Bone marrow fat accumulation after 60 days of bed rest persisted 1 year after activities were resumed along with hemopoietic stimulation: the Women International Space Simulation for Exploration study

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Bone marrow fat accumulation after 60 days of bed rest persisted 1 year after activities were resumed along with hemopoietic stimulation: the Women International Space Simulation for Exploration study. J Appl Physiol 107: 540–548, 2009. First published May 28, 2009; doi:10.1152/japplphysiol.91530.2008.—Immobility in bed and decreased mobility cause adaptations to most human body systems. The effect of immobility on fat accumulation in hemopoietic bone marrow has never been measured prospectively. The reversibility of marrow fat accumulation and the effects on hemopoiesis are not known. In the present study, 24 healthy women (age: 25–40 yr) underwent head-down bed rest for 60 days. We used MRI to noninvasively measure marrow fat accumulation, and marrow adiposity were associated with hemopoietic stimulation. One year after activities were resumed along with hemopoietic stimulation: the Women International Space Simulation for Exploration study. J Appl Physiol 107: 540–548, 2009. First published May 28, 2009; doi:10.1152/japplphysiol.91530.2008.—Immobility in bed and decreased mobility cause adaptations to most human body systems. The effect of immobility on fat accumulation in hemopoietic bone marrow has never been measured prospectively. The reversibility of marrow fat accumulation and the effects on hemopoiesis are not known. In the present study, 24 healthy women (age: 25–40 yr) underwent head-down bed rest for 60 days. We used MRI to noninvasively measure the lumbar vertebral fat fraction at various time points. We also measured hemoglobin, erythropoietin, reticulocytes, leukocytes, platelet count, peripheral fat mass, leptin, cortisol, and C-reactive protein during bed rest and for 1 yr after bed rest ended. Compared with baseline, the mean (± SE) fat fraction was increased after 60 days of bed rest (+2.5 ± 1.1%, P < 0.05); the increase persisted 1 yr after the resumption of regular activities (+2.3 ± 0.8%, P < 0.05). Mean hemoglobin levels were significantly decreased 6 days after bed rest ended (-1.36 ± 0.20 g/dl, P < 0.05) but had recovered by 1 yr, with significantly lower mean circulating erythropoietin levels (-3.8 ± 1.2 mU/ml, P < 0.05). Mean numbers of neutrophils and lymphocytes remained significantly elevated at 1 yr (+617 ± 218 neutrophils/μl and +498 ± 112 lymphocytes/μl, both P < 0.05). These results constitute direct evidence that bed rest irreversibly accelerated fat accumulation in hemopoietic bone marrow. The 2.5% increase in fat fraction after 60 days of bed rest was 25-fold larger than expected from historical ambulatory controls. Sixty days of bed rest accelerated by 4 yr the normal bone marrow involution. Bed rest and marrow adiposity were associated with hemopoietic stimulation. One year after subjects returned to normal activities, hemoglobin levels were maintained, with 45% lower circulating erythropoietin levels, and leukocytes remained significantly elevated across lineages. Lack of mobility alters hemopoiesis, possibly through marrow fat accumulation, with potentially wide-ranging clinical consequences.

Magnetic resonance imaging; erythropoietin; erythrocytes; leukocytes; deconditioning, muscular atrophy, osteoporosis, pressure sores, thromboembolic events, and joint contractures (2, 40, 47). However, the effect on bone marrow elements, specifically adipose and hematological lineages, has received little attention.

Decreased mechanical stress on bone may directly increase marrow adiposity (11). This has been documented in aging, paraplegia, and osteoporosis (all conditions associated with decreased mobility) as well as glucocorticoid administration and ovariectomy (21, 27, 37). Adipose volume in the bone marrow has been calculated to augment 6–7% per decade of life (32, 42). Although adipocytes are the most abundant cells in bone marrow, their function remains controversial. Adipocytes may occupy space no longer needed for hemopoiesis, play an active role in lipid metabolism as an energy reserve, and/or influence selected osteogenic and hematopoietic lineages (21). Clinically, changes in the fat content of bone marrow with prolonged immobility may adversely alter bone and bone marrow hematopoietic elements.

Adult bone marrow is also home to mesenchymal stem cells and hematoprogenitor cells for circulating erythrocytes and leukocytes. Hemopoiesis may be affected by decreased physical forces. People with paraplegia or tetraplegia secondary to spinal cord injury have demonstrated decreased erythrocyte mass (35, 37). Hemoglobin levels have been observed to drop by 9% and 10% in healthy men bedridden for 42 and 90 days, respectively (5, 19). A decrease in hemopoietic tissue has been documented in osteopenic patients (7), and Sonnenfeld (49) found an increased propensity to infection in bedridden subjects. Clinically, the longer-term effects of bed rest on erythroid and leukocytic lineages have not been thoroughly studied. Different hematological lineages each affect different functions. Anemia, leukocyte dysfunction, and hemostasis deficiencies may lead to decreased aerobic capacity, propensity to infection, abnormal control of autoimmunity, inflammation and allergy, impaired wound healing, and thromboembolism (32, 33, 55). These complications affect patients who are bedridden because of acute illness and people with a chronic disability limiting their mobility and also apply to astronauts living in a weightless environment. In the present experiments, we sought to better understand the effect of prolonged bed rest on fat accumulation in the bone marrow and on hemopoietic changes. We also wanted to detect the countereffects of a nutritional or an exercise countermeasure.

MOVEMENT IS NECESSARY to humans. The absence of physical forces alters the properties of essentially all organs and tissues (40). The clinical effects of bed rest include cardiovascular...
Twenty-four healthy female volunteers participating in the Women International Space Simulation for Exploration (WISE) study adopted the antioorthostatic −6° head-down tilt (HDT) position for 60 days. Our objectives were to measure prospectively 1) fat accumulation in the bone marrow during bed rest using MRI and its possible reversibility 1 yr after a return to normal activities and 2) the changes in hematological variables (hemoglobin, hematocrit, leukocytes, platelets, and erythropoietin) with bed rest and 1 yr after the resumption of activities.

METHODS

Ethics, Inclusion, and Exclusion

This study was approved by local and national ethics boards, and all participants gave written informed consent. Inclusion and exclusion criteria were extensive (see the APPENDIX). In brief, nonsmoking female volunteers aged 25–40 yr with a normal body mass index underwent a series of clinical evaluations and laboratory, fitness, and psychological testing. An abnormal result on one or more of these tests constituted an exclusion criterion.

Countermeasures

Participants were randomly allocated into one of three groups: bed rest + exercise (n = 8), bed rest + nutrition (n = 8), or bed rest only (control group; n = 8). These countermeasures were designed to prevent bone loss and muscle atrophy and weakness. The exercise countermeasure regimen consisted of resistive and aerobic exercises performed on separate days (14, 48, 54). The resistive exercise consisted of 19 sessions starting with 10 min of light supine cycling and submaximal thigh and calf presses on an inertial ergometer. Then, 4 sets of 7 maximal concentric and eccentric leg press repetitions were followed by 4 sets of 14 maximal concentric and eccentric calf press repetitions. Aerobic exercises consisted of 40 min of running on a vertical treadmill with lower body negative pressure (−48 to −55 mmHg). The nutrition countermeasure consisted of protein supplementation of 0.6 g·kg body wt⁻¹·day⁻¹ (14, 54). All participants received 24-h medical attention and psychological supervision and were constantly videomonitored to ensure compliance.

Schedule

The experiment was run at the Institut de Médicine et de Physiologie Spatiales in Toulouse, France, between January 2005 and December 2006. All participants stayed for 100 days: 20 days for baseline data collection (BDC), 60 days of −6° HDT bed rest, and 20 days for recovery (R). Participants underwent some recovery measurements 6, 45, 90, 180, and 360 days after bed rest was terminated. Times are noted accordingly, e.g., BDC16 stands for 16 days before bed rest was started, HDT1 stands for 1 day of HDT bed rest, and R90 stands for 90 days after bed rest had ended.

Magnetic Resonance Fat Fraction Protocol

MRI has long been used to measure the fat fraction (22, 30, 46, 52, 56, 57). It has the advantages of noninvasiveness and ease of measuring and the disadvantages of restricted availability and cost. Magnetic resonance images were acquired using a Phillips Intera 1.5-T unit with a sense-body coil. The lumbar spine was imaged in the coronal plane using T₁ WI SE sequences in and out of phase with a field of view of 400 mm, excitation times of 2.3 ms out of phase and 4.6 ms in phase, a relaxation time of 100 ms, and a flip angle of 25°. The lower lumbar/sacral vertebrae along with the pelvis are the predominant contributors to hemopoiesis in adults. We selected regular-shaped L3 and L4 for ease of reproducible repeated measurement as opposed to the pelvis. Signal intensities from the water image (Iw) and fat image (If) were processed according to the following formula: fat fraction = If/(If + Iw) × 100, using Image J (National Institutes of Health, Bethesda, MD) (56). To locate the region of interest, we identified the L3 and L4 vertebral bodies on DICOM images. We selected the image at which the vertebral bodies were the widest. A rectangular region of interest was drawn in the body of L3 and L4 exclusive of the endplates and cortices. The size of the region of interest was the same for both the in phase and out of phase images. The mean of L3 and L4 provided the vertebral fat fraction between 0% (no fat) and 100% (all fat).

Hematological Outcomes, Erythropoietin, Peripheral Fat Mass, Leptin, Cortisol, and C-Reactive Protein

Hemoglobin, hematocrit, reticulocytes, leukocytes, and platelet numbers were measured using an automated hematometry analyzer (Sysmex XE-2100, Sysmex, Kobe, Japan). Morning blood was collected for erythropoietin between BDC16 and R367. Erythropoietin was determined in duplicate using the 21-EPOHU-E01 enzyme immunoassay (EIA) kit (ALPCO Diagnostics, Salem, NH), with absorbance being measured with a SpectraMax 190 microplate reader (Molecular Devices, Sunnyvale, CA) using Softmax Pro 4.2 software. To allow comparisons with marrow fat and hematologic data, erythropoietin results were grouped into seven time intervals: BDC16 to the morning of HDT1, HDT12–HDT34, HDT41–HDT57, R5–R20, R42–R60, R89–R150, and R330–R367. The peripheral fat mass was measured by means of dual-energy x-ray absorptiometry (DXA) with a QDR 4500W bone densitometer (Hologic, Bedford, MA). Total plasma leptin concentrations were determined using the SLP010 EIA kit (R&D Systems, Minneapolis, MN). Fasting plasma cortisol levels were determined using the 11-CORHU-E01 EIA kit (ALPCO Diagnostics, Salem, NH). Finally, C-reactive protein was determined using the 30-9710s EIA kit (ALPCO Diagnostics). Fat mass, leptin, cortisol, and C-reactive protein were measured before bed rest was started and after the return to normal activities.

Data Analysis

Data were analyzed with SPSS 16.0 and are expressed as means ± SE. We determined the changes from baseline in the fat fraction, hematological outcome measures, erythropoietin, peripheral fat mass, leptin, cortisol, and C-reactive protein over the duration of the study and followup period using repeated-measures ANOVA with post hoc paired t-tests. We then tested for an effect of time in each group (exercise, nutrition, and bed rest only) using repeated-measures ANOVA with post hoc paired t-tests and for a countermeasure effect at each time using one-way ANOVA followed by Tukey’s tests.

RESULTS

All 24 participants (mean age: 32.1 yr, mean body mass index: 21.4) completed the 60 days of bed rest. Two missed MRI at R180 because of machine failure, and two others missed MRI at R360. For repeated-measures statistics, the missing data for these four participants were attributed the average value of the other participants in their group at that time point.

Fat Accumulation in Bone Marrow

Compared with baseline, the mean absolute vertebral fat fraction for the 24 participants was significantly increased at HDT57 (+2.5 ± 1.1%, P < 0.05) and R360 (+2.3 ± 0.8%, P < 0.05; Fig. 1). For the individual treatment groups, the fat fraction increases from baseline did not reach statistical sig-
Hemopoiesis

Hemoglobin, reticulocytes, and erythropoietin. Compared with baseline, the mean hemoglobin concentration for the 24 participants was significantly increased at HDT29 (+0.64 ± 0.12 g/dl, P < 0.05; Fig. 2) but had normalized at the end of bed rest. The cohort was anemic (hemoglobin concentration: <12 g/dl) at R6 (~13.6 ± 0.20 g/dl, P < 0.05; Fig. 2); this was so for all three treatment groups. Hemoglobin was still below baseline values at R45 and R90 (both P < 0.05; Fig. 2) but had normalized by R180.

The mean number of reticulocytes for the 24 participants was significantly increased at HDT57 and remained high at R6 and R45 (all P < 0.05) before returning to baseline levels 3 mo after bed rest had ended (Table 1 and Fig. 2).

The mean erythropoietin level for the 24 participants was significantly increased at HDT29, significantly increased at R6, and significantly decreased at R360 (all P < 0.05; Fig. 2). In the treatment groups, the decrease at R360 was significant in the nutrition and bed rest only groups (Table 1).

Leukocytes and platelets. The mean number of neutrophils for the 24 participants was significantly decreased at R6 and significantly increased at R45 and R360 (all P < 0.05; Fig. 3). The mean number of lymphocytes was significantly increased at R45, R90, R180, and R360 (all P < 0.05; Fig. 3). Statistically significant elevations in monocytes and eosinophils are also shown in Fig. 3. The mean platelet count for the 24 participants was significantly increased at HDT29, R6, R45, R90, and R180 (all P < 0.05; Table 1). In the treatment groups, the increase in neutrophils at R360 was significant in the bed rest only group, and the increase in lymphocytes at that time point was significant in the exercise and bed rest only groups (all P < 0.05; Table 1).

DISCUSSION

In the present study, 60 days of strict bed rest was associated with a relative increase of 9% of fat in the hemopoietically active lumbar vertebrae of premenopausal women. Fat accumulation persisted up to 1 yr after bed rest had ended, demonstrating the irreversibility of marrow adipogenesis over this period despite a return to normal activities. These changes were accompanied by hematological alterations across lineages: increased numbers of neutrophils (22%) and lymphocytes (26%) and hemoglobin levels maintained with reduced erythropoietin levels (43%) 1 yr after the resumption of normal activities.

Bone marrow fat has been measured histologically in post-mortem iliac crest biopsies (22, 30, 46, 52, 56, 57). Two independent groups (30, 39) have reviewed the available literature and calculated a 6–7% increase in marrow fat per decade, which translates into a yearly increase of ~0.6–0.7%, or 0.1% over 60 days. We prospectively measured repeat fat fraction from the same anatomic site noninvasively, meeting our first objective. The 2.5% increase in fat fraction we measured after 60 days of bed rest was 25-fold larger than expected from historical controls. Sixty days of bed rest accelerated by 4 yr the normal bone marrow involution that runs from birth to adulthood to older age (23, 36).

Cross-sectional studies (10, 27, 36, 37, 42, 52) have suggested a modulation of fat accumulation in the bone marrow with metabolic alterations (osteoporosis, endogenous or exogenous steroids, and alcohol). In the present study, after 60 days of bed rest, the lumbar spine bone density (as measured by DXA) had not changed (48), fasting plasma concentrations of cortisol were unchanged, and alcohol was prohibited. The increased adipose marrow contrasted with a global decrease in peripheral fat mass (as measured by DXA), which rules out a switch to systemic fat accumulation. We conclude that decreased mobility was the trigger for fat marrow accumulation.

One important finding was that marrow fat failed to return to baseline values 1 yr after participants had resumed their normal activities. By then, they had recovered their body fat mass (S. Blanc and A. Bergouignan, unpublished observations). Irreversibility of marrow fat accumulation has also been reported after exogenous corticosteroids were administered to rabbits, causing a loss of femoral epiphysis trabecular and growth plate bone and adipose marrow, as measured by magnetic resonance microimaging (51). In that study, discontinuation of steroids or treatment with bisphosphonates restored trabecular bone volume and thickness, but adipose marrow was not reversed 10 wk after cessation. Our results suggest that 60 days of bed rest may have irrevocably accelerated marrow involution, which was neither prevented by dietary or exercise countermeasures nor reversed by remobilization.

The mechanisms triggering fat accumulation in bone marrow are largely unknown. Mechanical stress on bone may drive...
Table 1. Effect of 60 days of bed rest on the fat fraction, hemopoiesis, and erythropoietin levels in subjects with the countermeasures of exercise and nutrition and those with bed rest only

<table>
<thead>
<tr>
<th></th>
<th>BDC</th>
<th>HDT29</th>
<th>HDT57</th>
<th>R6</th>
<th>R45</th>
<th>R90</th>
<th>R120</th>
<th>R180</th>
<th>R360</th>
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<tr>
<td><strong>Fat fraction,%</strong></td>
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<tr>
<td>Exercise</td>
<td>28.4±2.0</td>
<td>+1.4±1.1</td>
<td>+0.9±1.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+1.5±1.6</td>
<td>+1.8±1.4</td>
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<tr>
<td>Nutrition</td>
<td>28.1±2.7</td>
<td>+1.8±1.7</td>
<td>+4.3±2.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+2.3±2.3</td>
<td>+3.4±1.7</td>
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<tr>
<td>Bed rest only</td>
<td>30.5±2.1</td>
<td>+1.3±1.5</td>
<td>+2.3±2.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>-1.8±1.2</td>
<td>+1.7±1.1</td>
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<td><strong>Hemoglobin, g/dl</strong></td>
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<tr>
<td>Exercise</td>
<td>12.98±0.23</td>
<td>+0.33±0.28</td>
<td>-0.8±0.29*</td>
<td>-1.2±0.38*</td>
<td>-0.8±0.43</td>
<td>-0.41±0.47</td>
<td>+0.08±0.57</td>
<td>+0.24±0.12</td>
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<tr>
<td>Nutrition</td>
<td>13.53±0.33†</td>
<td>+0.63±0.09*</td>
<td>+0.11±0.13</td>
<td>-1.60±0.37*</td>
<td>-1.26±0.26*</td>
<td>-0.93±0.21*</td>
<td>-0.93±0.30*</td>
<td>-0.58±0.33†</td>
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<tr>
<td>Bed rest only</td>
<td>12.54±0.18</td>
<td>+0.98±0.18*</td>
<td>+0.36±0.21</td>
<td>-1.28±0.30*</td>
<td>-0.59±0.33</td>
<td>-0.04±0.34</td>
<td>+0.41±0.21</td>
<td>+0.69±0.26*</td>
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<tr>
<td><strong>Hematocrit,%</strong></td>
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<td></td>
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<tr>
<td>Exercise</td>
<td>38.43±0.63</td>
<td>+1.13±0.72</td>
<td>-2.70±0.83*</td>
<td>-3.28±1.05*</td>
<td>-1.09±1.16</td>
<td>-0.49±1.2</td>
<td>+0.43±1.55</td>
<td>+0.35±0.41</td>
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<tr>
<td>Nutrition</td>
<td>39.53±0.91</td>
<td>+1.89±0.55*</td>
<td>-0.45±0.51</td>
<td>-4.36±1.05*</td>
<td>-2.38±0.69*</td>
<td>-1.69±0.54*</td>
<td>-1.76±0.65*</td>
<td>-0.35±0.91†</td>
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<tr>
<td>Bed rest only</td>
<td>37.19±0.56</td>
<td>+2.78±0.51*</td>
<td>+0.26±0.53</td>
<td>-3.98±0.99*</td>
<td>-0.74±0.95</td>
<td>+1.20±0.89</td>
<td>+1.05±0.66</td>
<td>+1.41±0.84</td>
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<tr>
<td><strong>Neutrophils, cells/µl</strong></td>
<td>40,313±6,359</td>
<td>5,613±4,483</td>
<td>13,100±3,100*</td>
<td>13,600±3,493*</td>
<td>6,325±4,154</td>
<td>3,275±4,498</td>
<td>1,588±3,160</td>
<td>2,175±3,157</td>
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</tr>
<tr>
<td>Exercise</td>
<td>5,856±995</td>
<td>5,014±986</td>
<td>5,284±1,043</td>
<td>4,574±2,902</td>
<td>4,507±2,873</td>
<td>4,463±2,781</td>
<td>4,397±2,768</td>
<td>4,447±2,769</td>
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<tr>
<td>Nutrition</td>
<td>5,800±990</td>
<td>5,000±985</td>
<td>5,250±1,039</td>
<td>4,550±2,890</td>
<td>4,500±2,875</td>
<td>4,450±2,780</td>
<td>4,390±2,765</td>
<td>4,440±2,765</td>
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<tr>
<td><strong>Lymphocytes, cells/µl</strong></td>
<td>2,774±469</td>
<td>212±285</td>
<td>46±253</td>
<td>-325±346</td>
<td>+1,233±345*</td>
<td>975±445</td>
<td>+859±298*</td>
<td>+1,057±395*</td>
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<tr>
<td>Exercise</td>
<td>1,811±161</td>
<td>-106±100</td>
<td>-97±70</td>
<td>92±102</td>
<td>+314±101*</td>
<td>+682±187*</td>
<td>+395±152*</td>
<td>+502±198*</td>
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<tr>
<td>Nutrition</td>
<td>1,937±187</td>
<td>+26±120</td>
<td>+64±81</td>
<td>-24±65</td>
<td>+178±116</td>
<td>+317±152</td>
<td>+157±166</td>
<td>+476±213</td>
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<tr>
<td>Bed rest only</td>
<td>2,063±192</td>
<td>+48±84</td>
<td>+81±133</td>
<td>-11±115</td>
<td>+757±238*</td>
<td>+747±233*</td>
<td>+625±261*</td>
<td>+514±196*</td>
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<tr>
<td><strong>Platelets, cells × 10⁹/µl</strong></td>
<td>260±11</td>
<td>+28±13</td>
<td>+24±12</td>
<td>+33±16</td>
<td>+60±13*</td>
<td>+31±18</td>
<td>+23±13</td>
<td>-26±37</td>
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<tr>
<td>Exercise</td>
<td>256±13</td>
<td>+8±12</td>
<td>-1±11</td>
<td>+10±10</td>
<td>+51±19*</td>
<td>+16±17</td>
<td>+20±12</td>
<td>+7±11</td>
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<tr>
<td>Nutrition</td>
<td>210±11</td>
<td>+13±4*</td>
<td>+12±7</td>
<td>+19±11</td>
<td>+57±13*</td>
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<tr>
<td>Bed rest only</td>
<td>242±8</td>
<td>+17±6*</td>
<td>+12±6</td>
<td>+21±7*</td>
<td>+57±9*</td>
<td>+28±9*</td>
<td>+19±6*</td>
<td>-2±13</td>
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<tr>
<td><strong>Erythropoietin, mU/ml</strong></td>
<td>71.5±0.46</td>
<td>-0.44±0.49</td>
<td>+0.16±1.04</td>
<td>+12.45±1.53*</td>
<td>+2.80±1.75</td>
<td>+4.87±4.41</td>
<td>-1.00±1.57</td>
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<tr>
<td>Exercise</td>
<td>11.22±2.09</td>
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<td>-1.26±1.15</td>
<td>+15.02±2.07*</td>
<td>+3.04±3.86</td>
<td>-3.48±2.06</td>
<td>-5.89±2.46*</td>
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<td>+13.37±2.28*</td>
<td>+2.91±2.21</td>
<td>-0.45±1.21</td>
<td>-4.60±1.67*</td>
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</table>

Values are means ± SE; n = 8 subjects in each treatment group. Time periods were as follows: baseline data collection (BDC), head-down tilt bed rest (HDT), and recovery (R); numbers following the time periods are numbers of days. ND, not done. *P < 0.05 compared with baseline; †P < 0.05 compared with the bed rest only group.
the differentiation of mechanosensitive mesenchymal stem cells on the endosteal surface away from adipocytes and toward osteoblasts (21). In one study (11), cyclic stretching favored osteoblastogenesis at the expense of adipogenesis in stromal cells. Peroxysome proliferator-activated receptor (PPAR)-γ may be central to this differentiation, as cyclic loading induced lower levels of PPAR-γ, and antagonists of PPAR-γ potentiated osteoblastogenesis (11). Similar results have been found in vivo: mice deficient in PPAR-γ (PPAR-γ<sup>hyp/hyp</sup>) are lipodystrophic and show enhanced bone formation (8). Inversely, bed rest, through decreased mechanical forces, would favor differentiation toward adipocytes. PPAR-γ agonists have been found to increase the differentiation into adipocytes at the expense of osteoblasts in vitro (34, 45). Two recent reports (21, 43) have reviewed the evidence for a possible inverse relationship between bone and bony fat. In the present study, marrow fat accumulated without bone loss, as measured by DXA (48).

Paths of other than PPAR-γ may cause fat accumulation (21). Mice with mutated thyroid receptors-α and -β have been found to accumulate marrow fat (28), and inhibition of VEGF and PDGF receptors increased marrow fat in culture (15). Steroids cause bone marrow adiposity (38, 51, 52), and mice

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**Fig. 2.** Erythropoiesis with bed rest. The mean hemoglobin concentration for the 24 participants at baseline was 13.01 ± 0.16 g/dl, the mean number of reticulocytes was 36.07 ± 2.645 reticulocytes/μl, and the mean erythropoietin level was 8.8 ± 0.9 mU/ml. The fall in the hemoglobin level recovered 180 days after bed rest had ended. Reticulocytes were significantly elevated during and after bed rest. Erythropoietin levels were elevated after bed rest but reset at lower levels 1 yr after bed rest had ended. Values are means ± SE. *P < 0.05.

**Fig. 3.** Leukopoiesis with bed rest. The mean number of neutrophils for the 24 participants at baseline was 2,866 ± 199 neutrophils/μl, the mean number of lymphocytes was 1,937 ± 102 lymphocytes/μl, the mean number of monocytes was 406 ± 31 monocytes/μl, and the mean number of eosinophils was 162 ± 18 eosinophils/μl. Neutrophils and lymphocytes were significantly higher after bed rest. Values are means ± SE. *P < 0.05.
knocked out for 11β-hydroxysteroid dehydrogenase-1 have shown an absence of marrow adipocytes (26). Transforming growth factor (TGF)-β pathways have also been shown to suppress adipogenesis (17). Mechanistic experiments are needed to confirm the pathways of marrow fat accumulation in humans during bed rest.

Bone marrow is also home to hematopoietic stem cells. Our results confirm our second hypothesis: that bed rest affects hemopoiesis, possibly through localized fat accumulation. Fluid shifts have been used to explain erythropoietic adaptations during the first 10 days: blood volume contracts when the body is at −6° anteriothostatic and in microgravity (40). The resulting polycythemia would trigger hemolysis of younger erythrocytes until erythroid mass corresponded to the contracted circulatory volume (41). In our study, fluid shifts were estimated at 8% based on the drop in hematocrit between HDT57 and R6. Many hematological changes were consistent with fluid shift: HDT29 polycythemia, R6 anemia, R6 decreased neutrophils, R6 increased erythropoietin, and R6 increased reticulocytes in the context of low hemoglobin levels. However, other changes cannot be explained by fluid shifts alone: reticulocytosis at HDT57 without matching erythropoietin stimulation (suggesting that erythropoietin was not the stimulus for reticulocyte production) and increased platelets at R6. Furthermore, new findings have been identified: increased platelets from R6 to R180, resetting of neutrophils and lymphocytes at higher levels from R45 to R360, and decreased erythropoietin requirements at R360 in the context of recovered hemoglobin. These latter changes as well as those not explained by fluid shift were all in the direction of panhemopoietic stimulation, which suggest that factors other than fluid shifts account for the hematopoietic adaptations to bed rest. Increased amounts of metabolically active adipose tissue right at the most active hemopoietic sites may constitute the link to this multilineage hemopoietic stimulation.

Experimental and clinical data (3, 6, 8, 9, 20, 31, 50) have provided strong evidence for a direct stimulatory influence of marrow adipose tissue on hemopoiesis. Leptin stimulated multilineage marrow expansion of hematopoietic stem cells, maintained circulating lymphocyte levels, and, along with erythropoietin, played a role in erythropoiesis (6). Human CD34+ cells seeded onto marrow adipocytes completed their myeloid and B-lymphoid differentiation (9). Higher circulating levels of the adipokine leptin increased “eposensitivity” (defined as a lower requirement in erythropoietin to maintain hemoglobin levels) in dialysis patients (3, 50). Leptin, alone or in combination with other cytokines, increased the proliferation of cultured hematopoietic stem cells for lymphoid, myeloid, and erythroid lineages, suggesting a role in normal hemopoiesis (6, 29). Leptin stimulated marrow CD34+ progenitor cells to differentiate into granulocyte-macrophage colonies (31). Expression of the leptin receptor, normally absent in promyeloocytes, was increased in leukemic promyelocytes, which may inhibit their apoptosis (29), and patients with acute promyelocytic leukemia had an increased body mass index, which is closely correlated with leptin levels (18). Conversely, decreased leptin levels in lipodystrophic mice were associated with severe impediment to hemopoiesis (8), and truncation of the leptin receptor on hematopoietic stem cells in the db/db mouse resulted in reduced levels of B and T lymphocytes (6). Other marrow adipokines and pathways have been reported to encourage hemopoiesis. Adiponectin stimulated erythroid and lymphoid cell lines (58) and increased the proliferation of hematopoietic stem cells, possibly through a p38-dependent pathway (13). Abrogation of TGF-β signaling in Smad3+/− mice enhanced adipogenesis and hemopoiesis (17).

This panhemopoietic stimulation and eposensitivity up to 1 yr after 60 days of bed rest is a novel finding. It has been reported in obesity, hypercortisolemia, inflammation, and infection (12, 25, 53), conditions that were absent in the present study. Body mass and peripheral fat mass actually decreased in the participants. Participants were immunized with bacteriophage ΦX-174 at 21 and 42 days to test humoral immunity. This procedure is harmless to humans and does not alter leukocyte numbers (4). The inflammatory cytokine IL-6 predicted erythropoietin requirements in dialysis patients (3). In the present study, cortisol and C-reactive protein levels were unchanged, indicating no systemic inflammation from the bed rest or from the immunization procedure. In addition, panhemopoietic stimulation and eposensitivity cannot be attributed to increased peripheral fat mass or higher circulating leptin concentrations. In the absence of other factors, the hemopoietic stimulation with bed rest may be linked to marrow fat accumulation. The irreversible fat accumulation may have enhanced adipokine availability locally in the hemopoietic vertebrae. The increased marrow adipokine availability may, in turn, have directly and persistently stimulated multilineage hemopoiesis.

Antiorthostatic bed rest has been used in men as a model of microgravity, mimicking changes experienced during spaceflight (1, 40). The WISE study was the largest such study in women. Exercise and nutritional countermeasures have been demonstrated to have beneficial effects in preserving muscle, bone, and heart mass (14, 48, 54). In the present study, these countermeasures did not prevent marrow fat accumulation, and hemopoietic variations were comparable in the three treatment

Table 2. Effects of 60 days of bed rest on peripheral fat mass, serum leptin, serum cortisol, and C-reactive protein in the three groups

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Peripheral fat mass, kg</th>
<th>Leptin, ng/ml</th>
<th>C-reactive protein, mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDC</td>
<td>HDT57</td>
<td>Change</td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>14.55±1.1</td>
<td>12.42±1.3*</td>
<td>−2.13±0.30†</td>
</tr>
<tr>
<td>Nutrition</td>
<td>16.17±0.72</td>
<td>16.42±7.2</td>
<td>+0.25±0.33</td>
</tr>
<tr>
<td>Bed rest only</td>
<td>14.76±1.30</td>
<td>14.25±1.2*</td>
<td>−0.51±0.21*</td>
</tr>
<tr>
<td>All groups</td>
<td>15.16±0.61</td>
<td>14.37±0.70*</td>
<td>−0.80±0.26*</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>4.68±0.96</td>
<td>3.06±0.50</td>
<td>−1.62±1.07</td>
</tr>
<tr>
<td>Nutrition</td>
<td>5.79±1.31</td>
<td>7.64±1.52</td>
<td>+1.85±0.80</td>
</tr>
<tr>
<td>Bed rest only</td>
<td>4.68±0.81</td>
<td>4.77±0.66</td>
<td>+0.10±0.43</td>
</tr>
<tr>
<td>All groups</td>
<td>5.05±0.59</td>
<td>5.16±0.68</td>
<td>+0.11±0.54</td>
</tr>
<tr>
<td>Cortisol, μg/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>25.5±5.5</td>
<td>28.0±7.4</td>
<td>+2.5±5.2</td>
</tr>
<tr>
<td>Nutrition</td>
<td>29.5±3.2</td>
<td>28.9±5.4</td>
<td>+0.2±5.1</td>
</tr>
<tr>
<td>Bed rest only</td>
<td>15.4±3.9</td>
<td>16.3±3.0</td>
<td>+0.8±5.1</td>
</tr>
<tr>
<td>All groups</td>
<td>23.5±2.7</td>
<td>24.7±3.7</td>
<td>+1.2±2.8</td>
</tr>
<tr>
<td>C-reactive protein, mg/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>0.25±0.08</td>
<td>0.11±0.06</td>
<td>−0.14±0.07†</td>
</tr>
<tr>
<td>Nutrition</td>
<td>0.16±0.05</td>
<td>0.23±0.07</td>
<td>+0.08±0.06</td>
</tr>
<tr>
<td>Bed rest only</td>
<td>0.28±0.07</td>
<td>0.45±0.15</td>
<td>+0.17±0.12</td>
</tr>
<tr>
<td>All groups</td>
<td>0.23±0.04</td>
<td>0.26±0.06</td>
<td>+0.03±0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 compared with baseline; †P < 0.05 compared with the bed rest-only group.
groups. Effective countermeasures preventing marrow fat accumulation (e.g., passive mobilization, functional stimulation, and vibration protocols) remain to be tested. Clinically, our findings suggest that marrow fat can have a significant and lasting impact on patients who are bedridden, immobile in the intensive care unit, or have decreased mobility from chronic diseases. Abnormal hemopoiesis in these patients can affect many functions, including aerobic capacity, defense against infection, autoimmunity, control of inflammation, allergic reaction, asthma, and wound healing (32, 33, 55).

Limitations

The bed rest model allows participants to remain active in the antioorthostatic position. Complete bed rest, as in a critical illness, would further decrease physical loads and might increase marrow fat accumulation. Fat fraction at sites other than the L3/L4 vertebrae may differ, although fat fraction at sites other than the lumbar/sacral vertebrae and pelvis (e.g., in long bones) may not significantly impact hemopoiesis. The MRI fatty signal in the bone marrow does not distinguish between changes in adipocyte number or the size of existing adipocytes, and studies at the microscopic level are needed to explore the possible mechanisms responsible for fat accumulation in the marrow (38, 44). Iatrogenic venipuncture during the study (710 ml between BDC14 and R6) complicated the interpretation of the hemopoietic alterations but was identical for all participants. This report concentrated on the group of 24 participants since low final sample sizes in the countermeasure groups (n = 8) limited the identification of significant protective effects from the nutrition or exercise interventions. Finally, the effects of bed rest on men and postmenopausal women, both of whom have higher baseline marrow fat than our subjects (22), are unknown, as is evolution beyond 1 yr.

Conclusions

This study, conducted under highly standardized conditions, provides the first prospective evidence that 60 days of bed rest accelerated fat accumulation in the vertebral bone marrow of 24 healthy premenopausal women. The accumulation was not reversed 1 yr after a return to normal activities and was associated with normalized hemoglobin with decreased erythropoietin levels and with persistently elevated leukocyte numbers, with numerous potential detrimental effects. Further characterization is needed to help prevent and treat these complications in bedridden patients.

APPENDIX: INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria

Inclusion criteria were as follows:

- Age of 25–40 yr
- Nonsmoker
- No alcohol or drug dependence
- Height of <185 cm
- Body mass index between 20 and 25
- Regular menstrual cycles
- No personal or family past record of chronic or acute disease or psychological disturbances that could affect the physiological data and/or create a risk for the subject during the experiment
- Active and free from any orthopedic, musculoskeletal, or cardiovascular disorders
- Agrees to be vaccinated against influenza during the autumn before the hospitalization
- Agrees to participate in psychological investigations
- Covered by a Social Security system
- Free of any engagement during 4 consecutive months

Exclusion Criteria

Exclusion criteria were as follows:

- Having given >300 ml of blood within 3 mo before the start of the experiment
- Currently participating in a clinical research experiment
- In the exclusion period in the Healthy Participants National Register of the French Ministry of Health
- Poor tolerance to blood sampling
- Past record of orthostatic intolerance
- History of hiatal hernia or gastroesophageal reflux
- History of cardiac rhythm disorder
- History of inguinal hernia
- History of vestibular disease
- History of central or peripheral nervous system disease
- History of active claustrophobia
- History of severe hypersensitivity
- Known or suspected pregnancy
- Having given birth or undergone an abortion within 3 mo before the start of the study
- Currently breastfeeding or stopped breastfeeding within 2 mo before the start of the study
- Use of hormonal contraceptives within 2 mo before the start of the experiment
- Use of an intrauterine device at the beginning of the study
- Completely sedentary or extremely fit
- History of fracture or tendon laceration within the previous year
- History of genetic muscle and bone disease of any kind
- Presence of osteosynthesis material
- History of chronic back pain
- History of deep venous thrombosis or pulmonary embolus
- Presence of metallic implant
- Needs special diet
- Refusal to give permission for general practitioner to be informed of participation in the trial
- Incarcerated or patient in an emergency situation
- Unlikely to cooperate in the study, poor compliance anticipated by the investigator, or unable to cooperate because of a language problem
- Has received >3,800 Euros within 12 mo for being a research subject

Additional Possible Exclusion Criteria (Procedural)

In addition to the above exclusion criteria, the participants underwent the following investigations, and any abnormality constituted an exclusion criterion:

- Clinical examination
- Twelve-lead electrocardiography
- Measurement of orthostatic hypotension with a stand test and tilt test
- Gynecological examination with a cervical smear
- Serum chemistry, including urea, glucose, electrolytes (sodium, potassium, chloride, phosphorus, bicarbonates, and calcium), proteins, cholesterol, triglycerides, creatinine, total and free bilirubin, uric acid, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, γ-glutamyl transpeptidase, alkaline phosphatase, globular count, platelet count, leukocyte formula, hemoglobin, hematocrit, erythrocyte sedimentation rate, C-reactive protein, prothrombin time, partial thromboplastin time, fibrinogen, presence of procoagulant marker (antithrombin III, S-protein, C-protein, molecular marker for factor V Leiden mutation, and mutation 20210 of the prothrombin gene), thyroid-stimulating hormone, triiodothyronine, thyroxine, hep-
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adapts markers (B and C) and serological human immunodeficiency virus
Urineanalysis, including a toxicology screen for barbiturates, benzodiazepines, opiates, and cannabis
Echo-Doppler measurements of the lower limbs to screen for vein deficiencies
Chest radiography (anteroposterior and lateral)
Dental panoramic radiography
Abdominal ultrasonography
Dual-energy X-ray absorptiometry bone density measurement
Maximal oxygen consumption test

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