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ORIGINAL ARTICLE

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Short-term effects of glucocorticoid therapy on biochemical markers of bone metabolism in Japanese patients: a prospective study

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Abstract Glucocorticoid (GC) therapy induces rapid bone loss, but the early changes in calcium and bone metabolism in patients treated with GC have not been clarified. To investigate the changes in calcium and bone metabolism during the early stage of GC therapy, we analyzed various biochemical markers of bone metabolism. The serum levels of calcium (Ca), phosphorus, parathyroid hormone (PTH), osteocalcin (OC), bone alkaline phosphatase (BAP), and type I collagen cross-linked N-telopeptide (NTx), as well as the urinary levels of Ca, creatinine, and NTx, were measured on days 0, 3, 7, and 28 of GC therapy. The subjects were divided into the following four groups: 9 patients receiving pulse therapy (P), 18 patients receiving prednisolone (PSL) at doses ≥40 mg/day (H), 9 patients receiving PSL at doses ≥20 mg/day (M), and 11 patients receiving PSL at doses ≤10 mg/day (S). The serum OC level showed a marked decrease on day 3 of GC therapy (–41.2% ± 6.6%, $P < 0.01$), while the BAP level decreased gradually. Both serum and urinary NTx levels significantly increased on day 7 of GC therapy (9.9% ± 4.5%, $P < 0.05$, and 42.2% ± 10.6%, $P < 0.01$, respectively). Urinary Ca excretion was increased on day 3 of GC therapy and continued to increase until 4 weeks, while intact PTH showed an increase on day 3 and then remained constant until 4 weeks. In groups P and H, there were significant early changes in OC, BAP, NTx, and intact PTH levels, as well as urinary Ca excretion. Even a PSL dose of <10 mg/day caused a decrease in the serum OC level. In conclusion, the biochemical markers of Ca and bone metabolism showed different kinetics depending on the dose of GC, and it is important for patients on high-dose GC therapy to receive prophylaxis for bone loss from the start of GC treatment.

Key words glucocorticoid therapy · biochemical markers of bone metabolism · short-term effects · prospective study · glucocorticoid-induced osteoporosis · initial glucocorticoid dose

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Introduction

Glucocorticoids (GC) are used to treat a wide variety of allergic and inflammatory diseases [1]. Because GC affect almost all aspects of human metabolism, these drugs have a number of side effects such as impaired glucose tolerance [2], hyperlipidemia, and bone loss. GC induce secondary osteoporosis that is characterized by rapid bone loss and an increased risk of fracture [3]. The mechanism of GC-induced osteoporosis involves decreased bone formation, increased bone resorption, reduced intestinal calcium (Ca) absorption, reduced renal Ca reabsorption, increased parathyroid hormone (PTH) secretion, and sex hormone deficiency. Among these factors, the effect of GC on bone formation appears to be the most important [4], but the exact mechanism has not been fully clarified.

GC rapidly induce bone loss, but most previous clinical studies were performed in patients on long-term therapy. Thus, the early changes in Ca and bone metabolism as well as the cause of rapid bone loss remain unclear. Moreover, the relationship between changes in bone metabolism and the dose of GC has not been sufficiently investigated. Pred-

nisolone (PSL) is administered at daily doses of 5–30 mg for antiinflammatory therapy, whereas higher doses of 40–60 mg are required for immunosuppression [1,5]. In patients with rheumatoid arthritis, only 3 mg PSL daily is often enough to improve symptoms [6], although patients with collagen diseases sometimes require up to 1 g/day methylprednisolone as pulse therapy [7]. Therefore, GC therapy is started over a wide dose range from 1 mg to 1 g PSL, but the influence of the initial GC dose on calcium and bone metabolism remains unclear.

Biochemical markers of bone metabolism are useful for measuring bone turnover and assessing fracture risk [8–10]. Although biochemical markers may be useful for predicting future changes in bone mineral density (BMD) [11], there have been few longitudinal studies on the changes in biochemical markers from the beginning of GC therapy. To clarify the changes in Ca and bone metabolism during the early stage of GC therapy, the present study was performed to analyze biochemical markers of bone metabolism, PTH levels, and urinary Ca excretion on days 0, 3, 7, 14, and 28 of treatment in patients starting GC therapy. Then, we analyzed the relationship between the changes in these parameters and the initial dose of PSL.

Patients and methods

Patients and study protocol

Forty-seven patients (20 men and 27 women with a mean age of 58.1 ± 16.7 years) who were scheduled to start GC therapy were enrolled in this study. Fasting morning blood and urine samples were collected from the patients before they started treatment and on days 3, 7, 14, and 28 of PSL therapy. The serum levels of albumin, Ca, phosphorus, and creatinine (Cr), as well as urinary Ca and Cr, were measured on each day. Biochemical markers of bone metabolism were also measured on the indicated days. Ca supplements were administered to all patients, but no other drugs that could influence bone metabolism were taken. The subjects were divided into the following four groups: 9 patients receiving pulse therapy (P), 18 patients receiving PSL at doses ≥40 mg/day (H), 9 patients receiving PSL at doses ≥20 mg/day and <40 mg/day (M), and 11 patients receiving PSL at doses ≤10 mg/day (S). The gender ratio and menopausal status were 20 men and 11 premenopausal and 15 postmenopausal women. The underlying diseases were classified as collagen diseases ($n = 36$), hematopoietic diseases ($n = 6$), and neuroimmune diseases ($n = 5$). All 47 patients included in this study gave written informed consent for participation, and the study protocol was approved by the Institutional Review Board of each hospital.

Biochemical markers

As bone formation markers, serum osteocalcin (OC) and bone alkaline phosphatase (BAP) were measured. OC was determined in an immunoradiometric assay (BGP IRMA Mitsubishi, Tokyo, Japan) and BAP was also measured in an enzyme immunoassay (Osteolinks BAP, Quidel San Diego, CA, USA) [12]. As bone resorption markers, both the serum and urinary levels of type I collagen cross-linked N-telopeptide (NTx) were measured in an enzyme immunoassay (Osteomark NTX, Inverness, Princeton, NJ, USA) [13]. Intact PTH was measured in an electrochemiluminescent immunoassay (Eclusys PTH, Roche Diagnostics, Tokyo, Japan) [14]. The serum levels of OC, BAP, NTx, and intact PTH were measured on days 0, 3, 7, and 28, and the urinary NTx levels were measured on days 0, 7, and 28 of GC therapy.

BMD measurements

BMD values were measured by dual-energy X-ray absorptiometry using QDR-2000, QDR-4500SL, DELPHIQDR-4500Ck, or XR-30 (Hologic, Waltham, MA, USA) at the lumbar spine (L2–L4). BMD was automatically calculated from the bone area (cm^2) and bone mineral content (BMC) (g), and expressed absolutely in g/cm^2. The T-score is the number of SD by which a given measurement differs from the mean for a normal young adult reference population.

Statistical analysis

Statistical analysis of data was performed with the StatView ver. 6.0 software package (SAS Institute, Cary, NC, USA). The unpaired Student's t test was used to compare differences in patient profiles among groups. Values are expressed as mean ± SD. Assessment of the changes in bone metabolism markers during GC therapy was performed using the nonparametric Student's t test. Results are presented as mean ± SEM, and $P < 0.05$ was considered to indicate a statistically significant difference. The figures show data expressed as a percentage of the baseline value.

Results

Patient profiles

The clinical characteristics and the baseline values of Ca and bone metabolism markers are shown for each group in Table 1. The basal OC levels of groups H and M were significantly lower than that of group S, but the other baseline Ca and bone metabolism markers showed no significant differences among the four groups. The total PSL dose received over 28 days was 4669 ± 1851 mg in group P, 1323 ± 357 mg in group H, 604 ± 189 mg in group M, and 204 ± 66 mg in group S.

Short-term changes in bone formation markers

The serum OC level markedly decreased on day 3 of PSL administration (–41.7% ± 6.6%, $P < 0.01$) but then returned to baseline by day 28 whereas the serum BAP level

Table 1. Patient profiles

Initial dose of PSL Group Group No. of patients	Pulse therapy (P) 9	≧40 mg (H) 18	≧20 mg (M) 9	≦10 mg (S) 11
Age	55.8 ± 19.9	59.7 ± 15.1	60.7 ± 17.5	55.4 ± 17.7
M:F (postmenopausal)	1:8 (5)	11:7 (4)	3:6 (4)	5:6 (3)
Collagen diseases	6	10	9	11
Hematological diseases	3	3	0	0
Neuroimmune diseases	0	5	0	0
PSL dose (mg)				
3 days	2607 ± 92	136 ± 68	78 ± 27	23 ± 7
7 days	2831 ± 671	318 ± 132	175 ± 49	53 ± 11
28 days	4669 ± 1851	1323 ± 357	604 ± 189	204 ± 66
OC (ng/ml)	5.1 ± 3.6	3.6 ± 2.3**	4.1 ± 1.8*	6.8 ± 2.3
BAP (U/l)	29.75 ± 17.2	24.6 ± 13.6	19.8 ± 4.7	23.4 ± 7.9
sNTx (nmol/l)	23.6 ± 13.5	17.1 ± 6.4	18.1 ± 6.1	16.2 ± 6.4
uNTx (nm/mmcr)	74.0 ± 29.7	62.7 ± 63.2	73.1 ± 46.3	55.5 ± 36.9
Intact PTH (pg/ml)	42.6 ± 36.2	38.0 ± 22.8	28.8 ± 9.2	37.0 ± 10.3
Corrected sCa (mg/dl)	9.2 ± 0.4	9.1 ± 0.4	9.2 ± 0.6	9.2 ± 0.3
uCa/Cr	0.10 ± 0.12	0.12 ± 0.11	0.13 ± 0.13	0.13 ± 0.08
BMD (L2–L4) (T-score)	–1.3 ± 1.6	–0.32 ± 1.0	–0.99 ± 1.8	–0.89 ± 1.0

Values are expressed as the means ± SD
PSL, prednisolone; OC, osteocalci; BAP, bone alkaline phosphatase; NTx, type I cross-linked N-teleopeptide; s, seruni; u, urrary; PTH, parathyroid hormone; BMD, bone mineral density
** $P < 0.01$, * $P < 0.05$, compared with group S

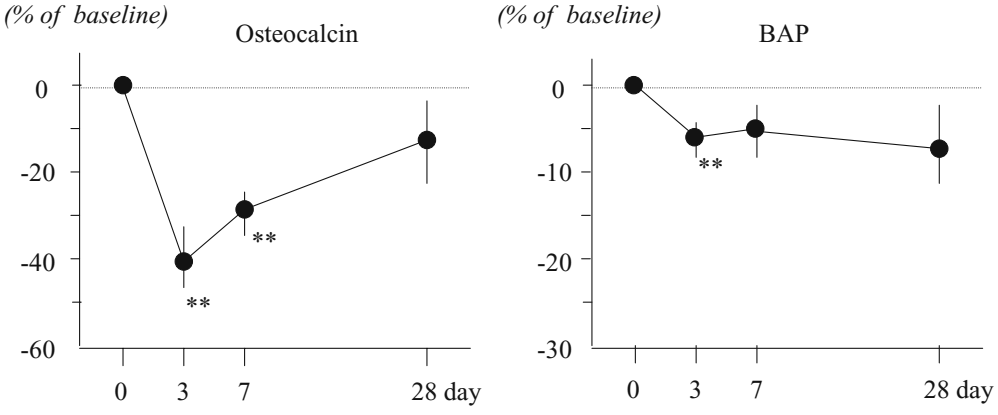

Fig. 1. Short-term effects of glucocorticoid (GC) therapy on bone formation markers. The serum osteocalcin (OC) level showed an immediate decrease on day 3 of prednisolone (PSL) administration, while the serum bone alkaline phosphatase (*BAP*) level decreased gradually over 28 days. *$P < 0.05$, **$P < 0.01$

decreased gradually over 1 month (Fig. 1). Analysis of differences among groups showed that the serum OC level significantly decreased soon after the start of GC therapy in all groups, but the decrease in BAP was less marked and was only significant in group P. In the patients who received pulse therapy, the serum OC level was significantly lower than that in the other groups on day 3 but recovered to a level similar to that in groups H and M by day 7 (Fig. 2).

Short-term changes in bone resorption markers

Both serum and urinary NTx levels showed significant increases on day 7 of 9.9% ± 4.5% ($P < 0.05$) and 42.2% ± 10.6% ($P < 0.01$), respectively (Fig. 3). Analysis of the differences among groups showed that the serum NTx levels increased on day 7 in group P by 26.2% ± 11.5% ($P = 0.053$) and in group H by 12.5% ± 4.9% ($P < 0.05$). The urinary NTx levels significantly increased on day 7 (56.8 ± 17.4, $P < 0.01$) and remained high until day 28 (38.9 ± 17.2, $P < 0.05$) in group H, whereas in groups P, M, and S, it had a tendency to increase but this effect was not significant (Fig. 4). Dose dependency was not apparent in serum and urinary NTx levels.

Short-term changes in Ca and intact PTH

The serum Ca level did not change markedly during GC therapy, but showed a significant increase on day 14 (1.9% ± 0.7%, $P < 0.05$). Urinary Ca excretion increased on day 3 (81.9% ± 35.7%, $P < 0.05$) and continued to increase until 4 weeks of GC therapy (Fig. 5). The extent of the increase in urinary Ca excretion appeared to be dose dependent (Fig. 6). PTH also showed an increase on day 3 (19.3% ± 7.6%, $P < 0.05$), and the level remained constant until 4 weeks (Fig. 5). In the patients receiving pulse therapy, the PTH level significantly increased on day 7 (46.0% ± 16.9%, $P < 0.05$), but then returned to baseline by day 28. In group H, the PTH level significantly increased on day 3 and then continued to increase until day 28. In both groups M and S, there were no significant changes in intact PTH (Fig. 6).

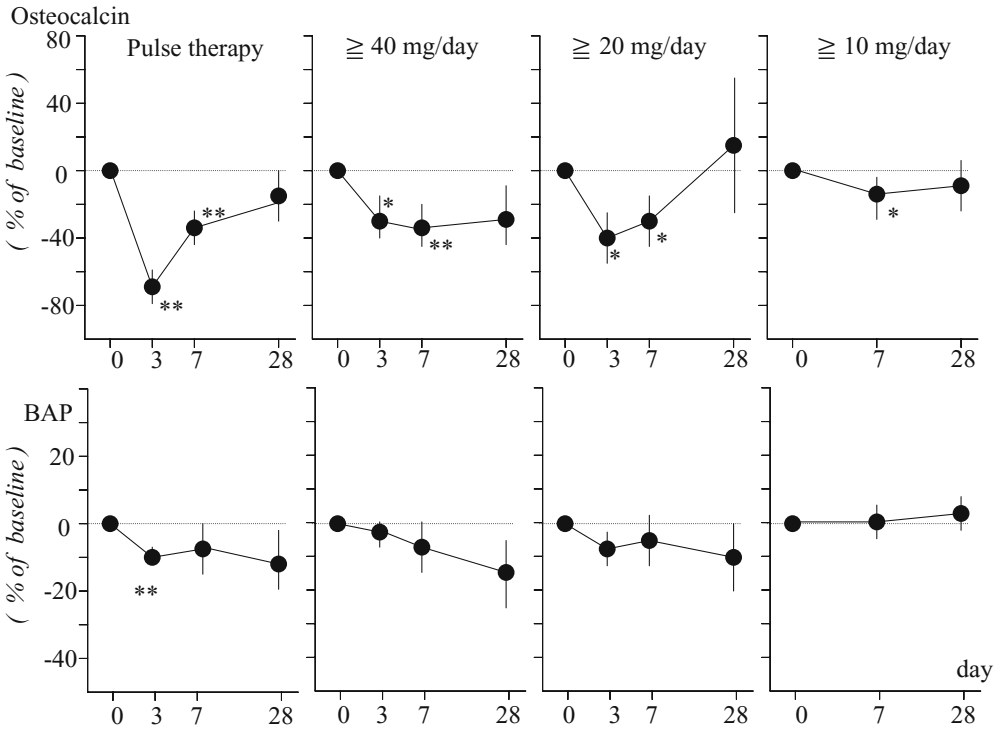

Fig. 2. Effects of various GC regimens on bone formation markers. The serum OC levels showed early and significant decreases in all groups, but the decrease in BAP was less and was only significant in group P. $*P < 0.05$, $**P < 0.01$

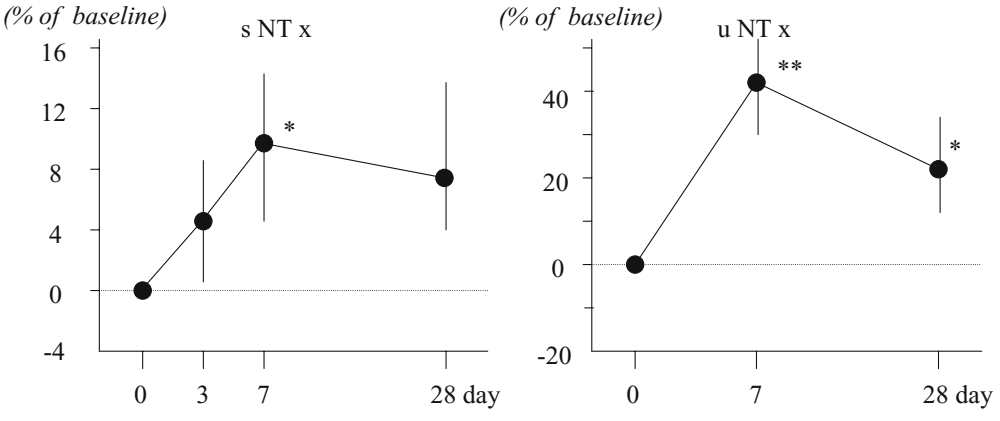

Fig. 3. Short-term effects of GC therapy on bone resorption markers. Both the serum and urinary type I collagen cross-linked N-telopeptide (NTx) levels (*sNTX*, serum NTX; *uNTx*, urinary NTx) were significantly increased on day 7 of PSL administration. $*P < 0.05$, $**P < 0.01$

Changes in bone metabolic indices in men and pre- and postmenopausal women

The changes in serum OC, PTH, and urinary levels of NTx and Ca/Cr showed a similar tendency in men and in pre- and postmenopausal women (Fig. 7).

Discussion

GC-induced osteoporosis is characterized by decreased bone formation and a relative increase in bone resorption. GC inhibits bone formation by suppressing osteoblastogenesis, as well as by promoting the apoptosis of both osteoblasts and osteocytes [4]. The present study revealed that bone formation markers were significantly decreased from the start of GC therapy, which was consistent with the results of previous studies [15–18]. In particular, the serum OC level showed a marked and rapid decrease compared with BAP and was also significantly decreased even in the group receiving <10 mg/day PSL. This difference in the profiles of these two markers can be explained by differences in their gene regulation and serum half-life. The OC gene has a GC response element (GRE) in the promoter region and thus is directly regulated by GC [18,19], whereas the serum half-life of OC is very short (about 5 min). In contrast, no GRE has been found in the BAP gene; therefore, BAP may be regulated indirectly by GC, and it also has a much longer half-life (about 3.5 days) than that of OC [20]. Moreover, OC and BAP reflect different steps of osteoblastogenesis. Because BAP reflects an early stage of this process and OC is produced by mature osteoblasts, it could be speculated that GC therapy specifically inhibits the later steps

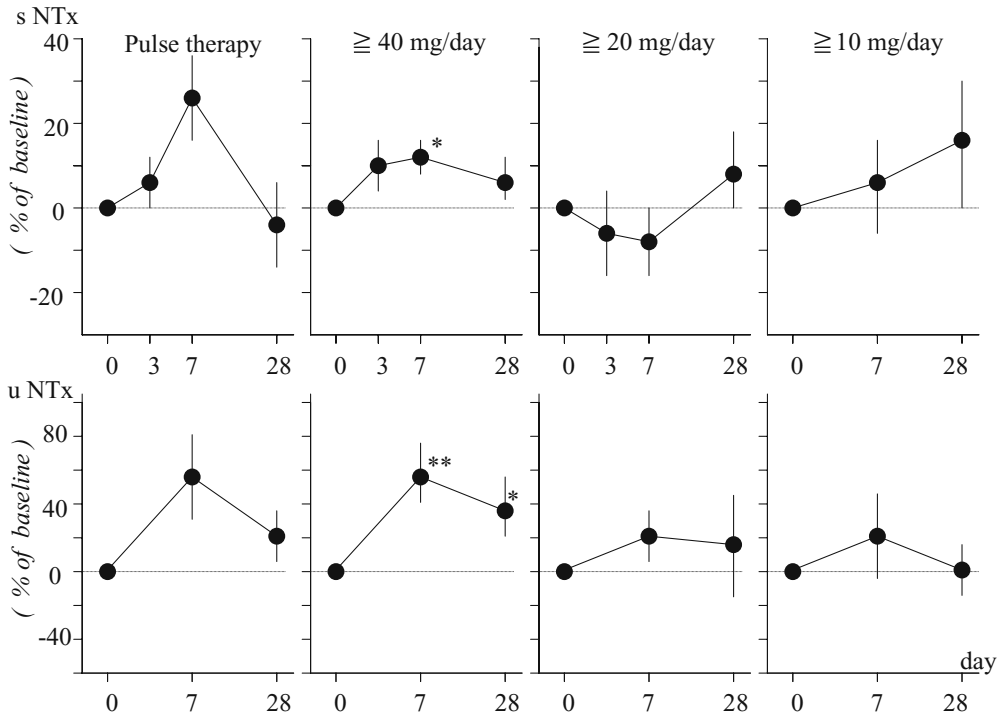

Fig. 4. Effects of various GC regimens on bone resorption markers. In group H, the serum (*sNTx*) and urinary (*uNTx*) NTx levels were significantly increased on day 7, and the urinary NTx levels remained high until day 28. $*P < 0.05$, $**P < 0.01$

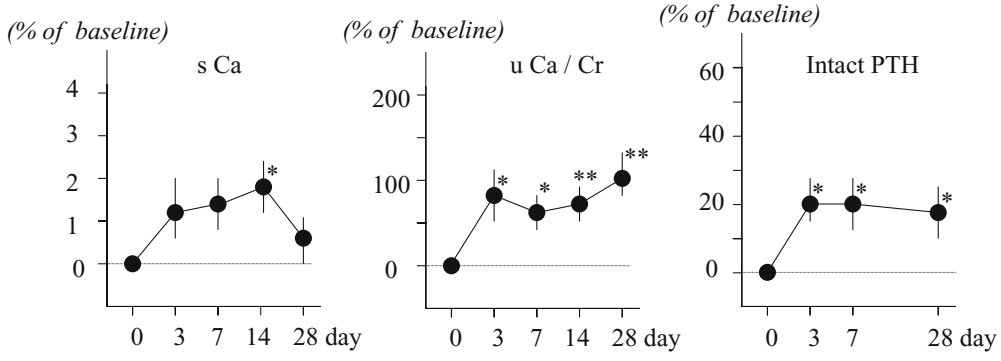

Fig. 5. Short-term effects of GC therapy on serum Ca (*sCa*), urinary Ca/creatinine (*uCa/Cr*), and intact parathyroid hormone (*PTH*). The serum Ca level (corrected for serum albumin) was not decreased, but instead was increased on day 14. Urinary Ca excretion was increased on day 3 and continued to increase until day 28. Intact PTH also showed an increase on day 3 and then remained constant until day 28. $*P < 0.05$, $**P < 0.01$

in the process of osteoblastogenesis, leading to impaired bone formation.

The present study showed that bone resorption markers (serum and urinary NTx) were also significantly increased soon after the start of GC therapy. The mechanisms accounting for the early increase in bone resorption are still unclear, but there have been reports of a direct effect on osteoclast activity and bone resorptive factors. Weinstein et al. reported that high-dose GC therapy inhibited apoptosis and prolonged the survival of osteoclasts [21]. Histomorphometric examination of vertebral cancellous bone showed an increase in osteoclasts after 7 days of GC therapy in mice [22]. These findings may be consistent with our present findings that high-dose GC therapy increased the levels of bone resorption markers on day 7. Increases in serum and urinary NTx levels in group P were not significant. The reason may be the small number of individuals or greater variability of bone response to high-dose GC in pulse therapy.

Although PTH does not appear to be primarily responsible for the pathogenesis of GC-induced osteoporosis [23–25], the present study revealed that high-dose GC therapy caused early elevation of the serum intact PTH level. Because high-dose GC therapy also increased the levels of bone resorption markers, GC may increase bone resorption by inducing PTH secretion. Secondary hyperparathyroidism can arise in response to diminished intestinal Ca absorption and increased urinary Ca excretion and is often cited as contributing to increased bone resorption in patients with GC-induced osteoporosis. However, serum Ca levels were not decreased but rather increased by GC therapy in the present study. Therefore, PTH might be responsible for the changes in bone metabolism, resulting in early bone loss during high-dose GC therapy, although the role of PTH appears to be less important in the chronic phase of GC-induced osteoporosis. PTH levels were decreased on day 28 in group P but not in group H. The reason for this was not

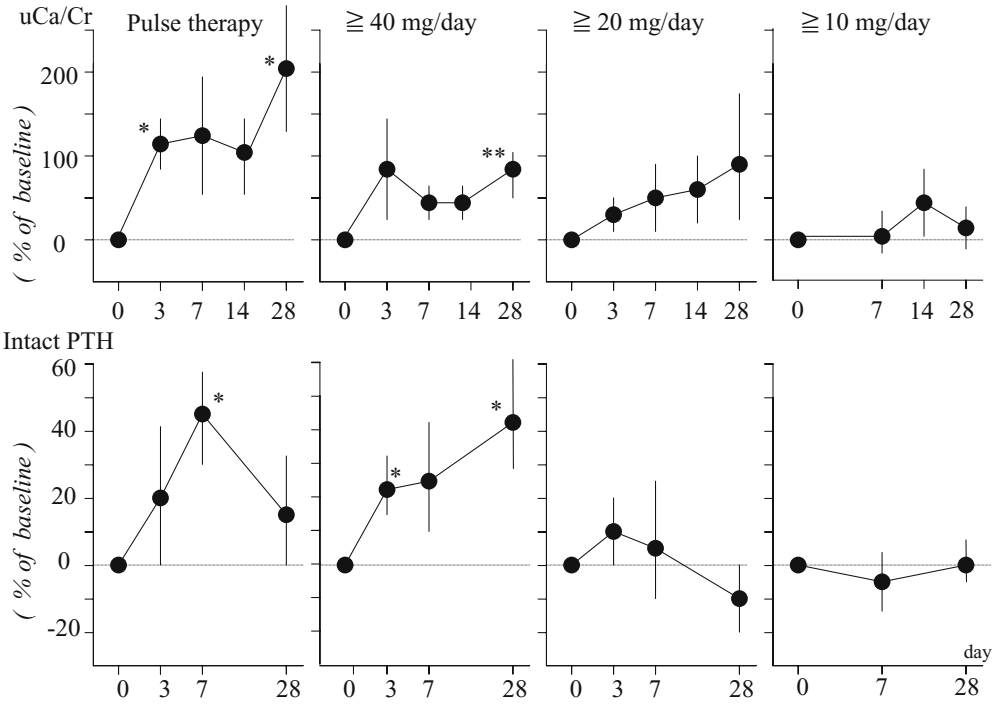

Fig. 6. Effects of various GC regimens on urinary Ca (*uCa*) excretion and *intact PTH*. Urinary Ca excretion continued to increase until day 28 in a dose-dependent manner. In group P, intact PTH was significantly increased on day 7, but then returned to baseline. In group H, PTH level significantly increased on day 3 and then continued to increase until day 28. In groups M and S, there were no significant changes. $^{*}P < 0.05$, $^{**}P < 0.01$

clear in the present study. Because increased urinary calcium excretion with higher-dose GC may be caused by calcium release into the blood from bone with enhanced bone resorption, a continued positive balance of blood calcium might suppress PTH secretion from the parathyroid in a negative-feedback mechanism more potently after pulse GC treatment. In addition, the peak of PTH level was higher on day 7 in group P compared with that in group H. We previously reported that GC directly stimulates PTH secretion from parathyroid cells in bovine parathyroid cell cultures [26]. Because the GC dose was higher in group P during the first 7 days, this higher peak of PTH level may be the result of the direct action of high-dose GC on parathyroid glands.

Previous studies revealed that bone formation markers were decreased transiently and soon recovered in patients receiving steroid pulse therapy [27,28]. However, taking these findings together, we can speculate that the increased bone resorption caused by GC therapy leads to a high bone turnover state that results in the early recovery of bone formation markers. This state leads to bone loss, and GC therapy also causes impaired bone formation, thus producing the characteristic clinical features of early bone loss and bone fragility in patients with GC-induced osteoporosis.

Chaki et al. reported that the initial NTx level was related to the lumbar spine BMD over the subsequent 3 years in postmenopausal women, although the relationship was stronger over a shorter period [29]. Christiansen et al. also reported that the initial levels of bone metabolism markers showed a stronger relation to the decrease in BMD in patients with rapid bone loss [11]. Furthermore, Iki et al. reported that urinary levels of bone resorption markers and serum OC had a strong relationship with the changes in BMD in healthy Japanese women [30]. As high-dose GC therapy causes rapid bone loss, the changes in bone metabolism markers in the early stage of treatment might predict the occurrence of rapid bone loss and subsequent fragility fracture.

Several studies [15–18,27,28] reported changes in bone metabolic indices after high-dose GC treatment or steroid pulse therapy. To the best of our knowledge, the present study is the first to compare the kinetics of PTH and bone metabolic markers in groups receiving different GC doses in a single study. Moreover, the present data first suggested that PTH is responsible for the changes in bone metabolism, which might result in early bone loss during high-dose, but not during low-dose, GC therapy, although a further, larger-scale study is necessary to obtain a concrete conclusion.

In the present study, the number of patients was insufficient to evaluate the differences among each of the four groups. Moreover, the gender ratio and underlying diseases of each group were not matched. Most of the patients in groups P and H were hospitalized, and therefore differences in the level of activity among the groups might have influenced the changes in bone metabolism. In patients with chronic inflammatory diseases such as rheumatoid arthritis or polymyalgia rheumatica, most of whom were in group S, GC therapy may actually inhibit bone resorption by its antiinflammatory effect. Although there were differences in the gender ratio and menopausal status among groups, the changes in bone metabolic indices showed a similar tendency in men, premenopausal women, and postmenopausal women. These findings suggest that the difference in gender or menopausal status did not markedly affect the early changes in bone metabolic indices after GC administration.

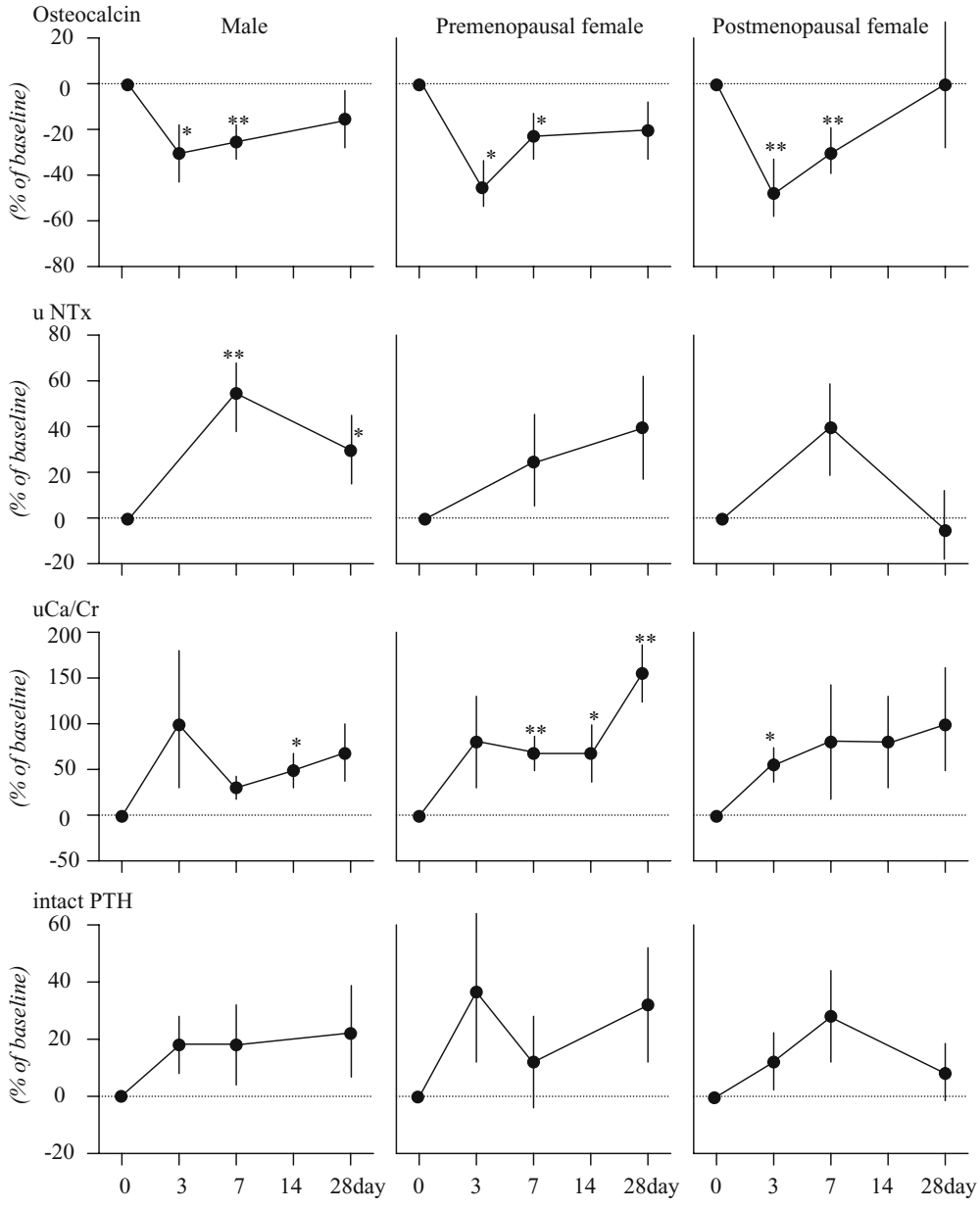

Fig. 7. Changes in bone metabolic indices in men and pre- and postmenopausal women. The serum OC levels showed an immediate decrease on day 3, and the urinary NTx levels showed an early increase in all groups. Urinary Ca excretion increased on day 3 and continued to increase until day 28 of GC therapy. PTH also showed an early increase in all groups. $*P < 0.05$, $**P < 0.01$

In our patients receiving steroid pulse therapy and high-dose PSL, there were significant early changes in bone formation markers, bone resorption markers, intact PTH, and urinary Ca excretion. In the patients receiving >20 mg/day PSL (group M), the serum OC level showed significant decreases and urinary Ca excretion tended to increase gradually, but serum BAP, serum NTx, urinary NTx, and serum PTH all showed no change. Even a PSL dose <10 mg/day (group S) caused a decrease in the serum OC level. Thus, the markers of bone metabolism investigated in this study showed different kinetics depending on the dose of PSL, and it is important for patients on high-dose GC therapy to receive prophylaxis for bone loss from the start of steroid treatment.

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