Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 1696–1703
Metabolism
Clinical and Experimental
www.metabolismjournal.com

# Triiodothyronine increases calcium loss in a bed rest antigravity model for space flight

Steven R. Smith*, Jennifer C. Lovejoy, George A. Bray, Jennifer Rood, Marlene M. Most, Donna H. Ryan

*Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA*

Received 15 September 2005; accepted 24 July 2008

**Abstract**

Bed rest has been used as a model to simulate the effects of space flight on bone metabolism. Thyroid hormones accelerate bone metabolism. Thus, supraphysiologic doses of this hormone might be used as a model to accelerate bone metabolism during bed rest and potentially simulate space flight. The objective of the study was to quantitate the changes in bone turnover after low doses of triiodothyronine ($T_3$) added to short-term bed rest. Nine men and 5 women were restricted to bed rest for 28 days with their heads positioned 6° below their feet. Subjects were randomly assigned to receive either placebo or oral $T_3$ at doses of 50 to 75 $\mu$g/d in a single-blind fashion. Calcium balance was measured over 5-day periods; and $T_3$, thyroxine, thyroid-stimulating hormone, immunoreactive parathyroid hormone, osteocalcin, bone alkaline phosphatase, and urinary deoxypyridinoline were measured weekly. Triiodothyronine increased 2-fold in the men and 5-fold in the women during treatment, suppressing both thyroxine and thyroid-stimulating hormone. Calcium balance was negative by 300 to 400 mg/d in the $T_3$-treated volunteers, primarily because of the increased fecal loss that was not present in the placebo group. Urinary deoxypyridinoline to creatinine ratio, a marker of bone resorption, increased 60% in the placebo group during bed rest, but more than doubled in the $T_3$-treated subjects ($P < .01$), suggesting that bone resorption was enhanced by treatment with $T_3$. Changes in serum osteocalcin and bone-specific alkaline phosphatase, markers of bone formation, were similar in $T_3$- and placebo-treated subjects. Triiodothyronine increases bone resorption and fecal calcium loss in subjects at bed rest.


## 1. Introduction

Hormonal and nutritional signals adjust bone mass to match the needs of the organism. Estrogen, for example, decreases bone turnover and is "anabolic" in bone [1]. In contrast, thyroid hormones enhance bone turnover; and hyperthyroidism has been long recognized as a cause of osteopenia if left untreated for a prolonged period [2-6]. Serum osteocalcin, a marker of bone turnover, showed a positive relationship with serum triiodothyronine ($T_3$) and thyroxine ($T_4$) levels; but alkaline phosphatase, urinary calcium, and urinary hydroxyproline did not [4-6]. Restoration of euthyroidism partly ameliorated these effects.

Bone is a tissue that also responds to the mechanical forces placed on it. There is good evidence that increased mechanical forces during growth and development increase bone size. Similarly, a lack of mechanical forces on bone results in loss of bone mass in both children and adults. Unloading of the skeleton is a particular problem for patients with spinal cord injury and debilitated patients after injury or fracture [7,8]. Inactivity is also a contributing factor to the bone loss that occurs with aging and bed rest; and skeletal unloading is a major problem for astronauts on long-duration space missions, where the lack of gravity results in bone loss [9-14].

When the skeleton is unloaded by bed rest [9-12,15] or space flight [13,14], bone loss occurs in specific regions, particularly in the axial skeleton and lower extremities. After 12 weeks of bed rest, bone mineral density at the spine and hip was significantly decreased; and bone markers of resorption were increased [15]. Unloading bone by bed rest

* Corresponding author. Tel.: +1 504 763 3028; fax: +1 504 763 2525.
*E-mail address:* smithsr@mhs.pbrc.edu (S.R. Smith).

0026-0495/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.07.026

increases urinary calcium excretion and decreases intestinal absorption of calcium [10,13,15]. The excess calcium released from the bone is cleared through the kidney, where the risk of renal calculi is increased.

Bed rest for weeks to months unloads the axial skeleton from gravitational forces and produces cephalad shifts in fluid, with headaches and backaches as common complaints [16]. This effect is enhanced by placing the subjects with their heads 6° below their feet [16]. The duration of the bed rest required to accelerate bone turnover and hypercalciuria makes testing countermeasures to prevent bone loss expensive and time consuming, although 1 study has shown that changes in calcium excretion and bone formation can be observed within 1 week [9]. As noted previously, hyperthyroidism shares features with immobilization in its effect on the skeletal system [5,6,17]. We hypothesized that treatment with low doses of thyroid hormone during bed rest would increase bone resorption with greater loss of calcium earlier than immobilization alone [18-21]. This strategy would require monitoring and readjustment of thyroid hormone doses after the normal physiologic response to lower thyroid-stimulating hormone (TSH). If successful, this human model could allow for testing new countermeasures to prevent bone loss in a more efficient and less costly manner.

## 2. Methods

### 2.1. Subjects

Nine healthy men and 7 healthy women were recruited for a protocol involving 28 days of complete bed rest. Two women dropped out of the study within the first week of bed rest, one because of social issues and one because of the difficulty of voiding while recumbent in bed; and these subjects were not included in the metabolic data. Written informed consent was obtained from each subject, and the protocol was approved by the Louisiana State University Institutional Review Board. All participants lived on the Metabolic Research Unit for the duration of the protocol. Data on leucine turnover, growth hormone pulsatility and secretion, insulin pulsatility, energy expenditure, and body composition in these subjects have been reported previously [18-20].

### 2.2. Protocols

The protocol for this study has been described previously [20]. Briefly, volunteers were admitted to the inpatient metabolic unit and remained there 24 hours per day during the 35-day study. Baseline measures were obtained on a defined metabolic diet (see below). The baseline metabolic balance period lasted 5 days before the recumbent (in bed) period. For the next 28 days, all subjects remained in bed with the head of the bed positioned 6° below the foot of the bed. All activities of daily living occurred in the recumbent position, including bathing, personal hygiene, voiding, and other activities. Bathing was performed in a specially designed shower in the level position. At meal times, subjects were allowed to prop themselves up on 1 elbow while eating. At all other times, the subjects were required to remain with the head on 1 pillow. No physical activity other than rolling over was allowed. Compliance was enforced by observation with infrared cameras in each room. Subjects were housed 2 to a room and gathered daily in a common area for socialization. Psychological and cognitive status was measured intermittently as a safety measure.

Subjects were randomly assigned to receive $T_3$ or placebo in a single-blind fashion. Blood samples for $T_3$, $T_4$, TSH, and other analytes were collected in the morning in the fasting state. The nursing staff was blinded to the treatment, but the medical staff reviewed and adjusted $T_3$ doses to maintain steady blood levels. Oral $T_3$ or placebo treatment was initiated after the 5-day baseline balance period and continued for 28 days. The goal was to administer a dose of $T_3$ that would suppress TSH levels but not produce symptoms of hyperthyroidism. All subjects initially received a loading dose of 100 $\mu$g for women and 75 $\mu$g for men (liothyronine sodium, Cytomel; Smith-Kline-Beecham, Philadelphia, PA). Thereafter, 10 $\mu$g of $T_3$ was administered every 4 hours during waking hours at 6:00 AM, 10:00 AM, 2:00 PM, 6:00 PM, and 10:00 PM. The 2:00 AM dose was omitted. If a subject became symptomatic (none did) or if serum $T_3$ levels exceeded 4 ng/mL, the $T_3$ dose was reduced. Caloric intake was set at 1.4 times the resting metabolic rate, which in our metabolic chamber studies is essentially the no-activity level. Food intake was increased by adding food in 100-kcal units that contained only fat and carbohydrate when total body weight decreased below baseline. These unit foods contained no significant amounts of calcium, phosphorus, or protein.

### 2.3. Calcium balance

All diets were designed on a 5-day rotating schedule. Duplicate portions of the food consumed were analyzed in the food-analysis laboratory for validation. All subjects received low-fat dairy products to provide a total daily calcium intake of approximately 1000 mg/d. Sodium intake was targeted for 3600 mg/d; magnesium, 300 mg/d; and potassium, 4000 mg/d [8]. All food not consumed was analyzed by identical methods and subtracted from the food intake.

All urine was collected in 24-hour pools, and calcium content was measured for balance studies. Accuracy of daily urine collections was determined by analysis of creatinine excretion. Feces were collected in 5-day pools, separated by administration of carmine red dye, an indigestible fecal marker. The final 3 days of bed rest were a short balance period. Corrections for fecal losses were carried out by quantifying the nonabsorbable marker polyethylene glycol (10% solution, given orally as 10 mL 3 times per day with meals). Values reported are based on creatinine-corrected urine and polyethylene glycol–corrected stool calcium.

Table 1
Baseline clinical characteristics of the subject population by sex and treatment group

| | Men (n = 9) | | Women (n = 5) | | *P* for sex differences |
|---|---|---|---|---|---|
| | Placebo | $T_3$ | Placebo | $T_3$ | |
| No. of subjects | 4 | 5 | 2 | 3 | |
| Age (y) | 36.4 ± 1.3 | | 34.2 ± 2.1 | | |
| Weight (kg) | 81.1 ± 3.8 | 81.3 ± 2.7 | 57.1 ± 1.1 | 58.1 ± 2.4 | $P < .05$ |
| BMI (kg/m$^2$) | 24.8 ± 0.7 | | 22.8 ± 0.5 | | |
| Lean body mass (kg) | 57.7 ± 1.6 | 57.9 ± 2.2 | 35.2 ± 0.2 | 34.3 ± 1.2 | $P < .05$ |
| Fat mass (kg) | 19.2 ± 1.7 | 18.7 ± 3.6 | 18.2 ± 0.2 | 20.6 ± 2.5 | NS |
| Fat mass (%) | 25.3% | 24.5% | 34.1% | 37.5% | $P < .05$ |
| CT total fat area (cm$^2$) | 298 ± 14.9 | 306 ± 71 | 257 ± 15.3 | 301 ± 58 | NS |
| Visceral fat (cm$^2$) | 92.8 ± 8.2 | 105 ± 27.2 | 42.3 ± 3.0 | 76.3 ± 34.8 | $P < .05$ |
| Subcutaneous fat (cm$^2$) | 205 ± 21.3 | 201 ± 50.1 | 215 ± 18.3 | 225 ± 60.6 | NS |
| $T_4$ (nmol/L) | 7.50 ± 0.20 | 7.19 ± 0.78 | 10.16 ± 0.46 | 9.52 ± 0.89 | NS |
| $T_3$ (nmol/L) | 0.92 ± 0.041 | 0.96 ± 0.081 | 1.258 ± 0.143 | 0.983 ± 0.162 | NS |
| Thyrotropin (mIU/mL) | 1.70 ± 0.43 | 1.61 ± 0.30 | 0.59 ± 0.03 | 0.992 ± 0.251 | $P < .05$ |
| Serum calcium (mg/dL) | 9.8 ± 0.12 | 9.72 ± 0.98 | 9.6 ± 0.30 | 8.67 ± 0.21 | NS |
| Immunoreactive PTH (ng/L) | 26.2 ± 5.53 | 23.1 ± 0.022 | 22.7 ± 7.15 | 26.0 ± 5.10 | NS |
| Serum osteocalcin ($\mu$g/L) | 3.7 ± 1.23 | 4.1 ± 0.61 | 2.2 ± 0.20 | 1.8 ± 0.41 | $P < .05$ |
| BAP (mg/L) | 8.5 ± 2.0 | 11.2 ± 1.8 | 9.9 ± 0.50 | 10.5 ± 1.60 | NS |
| DPD (mg/mg urinary creatinine) | 2.5 ± 0.63 | 3.4 ± 0.53 | 4.2 ± 1.28 | 4.5 ± 1.05 | NS |

BMI indicates body mass index; NS, not significant.

Dermal calcium losses were assumed to be constant at 60 mg/d for both groups based on the results of the $^{47}$Ca method of Charles et al [22] and Jensen et al [10,23,24].

### 2.4. Analytical methods

Calcium balance was determined from daily urinary collections, 5-day fecal pools, and 7-day food composites. All measures of calcium were converted to milligrams of Ca per day, and the weekly values were the mean of the 7-day values for intake or excretion. Calcium balance = (calcium intake) − (urinary Ca excretion + fecal Ca excretion + 60). Thyroid hormones were measured on an Abbott IMx analyzer using either a microparticle enzyme immunoassay ($T_3$ and TSH) or a fluorescence polarization immunoassay ($T_4$) (Abbott Laboratories, Abbott Park, IL). Samples for serum osteocalcin (Osteocalcin; Incstar, Stillwater, MN), bone-specific alkaline phosphatase (BAP) (Tandem-R Ostase; Hybritech, Fullerton, CA), and intact parathyroid hormone (N-tact PTH, Incstar) were collected, transported on ice, and processed immediately. Urinary deoxypyridinoline (DPD) to creatinine ratio was determined on a 24-hour urine sample according to the manufacturer's instructions (Pyrilinks-D; Metra Biosystems, Santa Clara, CA).

### 2.5. Statistical analysis

All data were analyzed for effects of sex. The change in thyroid hormone concentrations over time were plotted for each sex, but the other measures showing response to treatment were pooled by treatment group. Data were analyzed using JMP/SAS System for Windows version 7.0 (SAS, Cary, NC). For dependent variables determined at baseline and at 1, 2, 3, and 4 weeks, a repeated-measures analysis of variance (ANOVA) was used to assess changes over time; and post hoc multiple comparisons were performed using the Tukey-Kramer honestly significant difference procedures. Analysis of calcium balance data was performed on the 7-day pooled data using a repeated-measures ANOVA based on the maximum likelihood method. The relationships between immunoreactive parathyroid hormone (PTH), serum calcium, and urinary and fecal calcium were examined as Pearson correlation coefficients. All data are reported as mean ± SEM unless otherwise indicated, and an $\alpha$ not exceeding .05 was considered statistically significant.

## 3. Results

Data on the 9 men and 5 women who completed in this study are presented in Table 1. The average age was 36.4 ± 1.3 years for the men and 34.2 ± 2.1 years for the women (range, 30-50 years). At baseline, there were a number of sex differences, with women having lower body weight, lower visceral fat area, lower thyrotropin and osteocalcin, and higher percentage of body fat than men (all $P < .05$). During the 28 days of bed rest, the placebo group lost 1.7 kg and the T3-treated group lost 3.6 kg, a difference of 1.9 kg that was statistically significant ($P = .0015$).

The plasma $T_3$ values were approximately 1 nmol/L at baseline. By week 1, the $T_3$ was significantly higher in the treated group ($F_{1,12} = 15.34$, $P = .0020$) and remained significantly elevated throughout (Fig. 1B). Triiodothyronine increased to a peak of 2.2 nmol/L in the men and to a peak of 4.7 nmol/L in the women. Both $T_4$ (Fig. 1A) ($F_{1,66} = 9.62$, $P = .0028$) and TSH (Fig. 1C) ($F_{1,67} = 28.89$, $P < .0001$) declined during treatment with $T_3$, but not in the placebo-treated group, during the 4 weeks of bed rest. Thyroid-stimulating hormone had declined by nearly two

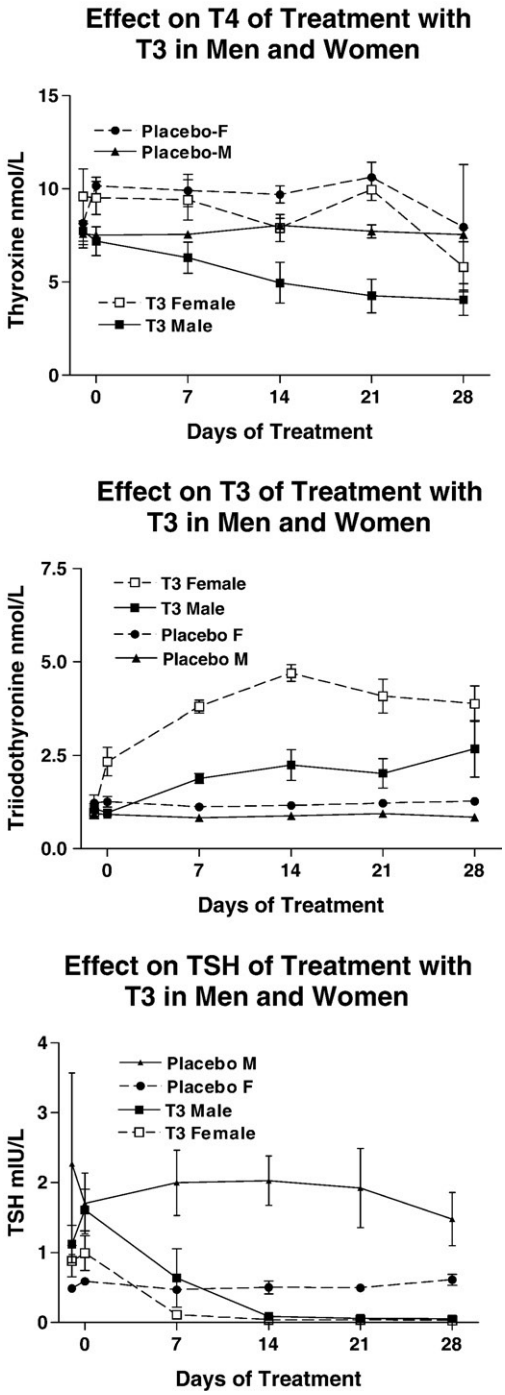


Fig. 1. Time course for the effect of treatment with $T_3$ or placebo on serum $T_3$, $T_4$, and TSH. The $T_3$ values at baseline and at the end of each week of treatment and during follow-up are shown in panel B. Corresponding data for $T_4$ and TSH are shown, respectively, in panels A and C. Data are mean ± SEM.

thirds within 1 day and, by 14 days, was almost undetectable. Thyroxine declined slowly to about 60% of the baseline value. There was prompt return to baseline values of $T_3$, $T_4$, and TSH during the follow-up period (data not included).

Fig. 2 shows both the immunoreactive PTH (top panel) and the serum calcium (lower panel). Baseline immunoreactive PTH did not differ between the sexes and was positively and significantly correlated with serum calcium ($F_{1,12}$ = 4.79, $P$ = .049) and negatively correlated with baseline osteocalcin ($F_{1,12}$ = 5.21, $P$ = .0415), but not with urinary DPD, BAP, or either fecal or urinary calcium. There was a significant decrease in immunoreactive PTH with bed rest in both groups ($F_{4,65}$ = 4.48, $P$ = .0029), but there was no significant effect of treatment with $T_3$ or of sex. Serum calcium was not different between men and women at baseline. Serum calcium showed a significant effect of time, with week 4 being significantly lower than the others ($F_{4,62}$ = 9.07 $P$ < .001). There was no effect of treatment with $T_3$.

Table 2 and Fig. 3 show calcium intake, calcium excretion, and calcium balance results. Low-fat dairy products were used to bring total calcium intake to approximately 1000 mg/d, and calcium intake remained constant between 800 and 1000 mg/d throughout the study. Urinary calcium excretion was not affected by bed rest (Table 2) or $T_3$. Fecal calcium was significantly increased by $T_3$ ($F_{1,68}$ = 11.89, $P$ = .0010), but did not increase over time. Calcium balance is shown in Fig. 3. Calcium balance was borderline significantly negative in the $T_3$ group ($F_{1,68}$ =

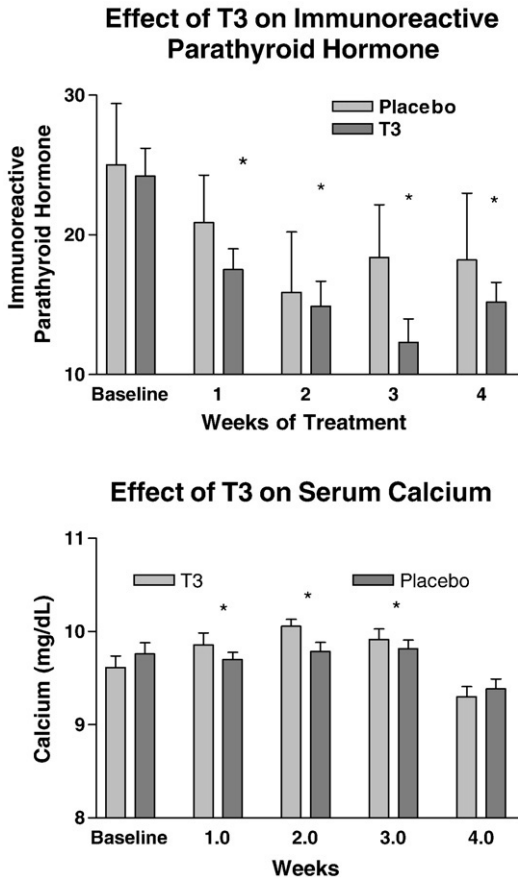


Fig. 2. Time course for the effect of $T_3$ and bed rest on serum calcium and immunoreactive PTH. The top panel shows the data for immunoreactive PTH, and the lower panel shows the serum calcium data. Data are the mean ± SEM. Immunoreactive PTH and serum calcium both showed significant changes from baseline as indicated by *$P$ < .05.

Table 2
Calcium intake and calcium excretion in the urine and feces

| Period | Calcium intake (mg/wk) | | Urinary calcium excretion (mg/wk) | | Fecal calcium excretion (mg/wk) | |
|---|---|---|---|---|---|---|
| | Placebo | $T_3$ | Placebo | $T_3$ | Placebo | $T_3$ |
| Baseline | 5789 ± 576 | 6657 ± 576 | 1174 ± 286 | 902 ± 185 | 4036 ± 843 | 6591 ± 790 |
| Wk 1 | 5777 ± 583 | 6475 ± 604 | 1122 ± 281 | 1143 ± 241 | 4854 ± 771 | 5995 ± 620 |
| Wk 2 | 5686 ± 542 | 6688 ± 584 | 1181 ± 309 | 1313 ± 187 | 4763 ± 857 | 6124 ± 817 |
| Wk 3 | 5658 ± 541 | 6451 ± 602 | 1317 ± 319 | 1473 ± 304 | 4327 ± 928 | 7330 ± 1094* |
| Wk 4 | 5700 ± 650 | 6590 ± 623 | 1486 ± 413 | 1684 ± 327 | 4986 ± 832 | 6164 ± 1114 |

Mean ± SEM.
* $P < .05$ compared with placebo.

3.70, $P = .0586$); and on day 4, the difference between groups was again borderline significant ($P = .0534$) (Fig. 3). Net calcium balance during the 28 days of bed rest was −218.5 ± 41.4 mg/d in the $T_3$-treated group.

Urinary excretion of creatinine was unaffected by treatment with $T_3$. Excretion of urinary DPD was corrected for creatinine excretion (DPD to creatinine ratio). Baseline excretion of DPD was not different between the sexes, but was significantly increased by treatment with $T_3$ ($F_{1,68} = 17.44$, $P < .0001$). The rise during bed rest ($F_{1,65} = 4.72$, $P = .0021$) (Fig. 4) was significantly greater in the $T_3$-treated subjects by the second week than in the placebo-treated group, and the difference widened from week to week. There was no correlation of baseline urinary DPD with immunoreactive PTH, serum calcium, urinary calcium, or fecal calcium.

Bone turnover was assessed by measuring BAP and osteocalcin. Bone-specific alkaline phosphatase did not differ between men and women at baseline and was not affected by treatment with $T_3$. Although the ANOVA did not show a significant effect of time, comparison of individual pairs showed that week 4 was significantly lower than each of the other time points ($F_{1,11} = 11.93$, $P = .0054$). There was no correlation of baseline BAP with baseline serum calcium, urinary calcium, fecal calcium, or week-4 BAP. Osteocalcin was significantly higher at baseline in men than women ($F_{1,12} = 5.22$, $P = .0413$). Baseline osteocalcin was inversely correlated with immunoreactive PTH ($F_{1,12} = 5.21$, $P = .0415$). Treatment with $T_3$ had no effect on osteocalcin. Analysis of variance showed no effect of time on osteocalcin; but matched-pair analysis showed baseline osteocalcin to be significantly lower than values in weeks 1, 2, and 3 ($P < .02$) but not week 4 (Fig. 5).

## 4. Discussion

It has been recognized for over 50 years that hyperthyroidism increases bone turnover and results in loss of bone mass [3-6,25-28]. Immobilization also affects bone resorption and calcium turnover, and results in loss of bone mass [4,10,11,13-15]. We hypothesized that small doses of $T_3$ would accelerate the changes in skeletal metabolism during immobilization that might serve as a model to test countermeasures that could be applied to space flight The purpose of this study was to determine the acute skeletal response to 28 days of bed rest in the presence or absence of pharmacologic doses of $T_3$.

Thyrotropin levels fell rapidly and $T_4$ levels fell gradually over the course of the study in the $T_3$-treated subjects and

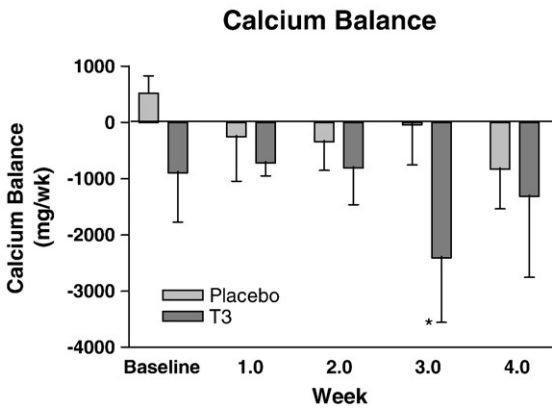


Fig. 3. Time course for the effect of $T_3$ and bed rest on calcium balance. The period numbers refer to the food pools, which were 5 days in duration. There was an overall borderline-significant effect of $T_3$ ($P = .586$) and at week 3 (*$P = .534$).

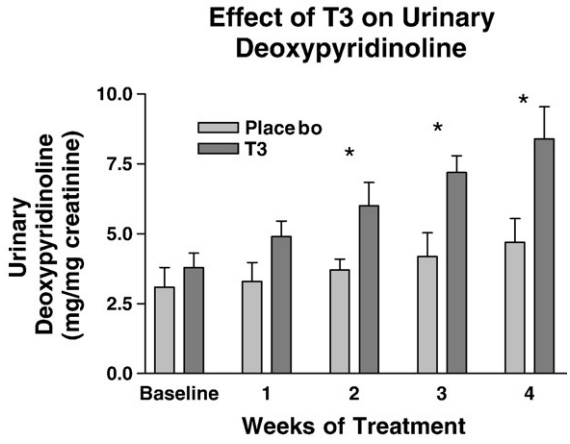


Fig. 4. Time course for the effect of $T_3$ and bed rest on the urinary excretion of DPD. The DPD to creatinine ratio was measured from a 24-hour urine collection. Data are mean ± SEM. Deoxypyridinoline rose significantly with time ($P = .0021$) and treatment with $T_3$ ($P < .0001$).

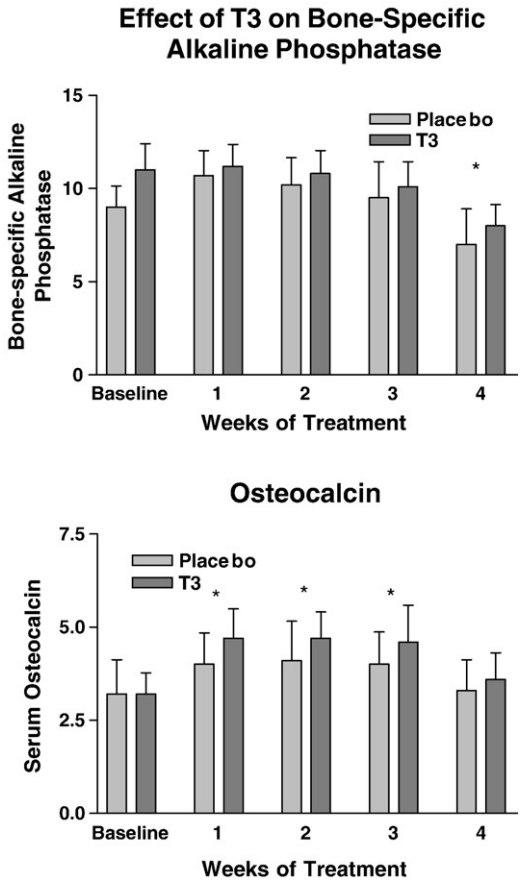


Fig. 5. Time course for the effect of $T_3$ and bed rest on BAP and osteocalcin. The top panel shows the BAP data, and the lower panel shows the osteocalcin data. Data are mean ± SEM. Differences from baseline ($P < .02$) are noted by "*."

reached a steady state at 2 to 4 weeks. The level of "nonsuppressible" $T_4$ concentration probably represents continued release of $T_4$ from the thyroid gland. All participants were asymptomatic in this study; but in an earlier ambulatory study, 1 subject displayed "nervousness" by pacing around the inpatient unit [20]. Bed rest was associated with no significant difference in measured thyroid hormone levels.

Triiodothyronine treatment resulted in a prompt increase in bone resorption, as evidenced by an increase in urinary excretion of DPD within the first 7 days of treatment. In comparison with the subjects in the placebo group, who were also at bed rest, this marker showed a greater increase during treatment with $T_3$. The rise of DPD in the placebo group in our study was slower than previously reported by Leuken and colleagues [11]. Previous studies of patients with hyperthyroidism have demonstrated increases in osteocalcin [5,6] that we did not find. Because DPD was the only marker showing a response, it would have been good to have other markers. The lack of these other markers for bone resorption is a weakness of our study, and the results must be interpreted cautiously.

In response to bed rest, blood calcium levels were unchanged. Immunoreactive PTH levels decreased in both groups with bed rest [29-31], and there was no effect of $T_3$. In other bed rest studies, immunoreactive PTH has been reported to decrease [9,12,14], as it did in this one, or to remain unchanged [10,13]. Blood levels of BAP and osteocalcin, markers of bone formation, showed similar values during bed rest whether they were treated with $T_3$ or placebo. Alkaline phosphatase was stable for the first 3 weeks, whereas osteocalcin increased slightly. Both appeared to decrease below baseline by the fourth week.

Fecal calcium showed a borderline-significant increase during treatment with $T_3$. This resulted in a small net negative calcium balance in the bed rest group and a borderline-significant negative calcium balance in those treated with $T_3$. Although not measured here, intestinal calcium absorption has been reported to be reduced during bed rest [10,13].

Deoxypyridinoline, a marker of bone resorption, was the only bone marker that showed a greater response to $T_3$ than to bed rest alone. Beginning in the second week, DPD significantly increased and continued to increase week by week for the 4 weeks of the study. The increase in those subjects treated with $T_3$ was significantly greater than that in the bed rest group. The increased rate of bone resorption is in harmony with that seen in patients with hyperthyroidism and in a longer study of healthy subjects [21] and in subjects at bed rest or in space flight [10,11,13,15]. These data are also consistent with earlier reports of decreased gut calcium absorption [32-36] and increased calcium excretion in hyperthyroid patients at steady state [5,6]. The change in immunoreactive PTH correlated with serum calcium, but not with the change in urinary calcium or fecal calcium.

Osteocalcin, a marker of bone turnover, rose slightly in the bed rest groups with and without $T_3$. Thyroid hormone has been demonstrated to increase osteocalcin in hyperthyroidism [4-6] and directly in cultured osteoblasts [37]. Classically, osteocalcin is associated with bone turnover by the osteoblast and has been demonstrated to correlate with osteoblast number and activity [38]. During bed rest, osteocalcin was reported to rise in 1 study [11] but was unchanged in most studies [10,13,14]. These clinical studies were performed at steady state. Our studies were performed under non–steady-state conditions. Osteocalcin increased with time but not with $T_3$ treatment. Bone-specific alkaline phosphatase level was significantly lower at 4 weeks, but was not affected by $T_3$ treatment.

How do these changes compare with those seen in immobilization and space flight? Immobilization results in increased bone resorption; normal or only slightly decreased PTH; decreased 1,25-$(OH)_2$ vitamin D; unchanged 25-OH vitamin D; increased urinary calcium; decreased intestinal calcium absorption; and increased fecal calcium loss. Bone mineral density is decreased to the greatest extent in the calcaneus and femur, with a lesser loss of bone in the spine. Similar changes occur in space flight [39], although after long-duration space flight, the rise in the biochemical markers of bone turnover appears to be attenuated. Calvarial bone mass may actually increase. These alterations are

similar in both magnitude and direction to our observations and previous observations of hyperthyroidism [4].

The similarities in the pathophysiology of immobilization, spaceflight, and mild hyperthyroidism can be summarized as follows: decreased mechanical stimulation, or $T_3$, activates osteoclasts, which increase bone resorption and may lower immunoreactive PTH and 1,25-$(OH)_2$ vitamin D. Urinary and fecal calcium excretion increases, and net calcium balance is negative. Bone formation is unchanged.

We conclude that the changes we observed in response to $T_3$ treatment in the bed rest model are similar in direction and of greater magnitude than those observed in bed rest. They are also similar in direction to those of space flight and immobilization, as shown by Smith et al [13], and by the comparison with the group at bed rest alone. We consider the addition of $T_3$ to the bed rest model a mechanism that more efficiently mimics the effect of space flight on bone and mineral metabolism.

## Acknowledgment

We thank Ms Susan Mancuso and the nursing staff for their attention to detail in this study. Peter Wickersham and Anthony Alfonso were very helpful with the initial statistical analyses. We also want to thank the participants who volunteered to spend time at bed rest in the metabolic unit.

## References

[1] Gallagher JC. Estrogen: prevention and treatment of osteoporosis. In: Marcus R, Feldman D, Kelsey J, editors. Osteoporosis. San Diego (Calif): Academic Press, Inc.; 1996. p. 1191-208.
[2] Aub JC, Bauer W, Heath C, Ropes M. Studies of calcium and phosphorus metabolism III. The effects of the thyroid hormone and thyroid disease. J Clin Invest 1929;7:97.
[3] Krane SM, Brownell GL, Stanbury JB, Corrigan H. The effect of thyroid disease on calcium metabolism in man. J Clin Invest 1956;35: 874-87.
[4] Garrel DR, Delmas PD, Malaval L, Tourniare J. Serum bone Gla protein: a marker of bone turnover in hyperthyroidism. J Clin Endocrinol Metab 1996;62:1052-5.
[5] Kisadol G, Kaya A, Gonen S, Runc R. Bone and calcium metabolism in subclinical autoimmune hyperthyroidism and hypothyroidism. Endocrinol Jpn 2003;50:657-61.
[6] Akalin A, Colak O, Alatas O, Efe B. Bone remodelling markers and serum cytokines in patients with hyperthyroidism. Clin Endocrinol 2002;57:125-9.
[7] Whalen RT, Carter DR, Steele CR. Influence of physical activity on the regulation of bone density. J Biomech 1988;21:825-37.
[8] Whedon GD. Disuse osteoporosis: physiological aspects. Calcif Tissue Int 1984;36:S146-50.
[9] Arnaud SB, Sherrard DJ, Maloney N, Whalen RT, Fung P. Effects of 1-week head-down tilt bed rest on bone formation and the calcium endocrine system. Aviat Space Environ Med 1992;63:14-20.
[10] LeBlanc A, Schneider V, Spector E, Evans H, Rowe R, Lane H, Demers L, Lipton A. Calcium absorption, endogenous excretion, and endocrine changes during and after long-term bed rest. Bone 1995;16: 301S-4S.
[11] Lueken SA, Arnaud SB, Taylor AK, Baylink DJ. Changes in markers of bone formation and resorption in a bed rest model of weightlessness. J Bone Miner Res 1993;12:1433-8.
[12] Scheld K, Zittermann A, Heer M, Herzog B, Mika C, Drummer C, Stehle P. Nitrogen metabolism and bone metabolism markers in healthy adults during 16 weeks of bed rest. Clin Chem 2001;47: 1688-95.
[13] Smith SM, Wastney ME, O'Brien KO, Morukov BV, Larina IM, Abrams SA, Davis, Stret JE, Oganov V, Shackelford L. Bone markers, calcium metabolism, and calcium kinetics during extended-duration space flight on the Mir space station. J Bone Miner Res 2005;20: 208-18.
[14] Smith SE, Heer M. Calcium and bone metabolism during space flight. Nutrition 2002;18:849-52.
[15] Zerwekh JE, Ruml LA, Gottschalk F, Pak CYC. The effects of twelve weeks of bed rest on bone histology, biochemical markers of bone turnover, and calcium homeostasis in eleven normal subjects. J Bone Miner Res 1998;13:1594-601.
[16] Giangregorio L, Blimkie CJ. Skeletal adaptations to alterations in weight-bearing activity: a comparison of models of disuse osteoporosis. Sports Med 2002;32:459-76.
[17] Mosekilde L, Eriksen E, Charles P. Effects of thyroid hormones on bone and mineral metabolism. Endocrinol Metab Clin 1990;19: 35-57.
[18] Lovejoy JC, Smith SR, Bray GA, et al. A paradigm of experimentally induced mild hyperthyroidism: effects on nitrogen balance, body composition, and energy expenditure in healthy young men. J Clin Endocrinol Metab 1997;82:765-70.
[19] Lovejoy JC, Smith SR, Bray GA, Veldhuis JD, Rood JC, Tulley R. Effects of experimentally induced mild hyperthyroidism on growth hormone and insulin secretion and sex steroid levels in healthy young men. Metabolism 1997;46:1424-8.
[20] Lovejoy JC, Smith SR, Zachwieja JJ, Bray GA, Most-Windhauser MM, Wickersham PJ, Rood J, Tulley R De La Bretonne JA. Low-dose T3 improves the bed rest model of simulated weightlessness in men and women. Am J Physiol 1999;277(Endocrinol Metabol 40): E370-9.
[21] Smith SR, Lovejoy JC, Roos J, Most M, Wickersham PJ, Volaufova J, Ryan D, Tulley R, Bray GA. The effects of triiodothyronine on bone metabolism in healthy ambulatory men. Thyroid 2003;13: 357-64.
[22] Charles P, Jensen FT, Mosekilde L, Hansen HH. Calcium metabolism evaluated by Ca-kinetics: estimation of dermal calcium loss. Clin Sci 1983;65:415-22.
[23] Jensen FT, Charles P, Mosekilde L, Hansen HH. Calcium metabolism evaluated by calcium-kinetics: a physiological model with correction for faecal lag time and estimation of dermal calcium loss. Clin Physiol 1983;3:187-204.
[24] Schneider V, McDonald J. Skeletal calcium homeostasis and countermeasures to prevent disuse osteoporosis. Calcif Tissue Int 1984;36 (Suppl 1):S151-4.
[25] Aurbach GD, Marx SJ, Spiegel AM. Parathyroid hormone, calcitonin, and the calciferols. In: Wilson JD, Foster DW, editors. Williams textbook of endocrinology. Philadelphia: W.B. Saunders Co.; 1992. p. 1397-476.
[26] Suwanwalaikorn S, Baran D. Thyroid hormone and the skeleton. In: Marcus R, Feldman D, Kelsey J, editors. Osteoporosis. San Diego: Academic Press; 1996. p. 855-61.
[27] Mundy GR, Shapiro JL, Bandelin JG, Canalis EM, Raisz LG. Direct stimulation of bone resorption by thyroid hormones. J Clin Invest 1976;58(3):529-34.
[28] Manicourt D, Demeester-Mirkine N, Brauman H, Corvilain J. Disturbed mineral metabolism in hyperthyroidism: good correlation with tri-iodothyronine. Clin Endocrinol 1979;10:407-12.
[29] Mosekilde L, Christensen MS. Decreased parathyroid function in hyperthyroidism: interrelationships between serum parathyroid hormone, calcium-phosphorus metabolism and thyroid function. Acta Endocrinol 1977;84:566-75.
[30] Bouillon R, DeMoor P. Parathyroid function in patients with hyper- or hypothyroidism. J Clin Endocrinol Metab 1974;38:999.

[31] Bouillon R, Muls E, DeMoor P. Influence of thyroid function on the serum concentration of 1,25-dihydroxyvitamin $D_3$. J Clin Endocrinol Metab 1980;51:793-7.
[32] Mindroiu TH, Esanu C. The intestinal calcium absorption in thyroid dysfunctions. Roum Med 1974;12:119-26.
[33] Shafer RB, Gregory DH. Calcium malabsorption in hyperthyroidism. Gastroenterology 1972;63:235-9.
[34] Bordier P, Miravet L, Matrajt H, Hioco D, Ryckewaert A. Bone changes in adult patients with abnormal thyroid function (with special reference to Ca-kinetics and quantitative histology). Proc R Soc Med 1967;60:26-8.
[35] Singhelakis P, Alevizaki CC, Ikkos DG. Intestinal calcium absorption in hyperthyroidism. Metabolism 1974;23:311-21.
[36] Haldimann B, Kaptein EM, Singer FR, Nicoloff JT, Massry SG. Intestinal calcium absorption in patients with hyperthyroidism. J Clin Endocrinol Metab 1980;51:995-7.
[37] Varga F, Rumpler M, Luegmayr E, Frantzl-Zelman N, Glantschnig H, Klaushofer K. Triiodothyronine, a regulator of osteoblastic differentiation: depression of histone H4, attenuation of c-fos/c-jun, and induction of osteocalcin expression. Calcif Tissue Int 1997;61(5):404-11.
[38] Charles P, Poser JW, Mosekilde L, Jensen FT. Estimation of bone turnover evaluated by Ca-kinetics. J Clin Invest 1985;76:2254-8.
[39] Caillot-Augusseau A, Lafage-Proust MH, Soler C, Pernod J, Duois F, Alexandre C. Bone formation and resorption biological markers in cosmonauts during and after a 180-day space flight (Euromir 95). Clin Chem 1998;44:578-85.