6% per year), there was no statistically significant difference between the RVE group and the RE group at baseline (30, 33, 36). Since all data are reported as change in VFF, correcting all data for age was not necessary. All MRI signal fat-fraction measures are affected by numerous technical factors, dependent on the platform and specific scan parameters used, including T_1 bias, T_2 bias, T_2* decay, fat spectrum, noise bias, eddy currents, inhomogeneous main magnetic field (B0), J-coupling, and field strength. These factors were optimized on the study scanner and were applied unchanged throughout the BBR2–2. While differences in scanner types and settings between the present study and prior work (48) may have influenced absolute VFF values, comparing changes in VFF between studies validates the results.

Conclusions

Bed rest at −6° HDT for 60 days increased lumbar VFF accumulation in men at a rate beyond natural bone marrow involution. These results confirmed similar findings in women (48), who have lower baseline VFF. Importantly, RE with and without whole body vibration effectively prevented VFF accumulation compared with inactivity. This first demonstration of an efficient intervention to prevent the accumulation of fat in vertebral bone marrow has important physiological implications and potential clinical applications.

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DISCLOSURES

D. Felsenberg acts as a consultant to the European Space Agency and Novotec Medical for the exploitation of the results of this study. All other authors have no conflict of interest.

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