Bone resorption is induced on the second day of bed rest: results of a controlled crossover trial

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Baecker, Natalie, Aleksandra Tomic, Claudia Mika, Andrea Gotzmann, Petra Platen, Rupert Gerzer, and Martina Heer. Bone resorption is induced on the second day of bed rest: results of a controlled crossover trial. J Appl Physiol 95: 977–982, 2003; 10.1152/japplphysiol.00264.2003.—The aim of the study was to analyze the kinetics of short-term changes in bone turnover. We studied in a randomized crossover design the effects of 6 days of bed rest on eight healthy male subjects (mean body wt: 70.1 ± 5.7 kg; mean age: 25.5 ± 2.9 yr). The metabolic ward period was divided into three parts: 4 ambulatory days, 6 days of either bed rest or non-bed rest periods, and 1 recovery day. The diet was identical in both bed rest and non-bed rest phases. Continuous urine collection started on the first day in the metabolic ward to analyze excretion of bone resorption markers, namely C-telopeptide (CTX) and N-telopeptide (NTX), creatinine, urea, and 3-methylhistidine. On the second ambulatory day and on the fifth day of bed rest or during the non-bed rest phase, blood was drawn to analyze bone formation markers and amino acid concentrations. Urinary calcium excretion was increased as early as the first day of bed rest (P < 0.01). CTX and NTX excretion stayed unchanged during the first 24 h of bed rest compared with the non-bed rest period. However, already on the second day, both resorption markers had increased significantly. NTX excretion increased by 28.7 ± 14.0% (P < 0.01), whereas CTX excretion rose by 17.8 ± 8.3% (P < 0.001). Creatinine, urea, and 3-methylhistidine excretion did not change. We conclude that 24 h of bed rest are sufficient to induce a significant rise in osteoclast activity in healthy subjects.

Osteoporosis shows an increase in incidence among the aging population of both genders (9). Concomitantly, aging people often suffer from different diseases and thus may be confined to bed for a short or long period of time. Long-term bed rest or any other kind of unloading of weight-bearing bones, for example microgravity, reduces bone density (14, 15, 23). Thereby, the above-mentioned positive effect of confinement for any other disease, especially in older patients, may exacerbate calcium loss and an already existing osteopenia or osteoporosis. In contrast to postmenopausal osteoporosis due to estrogen deficiency, immobilization causes bone density reduction by decoupling the bone turn-over process, i.e., unloading of weight-bearing bones increases bone resorption, whereas bone formation decreases (3, 4, 8, 19). Moreover, some studies have shown that the human skeleton undergoing immobilization rapidly starts the process of bone resorption (16, 25).

Several studies (7, 18) have provided evidence for a close correlation between bone mass and muscle mass. Recent human studies (2, 17) have also demonstrated that body nitrogen and muscle mass rapidly decrease with bed rest. Scheld et al. (17) have even shown that there is a rapid loss of body nitrogen during the first 2 wk of bed rest. However, the kinetics of changes in nitrogen metabolism compared with bone turnover during short-term bed rest are, to the best of our knowledge, not yet known.

In the present study, we therefore examined the kinetics of bone formation, bone resorption, and muscle mass changes in a short-term bed rest study by using a randomized crossover design.

MATERIALS AND METHODS

This study was approved by the ethics committee of the Aerztekammer Nordrhein, Duesseldorf, Germany. All subjects gave informed consent.

Volunteers. Eight healthy, male test subjects (mean age: 25.5 ± 2.9 yr; mean body mass 70.1 ± 5.7 kg) gave their written consent to participate in the bed rest study. The subjects were nonsmokers and did not have any physical activity before or during both study phases.

Study design. The study was performed in a randomized crossover design. Each of the two study phases consisted of 2 dietary adaptation days (ambulatory) and 11 days (24 h/day) in the metabolic ward [Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany]. The metabolic ward days were divided into a 4-day ambulatory period, a 6-day intervention period [either bed rest (−6° head-down tilt (HDT)] or non-bed rest], and 1 recovery day. During both 11-day periods in the ward, constant room temperature (24°C) and relative humidity (50%) were ensured. The eight subjects were randomly distributed into two groups. The first group started with the non-bed rest phase in the intervention phase and the second group with the bed rest period. During the bed rest phase, subjects were kept in bed for 24 h and

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were not allowed to elevate their heads >30° from horizontal. Horizontal movement was allowed. All activities, including showering and weighing, were carried out in the 6° HDT position. During the non-bed rest phase, the subjects were allowed to walk around in the ward. Both study phases were identical with respect to environmental conditions, study protocol, and diet.

**Diet.** Beginning with the dietary adaptation phase, the volunteers received a diet consisting of 10.03 MJ/day, 50 ml water · kg body wt⁻¹ · day⁻¹, 200 meq NaCl/day, 2,000 mg calcium/day, and 400 IU vitamin D/day provided by a multivitamin preparation. Dietary protein, fat, and carbohydrate intakes were calculated according to dietary reference intake values (24) (i.e., 15–20% of the daily energy intake was administered as protein, 30% as fat, and 50–55% as carbohydrates, respectively). All bread, cakes, and pastries were homemade and prepared in a metabolic kitchen according to predefined recipes. Ingredients of sodium contents usually >20 meq Na⁷/100 g were food chemically analyzed. Each ingredient was weighed on a laboratory scale. For the calculation of the nutrient content of each prepared food, PRODI 4.2 software (13) was used. The individual menu of each volunteer was also predefined with PRODI 4.2. During the daily preparation for each study day, the volunteers received the exact amount of food that was predefined in their individual menu. The evening before the ambulatory control days, the volunteers received packed meals from the metabolic kitchen for the following 2 days. To avoid any impact of other nutrients than the controlled foods, the menu as well as the meal frequency of the corresponding study days of both phases was identical. Because the volunteers fulfilled no physical workload during the non-bed rest phase, the additional energy requirement according to physical exercise was insignificant. Therefore, dietary energy intake was identical during the bed rest and the non-bed rest phases.

**Blood.** Blood samples were obtained from the subjects in the morning (7 AM) after overnight fasting in the recumbent body position during the ambulatory and the non-bed rest period, and in the 6° HDT position during the intervention period of HDT. On the third day in the metabolic ward and the fifth day of the intervention period, blood was drawn by a short catheter in serum monovettes from the antecubital vein (Sarstedt, Germany). Serum was distributed in small tubes and immediately frozen at −20°C until analysis. Total serum calcium concentrations were determined by the orthocresolphthalein method (Hitachi 747). Serum phosphate concentrations were analyzed by using the molybdate method (Hitachi 747). Serum protein concentrations were determined by the biuret method (Hitachi 747). Markers of bone resorption, C-telopeptide (CTX) and N-telopeptide (NTX), were analyzed by commercially available enzyme-linked immunosorbent assays (CTX: Crosslips, Osteometer BioTech, Herlev, Denmark, NTX: Osteomark, Ostex International, Seattle, WA). All samples for one subject (from pre-study phases HDT, non-bed rest, and recovery periods) were analyzed together in a single batch to avoid any interassay variation. The excretion rates of CTX and NTX were calculated by multiplying the urinary concentration in the 24-h pool with the 24-h urine volume and dividing it by the collection period in minutes.

Urinary 3-methylhistidine (3-Me-His) concentrations were quantified by HPLC using reversed-phase C18 separation after automated precolumn derivatization with orthophthalaldehyde/3-mercapto-propionic acid and fluorescence detection.

**Statistical analysis.** The anthropometric data presented are mean values ± SD. All results are presented as means ± SE. Statistical analyses were performed by SYSTAT software (21). Excretion data were compared by ANOVA (repeated-measure design) by using the intervention period as “grouping” factor and the sampling day as “within” factor. A significant effect of bed rest was accepted when the influence of the intervention on time (day) was evident. Post hoc testing was carried out by comparing the daily data of each phase by one-side paired Student’s t-tests. Serum concentrations were tested by paired Student’s t-tests that compared the bed rest phase with the relevant non-bed rest period measurement. P < 0.05 was considered to be the minimum level of significance.

**RESULTS**

Table 1 shows the body weight and urine volume data in the course of the study.

Urinary calcium excretion was examined from the urine taken consecutively for 24 h/day from day 1 to 11 in both study phases. During the first 4 days of the metabolic ward period of both phases (i.e., before the non-bed rest period and bed rest), urinary calcium excretion was almost identical (Fig. 1). But during the first 24 h in bed, calcium excretion already significantly increased (ANOVA, repeated-measure design: P < 0.01) by 22.7 ± 5.3% compared with the non-bed rest figures. Almost during the entire bed rest phase, urinary calcium excretion remained higher than the figures from non-bed-rest phase. On the first day of recovery, however, urinary calcium excretion descended to the level before bed rest and was almost the same as during the non-bed-rest phase.

Urinary excretion of the bone resorption marker NTX is shown in Fig. 2. During the first 4 days in the metabolic ward, urinary NTX excretion levels were almost identical and remained the same on the first bed rest day compared with the non-bed rest phase. But day 2 of bed rest, the NTX excretion levels significantly increased by 28.7 ± 14.0% (bed rest: 937.8 ±
This high level slowly decreased until day 5 of bed rest. On day 6, the urinary NTX excretion levels reduced to the non-bed rest levels and also remained in that range on the recovery day.

Urinary CTX excretion, another bone resorption marker, is also shown in Fig. 2. During both adaptation phases (before bed rest and non-bed rest phase) in the metabolic ward, the urinary CTX excretion levels were almost identical. CTX excretion, comparable with uri-

![Fig. 1. The 24-h calcium excretion in a crossover design of 8 healthy bed-rested and ambulatory male subjects. The 2 study phases consisted of a 4-day adaptation period and an 11-day intervention period (bed rest or non-bed rest). The black lines show the frame of the intervention period. ⊗, Intervention phase; ●, non-bed rest phase. *Significant difference between the 2 phases (P < 0.05).](http://jap.physiology.org/)

![Fig. 2. Bone resorption markers N-telopeptide (NTX; A) and C-telopeptide (CTX; B) in a crossover design of 8 healthy bed-rested and ambulatory male subjects. The 2 studies consisted of a 4-day adaptation period and an 11-day intervention period (bed rest or non-bed rest). The black lines show the frame of the intervention period. ⊗, Intervention phase; ●, non-bed rest phase. Significant difference between the 2 phases: *P < 0.05; **P < 0.01.](http://jap.physiology.org/)
Bone formation markers, type I procollagen bone alkaline phosphatase, calcium transformed by albumin, parathyroid hormone, serum protein, and albumin concentrations during a bed-rest experiment (crossover design) of 8 healthy, male test subjects.

Table 2. Bone formation markers, type I procollagen bone alkaline phosphatase, calcium transformed by albumin, parathyroid hormone, serum protein, and albumin concentrations during a bed-rest experiment (crossover design) of 8 healthy, male test subjects

<table>
<thead>
<tr>
<th></th>
<th>Pre-Bed Rest Phase (Day 3)</th>
<th>Pre-Non-Bed Rest Phase (Day 3)</th>
<th>Bed Rest Phase (Day 5)</th>
<th>Non-Bed Rest Phase (Day 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PICP, µg/l</td>
<td>140.11 ± 16.99</td>
<td>144.54 ± 8.94</td>
<td>122.55 ± 15.18</td>
<td>135.75 ± 8.93</td>
</tr>
<tr>
<td>bAP, µg/l</td>
<td>14.31 ± 1.71</td>
<td>14.35 ± 1.61</td>
<td>14.30 ± 1.58</td>
<td>14.30 ± 1.49</td>
</tr>
<tr>
<td>tCa, mmol/l</td>
<td>1.34 ± 0.06</td>
<td>1.34 ± 0.07</td>
<td>1.34 ± 0.06</td>
<td>1.32 ± 0.04</td>
</tr>
<tr>
<td>PTH, pg/ml</td>
<td>19.57 ± 4.60</td>
<td>18.06 ± 3.03</td>
<td>16.64 ± 2.78</td>
<td>17.86 ± 2.40</td>
</tr>
<tr>
<td>Protein, g/dl</td>
<td>7.63 ± 0.11</td>
<td>7.68 ± 0.15</td>
<td>7.56 ± 0.15</td>
<td>7.46 ± 0.11</td>
</tr>
<tr>
<td>Albumin, g/dl</td>
<td>4.83 ± 0.06</td>
<td>4.85 ± 0.07</td>
<td>4.73 ± 0.06</td>
<td>4.76 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE. PICP, type I procollagen; bAP, bone alkaline phosphatase; tCa, calcium transformed by albumine; PTH, parathyroid hormone. Short-term bed rest for 6 days did not lead to any changes regarding any of these parameters.

DISCUSSION

The presented data of our short-term bed rest study in a crossover design show that unloading of weight-bearing bones by bed rest increases calcium excretion as early as the first day in bed. Both excretions of bone resorption markers, namely NTX and CTX, increased on day 2 of bed rest. These results imply that even 1–2 days of bed rest are sufficient to exacerbate bone loss. In contrast, markers of muscle mass, like creatinine, or muscle breakdown, like urea and 3-Me-His excretion, did not increase during the 6 days of bed rest. Bone formation markers PICP and bAP did not decrease significantly in this short-term bed rest.

Bone adapts to its mechanical stimulus, i.e., change in mechanical stress. Thus unloading of weight-bearing bones and therefore a decrease in mechanical stress results in bone loss (3, 14, 15, 23). The presented data even show the kinetics of changes in bone resorption during 5 days of bed rest compared with the intraindividual non-bed rest period in the crossover design. Hence, calcium excretion increased as early as the first 24 h, whereas the increases in resorption marker were delayed until the second day of bed rest. One might argue that the increase in calcium excretion as early as the first day might be due to a decrease in the non-bed rest group. This might be true, but the...
following days show a continuous significant increase in calcium excretion so that calcium is certainly increased, together with the bone resorption markers, not later than the second day of bed rest. This effect of acute initiation of osteoclast activity has, to the best of our knowledge, been shown for the first time. Short-term bed rest rather than long-term bed rest should, from our point of view, therefore be the first step when countermeasures to prevent disuse osteoporosis are examined.

It is well recognized that the resorption markers we used have large within-subject and subject-to-subject coefficients of variation. These have been reported to be from 10 to 33% for NTX and as high as 48% for urinary CTX (11). In the present study, we therefore have chosen a study design with identical environmental study conditions to overcome these day-to-day variations. Both CTX and NTX excretion show almost identical values when the relevant 4 days of the well-standardized adaptation period are compared. We therefore suggest that in randomized, crossover study designs, including controlled activity and identical food intake, the variation in resorption markers is much lower than in day-to-day living situations.

Enhanced osteoclast activity is seen in astronauts or people in bed rest (3, 4, 17, 19). In the ambulatory control phase, the volunteers showed a daily calcium excretion of 206 mg. During bed rest, calcium excretion rose significantly to 240 mg/day while their diet was unchanged. Together with the increased calcium loss, CTX excretion rose by 18% on the second day, whereas NTX excretion, another bone resorption marker, rose by 29%. Therefore, calcium excretion as well as both resorption markers showed enhanced bone resorption during day 2 of bed rest. Although we cannot distinguish between the site or size of bone resorption, the results give a clear basis for demineralization of bone and loosening of bone matrix already at the beginning of immobilization. In accordance with our data, Inoue et al. (12) showed that NTX is increased by up to 50% within the first week of bed rest.

The results show that the rise in NTX on the second day of the intervention is greater than the rise in CTX. Moreover, until the end of immobilization, NTX excretion falls off, in contrast with CTX, which rises slowly and continues to increase even after the bed rest phase. Although CTX and NTX are believed to be derived from the same molecule and would be expected to vary in parallel, they did not. One explanation for this could be that there is a difference in the metabolism of these two fragments.

Our data on bone formation markers and bone resorption markers also support the uncoupling of bone collagen synthesis and breakdown, which have been shown after long-term bed rest (4, 17, 22, 25). Usually in healthy adults, bone mass is regulated through bone remodeling. In a first step, preexisting bone is resorbed by osteoclasts. Then the bone formation process follows by activation of osteoblasts. However, the bone formation marker PICP in the presented bed rest study tends to decrease by 11.2% (P = 0.09) as early as day 5 in bed. Because PICP is a biomarker for collagen synthesis (10), this suggests that the bone collagen biosynthesis is already reduced in the early days of bed rest.

3-Me-His excretion is a useful parameter to reflect myofibrillar protein breakdown. Additionally, creatinine excretion can be taken as a marker of muscle mass if the following requirements are fulfilled: 1) creatinine containing food consumption is almost zero or 2) the diet and amount of the respective food items during the respective ambulatory control, bed rest, and non-bed rest phases are identical. We have chosen the latter so that the subjects consumed the identical food composition as well as the identical amount of each food during both study phases. However, during the 6 days of HDT bed rest, neither 3-Me-His nor creatinine excretion nor urea-N increased. Our data are in accordance with results published by Stein and Schluter (20) and Scheld et al. (17). They also did not find a change in 3-Me-His during spaceflight or during 16-wk of HDT bed rest. Together with the unchanged excretion of these parameters, neither serum protein concentration nor serum amino acid concentration increased. Thereby, our results underline previous findings suggesting that 6 days of bed rest do not inevitably lead to a significant rise in muscle protein breakdown. We did not focus on changes in protein synthesis. However, protein synthesis is decreased during 14-day bed rest studies, as observed by tracer techniques (1, 5). Any change in muscle mass during bed rest is therefore most likely associated with a decrease in skeletal muscle protein synthesis rather than in protein breakdown. This resulting loss in lean body mass is evident within 7 days of bed rest as measured by MRI techniques (6). In future studies, MRI or dual-energy X-ray absorptiometry techniques should be included to analyze additional changes in lean body mass.

In contrast to the observations of Scheld et al. (17), higher serum concentrations of essential amino acids were not seen in our 6-day bed rest period compared with the non-bed rest phase.

In summary, our data indicate that bone resorption increases relatively quickly after bed rest. The acute rise in these markers at the beginning of bed rest also suggests that the development of possible countermeasures to prevent disuse osteoporosis does not require long-term studies but can be examined in short-term studies. Long-term studies are associated with several health risks, such as significant reduction in bone quality, danger of thromboembolism, or cardiovascular deconditioning. The aim of long-term bed rest studies in healthy subjects should thus be to stepwise extend the study duration with the help of countermeasures that work in short-term studies.

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DISCLOSURES

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