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Changes in bone turnover markers during 14-day 6° head-down bed rest

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Abstract Osteoporosis caused by exposure to microgravity represents a serious clinical concern, but the mechanisms have yet to be fully elucidated. The present research aimed to elucidate the effects of microgravity environments on bone turnover, with a specific focus on changes in bone resorption markers such as type I collagen cross-linked N-telopeptides (NTx) and deoxypyridinoline (Dpyr), for which scant data are available regarding detailed time course. Methods using 6° head-down bed rest were utilized to simulate a microgravity environment. Eleven adult male volunteers underwent 6° head-down bed rest for 14 days; measurements were made of serum and urine Ca concentrations, in addition to osteocalcin (OC), bone alkaline phosphatase (ALP), NTx, and Dpyr as bone turnover markers. By the end of bed rest, concentrations of bone ALP had significantly increased, but OC displayed a tendency toward decrease. Concentrations of Dpyr significantly increased from day 6, remaining elevated until the end of bed rest. Concentrations of NTx significantly increased on day 13 and at the end of bed rest. Serum and urinary concentrations of Ca increased significantly at the end of bed rest. Bone ALP represents a relatively early marker of osteoblast differentiation at the matrix maturation phase and OC is a late marker in osteoblast differentiation at the calcification phase. The present results therefore suggest an absolute increase in bone resorption and normal or reduced bone formation, together causing prominent uncoupling and rapid bone loss after simulated microgravity. Moreover, the present results suggest that bone resorption is enhanced at an early stage of exposure to microgravity environments.

Key words head-down bed rest · microgravity · bone formation marker · bone resorption marker

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Introduction

Exposure to microgravity is known to exert various effects on humans and animals [1–7]. These effects include the clinically significant problem of osteoporosis, caused by sustained reduction of the gravitational load on bone [2–4]. With the construction of the International Space Station proceeding in earnest, this effect represents a critical problem that must be solved. However, the mechanisms by which microgravity environments influence the human skeletal system and the onset of these influences are yet to be fully elucidated, which is attributable to the numerous difficulties associated with performing studies in microgravity environments. Several terrestrial experiments have therefore been devised to simulate physiological changes caused by reduced gravitational load. In laboratory animals, for example, some researchers have conducted tail suspension studies using rats [7]. In humans, head-down bed rest is widely used to simulate microgravity conditions [6]. This method involves subjects lying with the body inclined to leave the head as the lowest point, allowing the shift in body fluids [6] and changes in skeletal system induced by microgravity environments to be reproduced to a certain extent [5].

In the field of research into bone turnover, researchers have identified certain bone matrix proteins specifically synthesized or secreted during bone formation and various metabolites released into the circulation in association with bone resorption. Measurement of the concentrations of these substances in blood and urine is now possible, including osteocalcin (OC), bone alkaline phosphatase (ALP), deoxypyridinoline (Dpyr), and type I collagen cross-linked N-telopeptides (NTx). However, scant data are available regarding these substances during exposure of humans to microgravity environments.

The present work therefore sought to elucidate the effects of microgravity on human bone turnover and the

onset of associated effects by investigating the effects of 14-day 6° head-down bed rest on concentrations of bone turnover markers.

Materials and methods

Subjects

Subjects comprised 11 healthy adult males, with a mean (±SD) age of 22 ± 1 years, height 169.3 ± 1.3 cm, and weight 64.3 ± 1.9 kg. All candidates were thoroughly briefed regarding the objectives, significance, content, and potential adverse effects of the study. In addition, subjects were informed that they were free to withdraw from the study at their own discretion at any time whatsoever. Tests and measurements were initiated only after obtaining informed consent. Our series of gravitation studies [8,9], including the present study, were approved by the Institutional Review Boards of Nihon University School of Medicine.

Study protocol

A 14-day 6° head-down bed rest study protocol was implemented. All subjects received identical meals, with an intake of 2000–2100 kcal/day from 2 days before bed rest and during 2 weeks of bedrest. Urination and defecation were performed while inclined at −6° on the bed. Changes of position in bed, including supine, lateral, and prone positions, were permitted, but at no time was the head allowed to exceed the height of the heart.

Blood samples were withdrawn from supine subjects at 0700 before starting head-down bed rest and at the end of bed rest before ambulation. Urine was collected a total of nine times: before starting head-down bed rest, on days 1, 2, 4, 6, 9, 11, and 13 of head-down bed rest, and after completion of bed rest. First urine was collected immediately after subjects awoke at 0630; second urine samples were collected at 1100.

Sample assay

Blood samples were assayed for OC, bone ALP, and serum Ca. Urine samples were assayed for NTx, Dpyr, and Ca. Serum OC was measured using an immunoradiometric assay (BGP IRMA kit; Mitsubishi Kagaku, Tokyo, Japan) [10,11]. Serum bone ALP was determined using an immunoselective enzyme assay (Osteolinks BAP kit; Sumitomo Seiyaku, Tokyo, Japan) [12]. Urine Dpyr was determined using high performance liquid chromatography (Osteolinks DPD kit; Sumitomo Seiyaku) [11,13]. Urine NTx was determined using an enzyme immunoassay (Osteomark kit; Mochida, Tokyo, Japan) [14,15]. Serum and urine Ca were measured using orthocresolphthalein complexone (Mitsubishi Kagaku) [16].

Statistical analysis

Changes in urinary markers of bone turnover were tested for statistical significance using repeated one-way analysis of variance. If a significant difference was identified, Dunnett's multiple comparison tests were utilized to analyze differences from control values, which were defined as values measured before the start of head-down bed rest. Changes in blood levels of bone turnover markers and electrolytes were tested for significance using the paired t test. Threshold of significance was set at the level of $P < 0.05$.

Results

Bone formation markers

Bone ALP significantly increased at the end of bed rest (Fig. 1). OC tended to decrease at the end of bed rest,

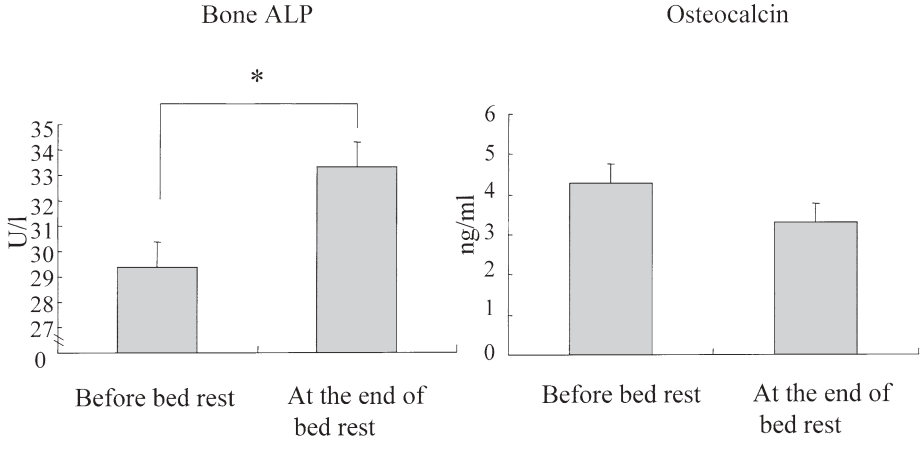

Fig. 1. Bone alkaline phosphatase (ALP) and osteocalcin concentrations before and at the end of 14-day 6° head-down bed rest (mean ± SEM). * $P < 0.05$

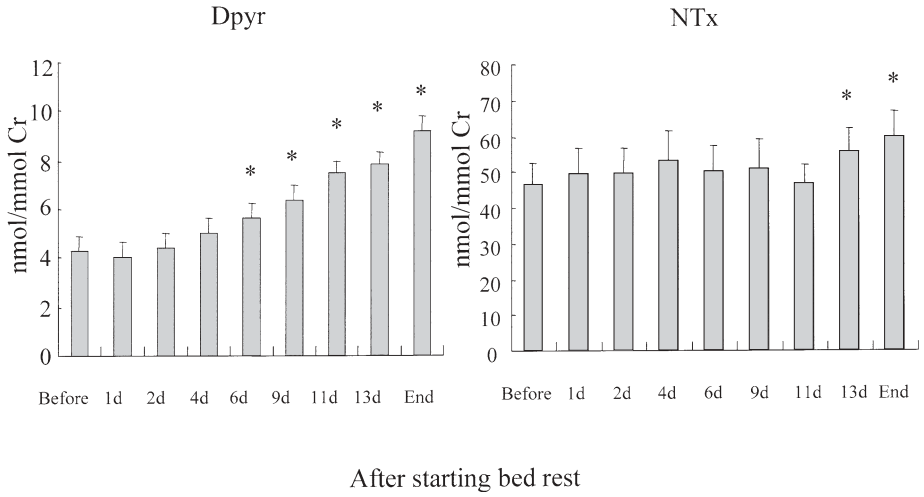

Fig. 2. Changes in urinary deoxypyridinoline (Dpyr) concentration and the urinary type I collagen cross-linked N-telopeptides (NTx) concentrations during 14-day 6° head-down bed rest (mean ± SEM). * $P < 0.05$ (relative to baseline)

Fig. 3. Serum corrected Ca and urinary Ca concentrations before and at the end of 14-day 6° head-down bed rest (mean ± SEM): serum corrected Ca (mg/dl) = total Ca (mg/dl) + [4.0 − serum albumin (g/dl)]. * $P < 0.05$

but no statistically significant differences were identified (Fig. 1).

Bone resorption markers

From day 6 of bed rest, concentrations of Dpyr increased significantly, rising to about twice baseline level by the end of bed rest (Fig. 2). NTx increased significantly compared with baseline on day 13 of bed rest and at the end of bed rest (Fig. 2).

Calcium

Both serum and urinary Ca significantly increased at the end of bed rest (Fig. 3).

Discussion

Microgravity and bone turnover

Several experiments in space and terrestrial simulations have been undertaken to reveal the effects of exposure to conditions of microgravity on bone turnover in humans [17–20]. In 1999, Smith and colleagues reported decreased calcaneal bone mineral density in astronauts who spent 3 months in space [17]. Evaluating the percentage decrease in bone mass is critical for predicting risk of bone fracture and abnormalities of Ca metabolism due to secondary osteoporosis when astronauts return to earth. Repetition of invasive bone biopsy is difficult for quantitative evaluation of the processes of bone formation and resorption and for estimation of percentage reduction in bone mass. Furthermore, radiographic methods of calculating bone mineral density by measuring Ca equivalents per unit present numerous

challenges: for instance, measurement sites are limited and only localized findings are available, and interpretation is required that includes information from radiographic imaging and other sources. In any event, due to these technical difficulties, hopes have been held for quantifiable markers of bone turnover that closely reflect bone resorption and bone formation. For example, some researchers have documented increased levels of bone resorption markers in human subjects during 120 days [18] and 16 weeks [19] of prolonged bed rest. However, the onset of influence from microgravity environments on the human skeletal system has not yet been fully elucidated.

Bone resorption markers

Dpyr and NTx represent highly specific markers of bone resorption. Dpyr is unaffected by diet, as it is not absorbed from the gastrointestinal tract. In addition, it is excreted in urine without being further metabolized within the body. Urinary NTx is claimed to represent a more specific marker than Pyr and Dpyr during bone resorption, because the immune response to nonbone NTx is lower than that to bone NTx. In light of these characteristics, we chose to evaluate Dpyr and NTx in the present study.

In the present study, Dpyr and NTx displayed a consistent tendency to increase during bed rest. In addition, Inoue et al. documented increased concentration of NTx in human subjects from day 7 of bed rest [18], and Kerstin et al. noted that NTx increased from week 10 in a 6° head-down bed rest study that lasted 16 weeks [19]. These findings in addition to our own indicate that bone resorption may be enhanced during microgravity simulations. In addition, serum and urinary concentrations of Ca significantly increased at the end of bed rest. These findings are consistent with increases in Dpyr and NTx. Moreover, Dpyr was significantly higher from day 6, and remained elevated for the remainder of the 14-day bed rest in the present study, suggesting that bone resorption may be enhanced from an early stage of exposure to microgravity environments. However, NTx significantly increased on day 13 and at the end of bed rest. These results suggest that NTx may be less sensitive than Dpyr for detecting increases in bone resorption [21].

Bone formation markers

Bone ALP and serum OC concentrations were selected because both are unlikely to be influenced by diet. The results showed conflicting trends: decreases in OC and increases in bone ALP. However, previous studies in humans have been consistent with the present results, showing conflicting increases in ALP [22,23] and decreases in OC [19,23]. A potential mechanism for these conflicting trends is worthy of consideration. During the progression from precursor cells to mature osteoblasts, cell products such as OC and ALP are produced at different times. Furthermore, although both are osteoblast products, OC and ALP probably reflect different aspects of osteoblast differentiation and function [24]. Bone ALP represents a relatively early marker of osteoblast differentiation at the matrix maturation phase, and may not always reflect the function of terminally differentiated osteoblasts [25]. On the other hand, OC is a late marker in osteoblast differentiation at the calcification phase [25]. Results such as those obtained in the present study may therefore suggest an increase in early differentiation, whereas function of terminally differentiated osteoblasts may decrease. According to a report by Kumei et al. [26], OC production and expression of the gene in osteoblasts are inhibited during space flight, and osteogenesis (calcification) may not be attained.

Conclusions

To investigate the effects of microgravity on bone, we measured concentrations of bone ALP, serum OC, Dpyr, and NTx in a 6° head-down bed rest study lasting 14 days. ALP and OC are bone formation markers, and Dpyr and NTx are bone resorption markers that are considered to represent excellent indices of bone turnover. ALP, as an early marker of osteoblast differentiation, was found to increase, although OC as a late marker of osteoblast differentiation tended to decrease at the end of bed rest. Moreover, concentrations of bone resorption markers increased significantly during bed rest. The present findings indicate that an absolute increase in bone resorption and normal or reduced bone formation may occur during simulated microgravity, together causing prominent uncoupling leading to rapid bone loss. Moreover, the increase in Dpyr from day 6 of bed rest suggests that bone resorption may be enhanced at an early stage of exposure to microgravity environments.

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