Acute effects of etidronate on glucocorticoid-induced bone degradation

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Abstract

Objectives. To study the acute short-term effects on the biochemical parameters of calcium and bone homeostasis in post-menopausal women treated with a high dose of prednisone alone or with additional etidronate, before and during 5 days of treatment.

Methods. Serum calcium, phosphorus, creatinine, alkaline phosphatase activity, osteocalcin, carboxy-terminal propeptide of type I procollagen (PICP), cross-linked carboxy-terminal telopeptide of type I collagen (ICTP), parathyroid hormone (PTH), 25-hydroxyvitamin D and urinary excretion of calcium over 24 h were measured before and during 5 days of treatment in 14 post-menopausal women treated with a high dose of prednisone (60 mg/day) alone (group A) or combined with cyclical etidronate (group B).

Results. Significant differences from baseline were found in osteocalcin and urinary excretion of calcium in both groups and for ICTP in group B. Significant differences between groups were calculated at day 5 of the study for osteocalcin, ICTP and 24 h urine calcium excretion ($P < 0.01$). Urinary excretion of calcium over 24 h increased in group A ($+14.7\%$; $P < 0.05$) and decreased in group B (-22.1%; $P < 0.01$). Osteocalcin levels decreased in group A (-38.1%) and increased in group B ($+27.4\%$; both $P < 0.01$). ICTP decreased only in group B (-19.4%; $P < 0.01$).

Conclusions. The results are consistent with the fact that etidronate is acutely able to prevent bone resorption due to corticosteroids. The increase in osteocalcin in the etidronate-treated group is a new feature. A direct or indirect (PTH, 1,25 vitamin D?) stimulatory effect of etidronate on the osteoblast cannot be excluded.

KEY WORDS: Prednisone, Corticosteroid, Glucocorticoid, Etidronate, Bisphosphonate, Osteoporosis, Calcium metabolism, Bone markers.

Osteoporosis and pathological fractures due to use of prednisone, in a daily dosage of more than 7.5 mg for periods longer than 6 months have been recognized for a long time [1–3]. However, the contribution of different pathophysiological mechanisms is still unclear. Supraphysiological doses of glucocorticosteroids given to animals and men, result in a negative calcium balance [4–7] which is associated with secondary hyperparathyroidism contributing to further calcium and bone loss [7–9]. Also direct effects of glucocorticosteroids on osteoblasts have been described [10–12]. Nowadays it

becomes clear that with the use of glucocorticosteroids for shorter or longer time periods [13], an uncoupling between bone resorption and formation occurs. Chronic use of glucocorticosteroids increases resorption and decreases formation, which leads to the development of osteopenia and fractures [2, 3, 14]. Different treatment modalities such as hormone replacement therapy, calcitonin, fluor and bisphosphonates have been used to prevent or treat low bone mass due to glucocorticosteroids [15–24]. We and others found a pronounced increase of bone mineral density (BMD) in patients with steroid-induced osteoporosis treated with etidronate or alendronate [21–24]. The results suggested an additional effect of bisphosphonates apart from decreased resorption alone. No data, however, have been published dealing with the acute effect of bisphosphonates on

glucocorticoid-induced changes in circulating bone markers. The objective of the current study was to determine the short-term effect of the bisphosphonate etidronate on glucocorticoid-induced changes in biochemical parameters of calcium and bone homeostasis in post-menopausal women with temporal arteritis treated with a high dosage of glucocorticosteroids.

Patients and methods

Study design

This prospective randomized open-label study was conducted in an out-patient clinic. Within a time period of 12 months 14 post-menopausal patients with histologically proven temporal arteritis were randomly allocated to the following treatments: high dose of prednisone (60 mg/daily) alone (group A) or high dose of prednisone (60 mg/daily) with etidronate (400 mg daily) (group B) for 5 days. Patients with any disease or medication that could interfere with calcium or bone metabolism were excluded from the study. All patients gave informed consent to the study. The procedures followed adhered to the guidelines of the Helsinki declaration for physician-initiated studies.

The study participants took their prednisone at 8.00 a.m. The patients in group B received 400 mg etidronate 1 h before prednisone intake. Breakfast was served at 9.00 a.m. The patients had a dietary calcium intake of 400–800 mg before the study and they were kept on this level during the study.

Measurements

Blood and urine were collected at 7.00 a.m. daily for 5 days starting 1 day before any medication was taken. The following biochemical parameters were evaluated before and during the first 5 days of treatment: serum calcium, phosphorus, creatinine, alkaline phosphatase activity, osteocalcin, carboxy-terminal propeptide of type I procollagen (PICP), cross-linked carboxy-terminal telopeptide of type I collagen (ICTP), parathyroid hormone (PTH) and 25-hydroxyvitamin D. Urinary excretion of calcium over 24 h was measured daily from day −1 to day 5. The serum and urine calcium, serum phosphorus, alkaline phosphatase and creatinine were measured by autoanalyser techniques. The samples for hormonal measurements were immediately frozen and stored at −20°C until use. All hormonal measurements were performed in a one assay session.

The radioimmunoassay (RIA) used for osteocalcin measurements was the commercially available kit of Incstar Corporation [Stillwater, MN, USA; intra- and inter-assay coefficient of variation (CV) 3.8% and 4.3%, respectively; sensitivity of the assay 0.5 μg/l; normal values 1.8–6.6 μg/l]. PICP and ICTP results were obtained with the RIA produced by Farmos Diagnostica (Orion Corporation Farmos, Turku, Finland). The intra- and inter-assay CV were 4.7% and 5.3% for PICP and 6.2% and 7.9% for ICTP. Sensitivity was 1.2 μg/l for PICP and 0.43 μg/l for ICTP; normal values PICP,

50–170 μg/l and ICTP 1.4–5.4 μg/l. PTH was measured using a two-sided RIA. The first step involved extraction and concentration of plasma PTH using solid-phase anti-amino-terminal antibodies. After elution, the PTH immunoextract was analysed using a sensitive mid- and C-region immunoassay [25]. The intra- and inter-assay CV were 8.3% and 10.2%, respectively; the sensitivity of the assay was 0.8 pmol/l (normal values 0.8–5.0 pmol/l).

Serum 25-hydroxyvitamin D was quantified, after acetonitrile extraction, using a direct commercial RIA (Incstar Corporation). The intra- and inter-assay CV were 6.3% and 13.0%, respectively (normal values 25–125 nmol/l).

Statistics

Data were expressed as mean ± standard error of the mean (S.E.M.), with 95% confidence intervals of the mean. The significance of change from baseline was determined by a paired t-test, SPSS Windows version 8.0 (P denoted by P). The significance of difference between means of biochemical parameters, at the end of the study (day 5), was determined by an independent two-sample t-test (P denoted by P^*). Also analysis of covariance (ANCOVA, with mean pre-treatment value determinations and time as covariables) was performed to compare the differences between groups at day 5 of the study. Differences were considered statistically significant when $P < 0.05$.

Results

Fourteen post-menopausal women were randomized to group A and group B. Each group comprised seven women. At baseline the two treatment groups were comparable in age and biochemical parameters. Group A: $n = 7$; mean age 72 yr, range 59–82 yr. Group B: $n = 7$; mean age 74 yr, range 63–82 yr. The results are given in Table 1 and illustrated for osteocalcin (Fig. 1), urinary excretion of calcium over 24 h (Fig. 2) and the telopeptide ICTP (Fig. 3). In both groups of prednisone alone and combined prednisone and etidronate treatment, serum calcium and phosphate levels, alkaline phosphatase, PTH and 25-hydroxyvitamin D levels virtually remained unchanged.

In the prednisone alone group, serum osteocalcin levels significantly decreased from 2.7 ± 0.3 mg/l at baseline to 1.7 ± 0.1 mg/l after 5 days (−38.1%, $P < 0.01$). In contrast, with the combined treatment group, serum osteocalcin levels steadily increased from 2.4 ± 0.3 mg/l at baseline to 3.0 ± 0.4 mg/l after 5 days (+27.4%, $P < 0.01$). The change in osteocalcin between the two groups was statistically significant from day 3 ($P^* < 0.05$). The procollagen I levels remained unchanged in both treatment groups.

The bone resorption marker ICTP remained virtually unchanged during prednisone treatment alone, but gradually decreased in the bisphosphonate group to levels 19.4% below baseline at day 5 ($P < 0.01$). At that time the difference between both groups was statistically

TABLE 1. Biochemical markers of calcium and bone homeostasis (mean ± s.e.m. and 95% confidence interval) (group A, prednisone; group B, prednisone and etidronate)

	Group	Before	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
SeCa (mmol/l)	A	2.36 ± 0.04 (2.26–2.46)	2.35 ± 0.04 (2.26–2.43)	2.37 ± 0.04 (2.26–2.47)	2.35 ± 0.05 (2.24–2.46)	2.35 ± 0.04 (2.26–2.43)	2.34 ± 0.04 (2.24–2.44)	2.34 ± 0.04 (2.25–2.44)
	B	2.34 ± 0.05 (2.23–2.45)	2.32 ± 0.05 (2.20–2.44)	2.30 ± 0.04 (2.21–2.39)	2.33 ± 0.04 (2.23–2.41)	2.34 ± 0.04 (2.24–2.43)	2.33 ± 0.04 (2.23–2.42)	2.34 ± 0.05 (2.23–2.45)
SePO$_4$ (mmol/l)	A	1.12 ± 0.07 (0.95–1.28)	1.12 ± 0.06 (0.98–1.26)	1.14 ± 0.06 (1.00–1.28)	1.18 ± 0.06 (1.02–1.33)	1.10 ± 0.07 (0.94–1.27)	1.12 ± 0.07 (0.95–1.29)	1.11 ± 0.07 (1.00–1.21)
	B	1.10 ± 0.08 (0.91–1.28)	1.08 ± 0.07 (0.91–1.25)	1.10 ± 0.06 (0.94–1.26)	1.07 ± 0.07 (0.91–1.24)	1.11 ± 0.07 (0.93–1.29)	1.08 ± 0.06 (0.92–1.24)	1.03 ± 0.06 (0.88–1.17)
Uca (mmol/l)	A	6.5 ± 0.6 (5.1–7.9)	6.6 ± 0.7 (4.8–8.4)	6.4 ± 0.8 (4.4–8.4)	7.2 ± 0.7 (5.4–8.9)	7.1 ± 0.9 (4.9–9.3)	7.2 ± 0.9 (5.0–9.3)	7.5 ± 0.8 (5.5–9.4)[b]
	B	6.2 ± 0.8 (4.3–8.1)	6.2 ± 0.7 (4.3–8.0)	5.9 ± 0.9 (3.7–8.1)	5.6 ± 0.8 (3.7–7.4)	5.0 ± 0.6 (3.5–6.6)	4.9 ± 0.7 (3.1–6.7)	4.8 ± 0.6 (3.4–6.1)[a,c]
Alkaline phosphatase (U/l)	A	62 ± 6.3 (46–77)	61 ± 5.8 (47–75)	61 ± 5.0 (49–73)	62 ± 5.7 (49–77)	62 ± 6.2 (46–77)	60 ± 5.9 (46–75)	61 ± 5.8 (47–75)
	B	61 ± 4.3 (51–72)	61 ± 3.9 (52–71)	62 ± 4.3 (52–79)	62 ± 5.1 (51–76)	61 ± 4.7 (50–73)	63 ± 3.9 (53–72)	64 ± 4.1 (54–74)
Osteocalcin (mg/l)	A	2.7 ± 0.3 (1.9–3.5)	2.7 ± 0.3 (1.9–3.5)	2.6 ± 0.3 (1.9–3.3)	2.0 ± 0.2 (1.4–2.6)	1.9 ± 0.2 (1.5–2.2)	1.7 ± 0.2 (1.3–2.1)	1.7 ± 0.1 (1.4–2.0)[a,c]
	B	2.4 ± 0.3 (1.6–3.2)	2.3 ± 0.3 (1.6–3.0)	2.4 ± 0.3 (1.7–3.1)	2.5 ± 0.3 (1.7–3.2)	2.8 ± 0.3 (1.9–3.6)	3.0 ± 0.4 (2.0–4.0)	3.0 ± 0.4 (2.2–3.9)[a,c]
Procollagen 1 (μg)	A	217 ± 41 (116–318)	213 ± 38 (119–307)	207 ± 35 (120–293)	206 ± 43 (102–310)	213 ± 37 (123–303)	220 ± 45 (108–331)	219 ± 36 (131–307)
	B	266 ± 53 (136–395)	260 ± 52 (133–388)	268 ± 54 (136–400)	275 ± 49 (155–395)	277 ± 53 (148–405)	272 ± 49 (153–391)	279 ± 54 (144–409)
Telopeptide (μg)	A	3.0 ± 0.4 (2.0–4.1)	3.0 ± 0.4 (2.1–3.9)	3.0 ± 0.4 (2.1–4.0)	3.1 ± 0.4 (2.2–4.0)	3.1 ± 0.4 (2.1–4.1)	3.1 ± 0.3 (2.3–4.0)	3.2 ± 0.3 (2.4–4.0)[c]
	B	3.0 ± 0.6 (1.5–4.4)	3.0 ± 0.6 (1.4–4.5)	2.8 ± 0.5 (1.5–4.2)	2.9 ± 0.6 (1.5–4.3)	2.4 ± 0.6 (1.4–4.1)	2.7 ± 0.6 (1.3–4.1)	2.4 ± 0.5 (1.2–3.7)[a,c]
PTH (pmol/l)	A	1.9 ± 0.3 (1.1–2.6)	2.0 ± 0.3 (1.1–2.8)	2.0 ± 0.4 (1.1–2.8)	2.1 ± 0.3 (1.3–2.9)	2.0 ± 0.3 (1.2–2.8)	2.2 ± 0.4 (1.3–3.1)	2.1 ± 0.4 (1.2–3.0)
	B	1.6 ± 0.2 (1.0–2.1)	1.6 ± 0.2 (1.1–2.1)	1.6 ± 0.2 (1.1–2.2)	1.5 ± 0.2 (1.1–1.9)	1.6 ± 0.2 (1.2–2.1)	1.7 ± 0.2 (1.3–2.2)	1.9 ± 0.2 (1.5–2.4)
Creatinine (μmol/l)	A	94 ± 4.5 (83–105)	95 ± 4.7 (83–106)	93 ± 5.4 (86–101)	93 ± 3.1 (86–101)	92 ± 4.9 (80–105)	91 ± 4.8 (78–103)	95 ± 4.3 (84–105)
	B	93 ± 4.2 (83–104)	90 ± 3.4 (82–99)	93 ± 3.7 (84–102)	90 ± 3.8 (81–100)	94 ± 4.2 (84–104)	93 ± 4.4 (82–104)	92 ± 3.2 (85–100)
25 hydroxyvitamin D (nmol/l)	A	42 ± 7.4 (24–60)	41 ± 6.9 (23–58)	42 ± 6.4 (27–58)	42 ± 7.1 (24–59)	42 ± 7.4 (24–60)	42 ± 7.8 (23–61)	43 ± 7.5 (25–62)
	B	43 ± 4.7 (31–54)	44 ± 4.7 (32–55)	44 ± 4.4 (33–54)	43 ± 5.4 (30–56)	41 ± 4.6 (30–52)	42 ± 5.5 (28–56)	43 ± 4.6 (31–54)

[a] $P < 0.01$ difference from baseline after 5 days.
[b] $P < 0.05$ difference from baseline after 5 days.
[c] $P* < 0.01$ difference between groups after 5 days.
SeCa, serum calcium; SePO$_4$, serum phosphate; Uca, urine calcium excretion.

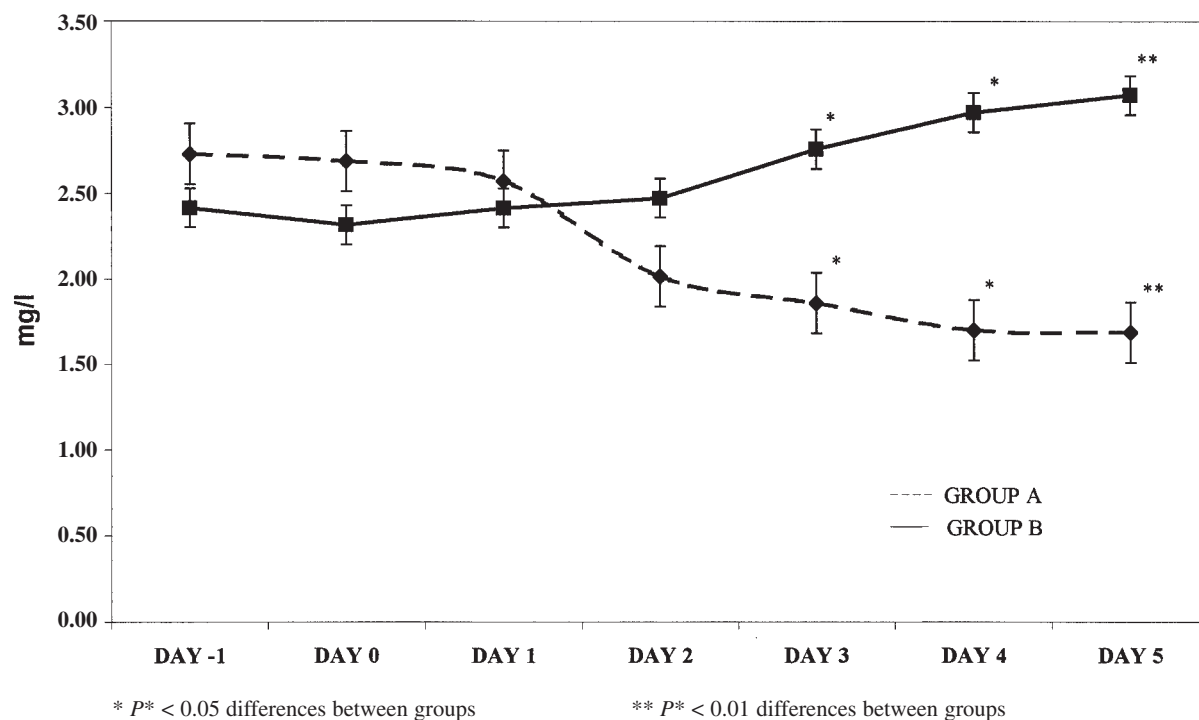* *P** < 0.05 differences between groups   ** *P** < 0.01 differences between groups

FIG. 1. Time course of serum osteocalcin (mean ± S.E.M.). Group A, prednisone; group B, prednisone and etidronate.

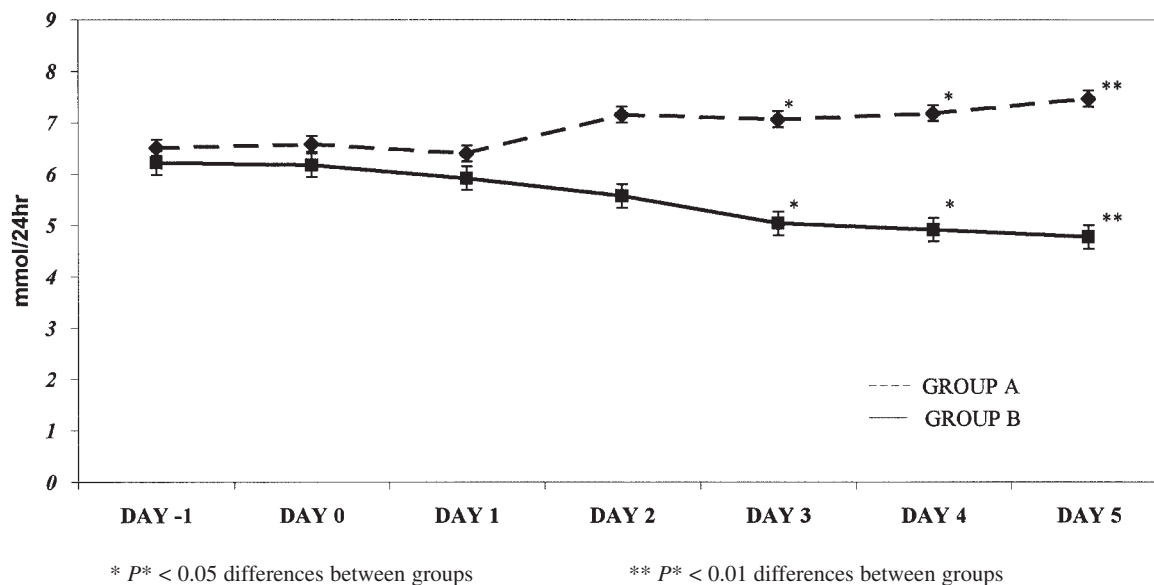* *P** < 0.05 differences between groups   ** *P** < 0.01 differences between groups

FIG. 2. Time course of 24 h urinary calcium excretion (mean ± S.E.M.). Group A, prednisone; group B, prednisone and etidronate.

significant $(P^* < 0.01)$. Urinary calcium excretion gradually increased over 5 days in the prednisone alone group $(+14.7\%, P < 0.05)$ but decreased in the combined prednisone and etidronate group $(-22.1\%,$ $P < 0.01)$. The difference in urinary calcium excretion between both groups was already statistically significant from day 3 on. Every patient completed the study and the study drug was well tolerated.
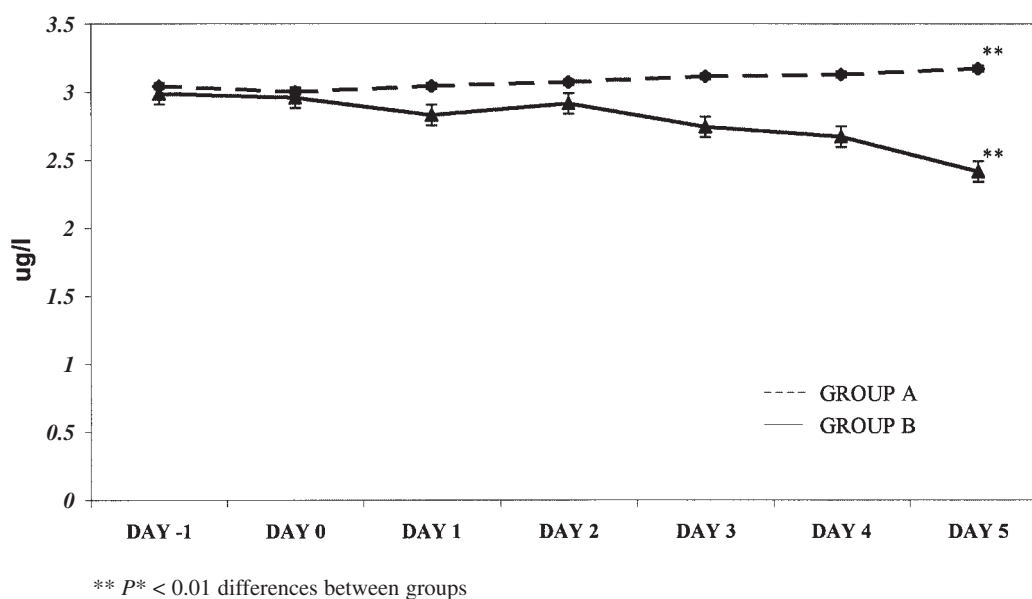
** *P* < 0.01 differences between groups

FIG. 3. Time course of serum telopeptide (mean ± S.E.M.). Group A, prednisone; group B, prednisone and etidronate.

Discussion

Short-term high-dose glucocorticoid therapy in post-menopausal women with fluoride arteritis temporalis resulted in acute changes in bone formation and resorption as reflected by a 38% decrease of osteocalcin—not PICP or alkaline phosphatase—and a 15% increase in urinary calcium excretion. The glucocorticoid-induced changes in bone remodelling markers have been attributed to a decrease in differentiated function of mature osteoblasts leading to a decrease in osteocalcin transcription and—not in this study—a decrease in type I collagen expression and increase in its degradation [10–12, 26, 27]. Other studies administering 10–20 mg of prednisone daily for 1 week to healthy men found a similar decrease in osteocalcin (−35%) and increase in urinary calcium (+45%), however, they reported a decrease in PICP [28, 29] and both decreased [28] and increased [29] ICTP has been reported. This could be explained by the different dosages of prednisone and/or the difference in study population. Similar results were obtained in patients with rheumatoid arthritis given a high dose of dexamethasone [13], in patients with multiple sclerosis given huge intravenous doses of methyl prednisolone [30] and after high-dose corticosteroid inhalation [31].

From the present study it appears that combined etidronate and glucocorticoid administration acutely reversed glucocorticoid-induced suppression of bone formation as reflected by a 27% increase in osteocalcin within 5 days. Urinary calcium excretion decreased from +14.7% in the prednisone alone group to −22.1% in the combined therapy group, indicating attenuation of bone resorption. This was also illustrated by a decrease in ICTP. This decrease of bone resorption markers is well known in chronically bisphosphonate-treated patients on glucocorticoids, but has never been reported after such a short time interval as in this study.

The increase in osteocalcin levels during short-term etidronate administration in glucocorticoid-treated patients is, however, remarkable, although we must be careful when extrapolating our short-term study results to the long-term benefits.

There are five possible mechanisms by which this increase in osteocalcin could be explained: first, decreased elimination of osteocalcin by the kidney during etidronate therapy. This is unlikely since in most studies on post-menopausal osteoporosis etidronate induces a decrease in osteocalcin. Second, altered degradation, third, increased production of osteocalcin, fourth, an indirect effect of etidronate on bone turnover by influencing the inflammatory process [32] and, fifth, initial displacement of osteocalcin from hydroxyapatite by EHDP as shown by Price *et al.* in rats [33]. This could also explain the initial rise in osteocalcin found by Tobias *et al.* in the first 2 weeks of treatment of post-menopausal women with hormone replacement therapy or a bisphosphonate [34].

A direct or indirect stimulatory effect of bisphosphonates on the osteoblast could be postulated, especially in high turnover bone disease as in patients with Paget's disease, in whom an increase in osteocalcin occurs after APD treatment [35]. Also, in vitamin D-deficient post-menopausal osteoporotic women, chronic etidronate therapy has been reported to induce a rise in osteocalcin [36]. Bisphosphonates may exert their effects on the osteoblast indirectly by at least two different mechanisms. One is the modulation in the release of cytokines by the osteoclast and the release of local signals due to the effects on the osteoclast function, resulting in decreased resorption [37]. The other is through stimulation of the production of the calciotropic hormones 1,25-dihydroxyvitamin D and PTH, which could increase during bisphosphonate treatment [6, 38, 39]. Recently Staal *et al.* [40] and Zhang *et al.* [41] reported

increased expression of plasma osteocalcin by 1,25-dihydroxyvitamin D_3 which is inhibited by glucocorticoids [42]. Our hypothesis is that etidronate could reverse this mechanism. Unfortunately we have no data on plasma 1,25 vitamin D_3 levels during the short-term administration of glucocorticoid alone or combined glucocorticoid and etidronate. During the short-term treatment period, serum phosphate and PTH levels remained virtually unchanged in both groups. Therefore, it seems unlikely that they trigger changes that could explain the increase in osteocalcin. Finally, etidronate could have a direct stimulatory effect on osteoblast function in a state of glucocorticoid-induced high bone turnover.

In conclusion, the present study is the first demonstrating an acute beneficial effect of etidronate on glucocorticoid-induced bone degradation as reflected by prevention of bone resorption and in addition stimulation of the bone formation marker osteocalcin by a thus far unknown direct or indirect effect (PTH, 1,25-dihydroxyvitamin D_3) on the osteoblast. A study with more individuals, a longer follow-up period with new more specific markers and vitamin D metabolites is warranted.

Acknowledgements

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