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The Effect of Long- and Short-Term Corticosteroids on Plasma Calcitonin and Parathyroid Hormone Levels

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Abstract. The role of calcitonin and parathyroid hormone (PTH) in corticosteroid-induced osteoporosis is controversial. We therefore measured plasma calcitonin and PTH levels in 34 adults receiving chronic pharmacological corticosteroids for obstructive airways disease, and in controls matched for age, sex, menopause, and disease. In addition, the acute effect of a 7-day course of 15 mg prednisolone daily on fasting and calcium-stimulated calcitonin was studied in 10 normal male volunteers. There was no difference in calcitonin and PTH levels in the corticosteroid-treated patients when compared with controls. The corrected serum calcium was significantly higher in the steroid-treated patients (patients mean 2.40 (SEM 0.01) mmol/liter; controls mean 2.33 (SEM 0.01) mmol/liter; $P < 0.001$). The short course of corticosteroids in volunteers did not alter basal or stimulated calcitonin, PTH, or calcium levels. These results suggest that neither calcitonin deficiency nor PTH excess is a feature of corticosteroid-induced osteoporosis.

Key words: Corticosteroids — Osteoporosis — Parathyroid hormone — Calcitonin

Osteoporosis is a well-recognized complication of therapy with pharmacological doses of corticosteroids [1, 2]. The etiology of corticosteroid osteoporosis is unknown and probably multifactorial [3]. A variety of mechanisms have been postulated and these related to either a suppression of bone formation [4, 5] or an increase in resorbing influences, possibly by a direct action on bone [6] but also indirectly through an elevation of parathyroid hormone (PTH) [7] or a reduction in calcitonin (CT) levels. CT reduces bone resorption by inhibiting osteoclast function [8]. The effect of acute or chronic corticosteroids on CT levels in man is controversial. Reduced [9, 10] or normal [11] levels of CT have been reported in patients on chronic steroids.

We studied serum CT and PTH levels in adults receiving chronic corticosteroids for asthma and chronic obstructive airways disease (COAD) and in age, sex, menopause, and disease-matched controls. In addition, we looked at the acute effect of a 7-day course of prednisolone on fasting and calcium-stimulated calcitonin levels in normal subjects.

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Patients and Methods

Thirty-four adult outpatients from chest clinics in London with symptomatic asthma or COAD on long-term supraphysiological treatment with prednisolone were studied (Table 1). Long-term treatment was defined as daily or alternate day prednisolone for at least 1 year. The mean dose for the preceding 3 months was 10.1 mg/day. All were on a dose of at least 5 mg daily (or 10 mg on alternate days) and most (30/34) were on at least 7.5 mg daily. For comparison, we took 34 control patients with asthma or COAD from the same clinics, who had taken no prednisolone in the preceding 3 months and who had never had continuous treatment with corticosteroids. The controls were individually matched for age (within 5 years), sex, and where appropriate, years since menopause. Patients with known causes of bone disease such as renal failure, thyroid dysfunction, alcoholism, and long-term anticoagulants were excluded. All patients were assessed clinically for possible adverse effects of corticosteroids including spontaneous vertebral and rib fractures, objective spontaneous bruises, and inability to rise unaided from squatting as a result of muscular weakness. Skin thickness was measured using Harpendon spring-loaded callipers. The skin was considered significantly thin if it was two standard deviations less than the mean thickness for age and sex-matched normal subjects [12].

Blood samples were drawn from all patients between 2 p.m. and 4 p.m. Plasma was separated, frozen immediately in dry ice, and stored at −20°C.

In a parallel study of 10 healthy male volunteers (age range 25–46 years), the same biochemical indices were measured before and at the end of a 7-day course of prednisolone (daily dose 15 mg). Fasting serum CT was also measured at these times and also on each occasion at 5, 10, 20, and 30 minutes after a slow (1 minute) bolus infusion of calcium gluconate (Ca 2 mg/kg). These studies were approved by the Hospital Ethics Committee and full informed consent was obtained.

Assays

Samples were analyzed without any knowledge of patient details. Plasma CT was measured by a well-established, nonextracted, single-site radioimmunoassay [13]. This uses a sensitive antiserum raised in rabbit against synthetic human calcitonin (hCT) conjugated to ovalbumin. The antiserum was used in a dilution of 1:36,000. A 7-day protocol was used involving preincubation of the sample with the polyclonal antibody for 4 days followed by the addition of radiolabeled hCT and a further 3-day incubation. Using this protocol, the lower limit of detection of the assay was 4 pg/ml. Intra- and interassay coefficients of variation at 100 pg/ml were 9% and 13%, respectively. Plasma PTH was measured using a 2-site immunoradiometric assay for the biologically intact 84 amino acid chain of PTH (PTH Allegro, Nichols Institute, California, USA). The between-batch coefficient was 10.8% and 6.1% at levels of 42 and 248 pg/ml,

Table 1. Clinical characteristics of asthmatic subjects treated with long-term prednisolone and controls

	Steroid treated Patients (n = 34)	Controls (n = 34)
Mean age (years)	55.7 (14.7)	55.4 (15.1)
Sex		
Male	13	13
Female (premenopausal)	9	9
Female (postmenopausal)	12	112
Years since menopause	13.3 (11.3)	13.8 (7.2)
Severity		
Duration (years)	19.4 (10.9)	13.4 (11.48)
FEV 1 (liters)	1.41 (0.76)	1.58 (0.68)
Breathless at rest	7	5
Prednisolone dose	Mean (SD)	
Current (last 3 months) (mg)	10.1 (3.8)	0
Duration therapy (years)	9.3 (7.5)	0

Data is shown as mean (SD). Statistical analysis by the nonpaired Student's *t*-test showed no significant difference between the two groups

respectively. Serum samples were analyzed for calcium, albumin, creatinine, alkaline phosphatase, and phosphate using routine Technicon SMAC methods. The albumin-adjusted (corrected) calcium (Ca corr) was calculated from the formula:

$$\text{Ca corr} = \text{total calcium} - [0.02 \times \{\text{albumin (g/l)} - 40\}] \text{ nmol/l.}$$

Statistics

For the patient study, variables measured in the patients on chronic corticosteroids were compared with those from the control group using a two-sample *t*-test. The incidence of steroid-related clinical signs in the two groups was compared using the Chi-squared test. The volunteer study was analyzed using a paired *t*-test.

Results

Patient Study

The patients on long-term pharmacological prednisolone had clinical evidence of side effects from their treatment (Table 2). They were significantly more likely to have thin skin, spontaneous bruising, and a history of fractured ribs or vertebrae.

The biochemical parameters in the prednisolone-treated patients and the control patients are shown in Table 3. There was no significant difference in the mean CT (patients 13.8 ng/liter; controls 13.2 ng/liter) or PTH levels (patients 33.8 ng/liter; controls 34.6 ng/liter). There was no correlation between the calcitonin or PTH levels and the dose of prednisolone or its duration.

The mean serum-corrected calcium was significantly higher in patients (2.40 mmol/liter) on long-term prednisolone than controls (2.33 mmol/liter) $P < 0.001$) but there was no associated difference in phosphate, creatinine, or alkaline phosphatase.

Volunteer Study

The results of the calcium infusion study are shown in Figure 1. There was a marked rise in CT in response to the calcium infusion in all volunteers. The 1-week course of prednisolone in normal subjects caused no side effects and did not change the fasting or the calcium-stimulated calcitonin levels (either maximum or area under the curve, Table 4). Other fasting indices, including serum-corrected calcium, plasma PTH, and testosterone, were all unaltered (Table 4).

Discussion

This study has shown no alteration in PTH or CT levels with long- and short-term corticosteroid treatment. These results do not support the hypothesis that corticosteroids cause an increase in bone resorption mediated by either increased PTH or reduced CT levels.

To examine the chronic effect of pharmacological doses of corticosteroids we studied large groups of subjects with obstructive lung disease. Patients with obstructive lung disease are the most appropriate steroid-treated patient group to study as they probably do not have altered bone metabolism or increased fractures as a result of their disease [1, 14]. Many systemic diseases treated by steroids, such as rheumatoid arthritis and sarcoidosis, are not suitable to study as they directly alter bone density and calcium metabolism. In cross-sectional studies, careful matching of controls is important. We chose controls with obstructive lung disease who were individually matched for age, sex, and where appropriate, menopausal status. Perfect matching in steroid-treated disease is difficult as steroid treatment is reserved for the more severe cases. Our subjects treated with steroids did have evidence of more severe lung disease (a lower FEV 1) than controls, but these did not reach significance.

We found no reduction in basal CT in patients on long-term corticosteroids compared with matched controls nor in the calcium-stimulated CT response following short-term, high-dose prednisolone in normal volunteers. Lo Cascio et al. [9] reported a more than 50% reduction in mean plasma calcitonin in seven patients treated with corticosteroids for 8 months. Four of these patients with pituitary failure received replacement doses of steroids only. One patient had sarcoidosis and another had nephrotic syndrome, both disorders known to affect calcium metabolism. Blunting of calcium-stimulated CT release was also reported [10] in three patients given 20 mg prednisolone daily. However, in keeping with our study, a controlled calcium clamp study in nine patients

Table 2. Clinical evidence of corticosteroid side effects in asthmatic subjects treated with long-term corticosteroids and controls

	Steroid treated Patients (n = 34)	Controls (n = 34)	Significance
Thin skin for age and sex	15	0	$P < 0.001$
Spontaneous bruising	24	4	$P < 0.001$
Proximal muscular weakness	6	3	n.s.
Fractures of ribs and vertebrae	7	1	$P < 0.01$

Statistical analysis was by the Chi-squared test

Table 3. Biochemical parameters in asthmatic patients on long-term prednisolone and controls

	Patients	Controls	Significance
Serum calcium (mmol/liter)	2.43 (0.02)	2.38 (0.01)	$P < 0.001$
Phosphate (mmol/liter)	1.11 (0.04)	1.09 (0.03)	ns
Serum albumin (g/dl)	41.5 (0.6)	42.2 (0.4)	ns
Corrected calcium (mmol/liter)	2.40 (0.01)	2.33 (0.01)	$P < 0.001$
Serum creatinine (μmol/liter)	92 (4)	84 (3)	ns
Alkaline phosphatase (IU/liter)	91 (6)	83 (6)	ns
Plasma testosterone (ng/liter) (n = 13)	11.6 (1.6)	13.8 (1.1)	ns
Calcitonin (ng/liter)	13.8 (1.6)	13.2 (1.2)	ns
PTH (ng/liter)	33.8 (3.2)	34.6 (3.2)	ns

Data presented as mean (SEM). Statistical analysis was by the nonpaired Student's *t*-test

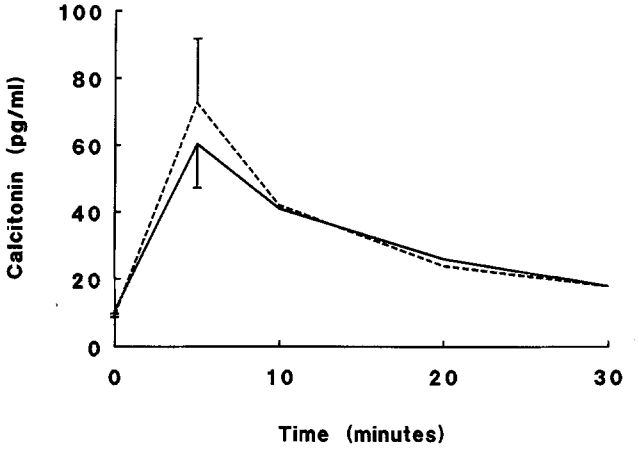

Fig. 1. Plasma CT levels following a calcium bolus, before (solid line) and after (dotted line) 7 days of 15 mg prednisolone/day. Error bars represent the SEM.

with endogenous Cushing's syndrome failed to show any abnormality in basal or stimulated calcitonin levels [11].

Experimental animal and *in vitro* studies have also failed to show a reduction in calcitonin with corticosteroids. In adrenalectomized rats, there was a fivefold increase in thyroid CT mRNA when treated with dexamethasone [15]. In the TT cell line, derived from medullary carcinoma, dexamethasone was again shown to increase calcitonin mRNA [16]. These results need to be interpreted cautiously as tumor cell lines and rat models may not represent the position in man.

Despite our failure to demonstrate CT deficiency in corticosteroid-treated patients, CT may still have a useful therapeutic role in preventing or treating osteoporosis. In postmenopausal osteoporosis [17], where plasma CT is probably normal [18, 19] and bone turnover is increased [20], intranasal CT therapy has significantly reduced the rate of bone loss [21]. Intramuscular CT has been shown to reduce the rate of bone loss in patients on chronic corticosteroids for sarcoidosis [22] and asthma [23]. Therapeutic doses of CT may increase bone mass by reducing bone resorption but will not be correcting an underlying CT deficiency.

Increased secretion of PTH has been postulated to play a role in causing steroid osteoporosis. Cortisol stimulates PTH secretion by a direct action on the parathyroid glands *in vitro* [3, 7]. Our study using an intact PTH assay failed to confirm elevated levels of PTH. Of nine reports since 1975, four report an increase in PTH (supported by cAMP or phosphate data in three) [7, 24–26] and in four, the levels were found to be unchanged [28–30].

The serum calcium was raised in patients on chronic steroids compared with the matched controls. There was no change in serum calcium in the volunteers on a 7-day course of steroids. A recent longitudinal study by Prummell et al. [30] also found a significant increase in mean-corrected serum calcium with a 12-week course of prednisolone given in a decreasing daily dose of between 60 mg and 20 mg. Six other studies have also shown an increase in calcium in subjects on corticosteroids but none reached significance [7, 24–26, 31, 32]. There was a nonsignificant fall in calcium in one study [27].

The raised serum calcium seen in our study is not explained by alterations in calcitonin, PTH, or renal function, and its mechanism is uncertain. A direct effect of cortisol on bone resorption has been seen *in vitro* [33], which is independent of PTH. If the increase in calcium resulted from such a PTH-independent effect, one would expect lower than normal PTH levels. If on the other hand, the rise in calcium is PTH dependent, a possible explanation is that there is enhanced responsiveness of the resorbing process to these normal PTH levels. This relative hyperresponsiveness could explain both bone loss and the elevation of serum calcium without an increase in PTH levels in the steroid-treated group. Studies have confirmed facilitation of PTH-

Table 4. Biochemical parameters in normal subjects before and after a 7-day course of 15 mg prednisolone daily

	Before prednisolone	After prednisolone	Significance
Fasting serum values			
Calcium (mmol/l)	2.43 (0.02)	2.45 (0.02)	ns
Albumin (g/dl)	45.6 (0.53)	46.3 (0.84)	ns
Corrected calcium	2.31 (0.02)	2.32 (0.02)	ns
Fasting plasma values			
PTH (ng/l)	29.4 (4.6)	29.6 (3.6)	ns
Testosterone (ng/liter)	23.3 (1.88)	23.7 (1.57)	ns
Basal CT (ng/liter)	10.3 (1.1)	9.1 (0.6)	ns
Peak CT (ng/liter)	60.3 (12.5)	72.6 (18.3)	ns
Area under curve (min ng/liter)	948 (227)	1008 (245)	ns

Data presented as mean (SEM). Statistical analysis was by the paired Student's *t*-test

mediated bone resorption by corticosteroids *in vitro* [34, 35]. A reduction of bone accretion of calcium might cause hypercalcemia if resorption continued. The renal action of glucocorticoids results in increasing calcium excretion and makes this explanation unlikely [25].

In conclusion, in doses commonly used to treat chronic respiratory disease, prednisolone does not reduce plasma CT levels or increase PTH levels when given in short- or long-term courses. The beneficial effect of therapeutic CT in corticosteroid-induced osteoporosis is not dependent on pre-existing CT deficiency.

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References

1. Adinoff AD, Hollister JR (1983) Steroid-induced fractures and bone loss in patients with asthma. N Engl J Med 309:265–268
2. Ruegsegger P, Medici TC, Anliker M (1983) Corticosteroid-induced bone loss. A longitudinal study of alternate day therapy in patients with biochemical asthma using quantitative computed tomography. Eur J Clin Pharmacol 25:615–620
3. Reid IR (1989) Pathogenesis and treatment of steroid osteoporosis. Clin Endocrinol 30:83–103
4. Meunier PJ, Dempster DW, Edouard C, Chapuy MC, Arlot M, Charhon S (1984) Bone histomorphometry in corticosteroid-induced osteoporosis and Cushing's syndrome. Adv Exp Med Biol 171:191–200
5. Reid IR, Chapman GE, Fraser TRC, Davies AD, Suras AS, Meyer J, Huq NL, Ibbertson HK (1986) Low serum osteocalcin levels in glucocorticoid-treated asthmatics. J Clin Endocrinol Metab 62:379–383
6. Reid IR, Katz J, Ibbertson HK, Gray WJ (1986) The effects of hydrocortisone, parathyroid hormone and the bisphosphonate APD on bone resorption in neonatal mouse calvaria. Calcif Tissue Int 38:38–43
7. Fucik RF, Kukreja SC, Hargis GK, Bowser EN, Henderson WJ, Williams GA (1975) Effect of glucocorticoids on function of the parathyroid glands in man. J Clin Endocrinol Metab 40:152–155
8. Chambers TJ, Moore A (1983) The sensitivity of isolated osteoclasts to morphological transformation by calcitonin. J Clin Endocrinol Metab 57:819
9. Lo Cascio V, Adami S, Avioli LV, Cominacini L, Galvanni G, Gennari C (1982) Suppressive effect of chronic glucocorticoid treatment on circulating calcitonin in man. Calcif Tissue Int 34:309–310
10. Gennari C, Civitelli R (1986) Glucocorticoid-induced osteoporosis. Clin Rheum Dis 12:637–654
11. Toring O, Bucht E, Thorer M, Sjoberg HE (1986) Plasma calcitonin response to a calcium clamp in endogenous Cushing's syndrome. Acta Endocrinol 111:258–263
12. Wright AD, Joplin GF (1969) Skin-fold thickness in normal subjects and in patients with acromegaly and Cushing's syndrome. Acta Endocrinol 60:705–711
13. Lynch C, Seth R, Bates DL, Self CH (1988) Calcitonin determination by a fast and highly sensitive enzyme-amplified immunoassay. J Immunoassay 9:179–192
14. Riancho JA, Gonzales-Macias J, Del Arco C, Amado JA, Freijanes J, Anton MA (1987) Vertebral compression fractures and mineral metabolism in chronic obstructive lung disease. Thorax 42:962–966
15. Bernard P, Jonsset V, Gorel JM (1989) Calcitonin gene expression in adrenalectomised rats: effects of dexamethasone and calcium. J Bone Miner Res Joint Meeting ASB and ICCRH, abstract 115
16. Cote JC, Gagel RF (1986) Dexamethasone differentially affects the levels of calcitonin and calcitonin gene-related peptide mRNA expressed in human medullary thyroid carcinoma cell line. J Biol Chem 261:15524–15528
17. Riggs BL, Melton LJ III (1986) Involutional osteoporosis. New Engl J Med 314:1676–1686
18. Tiegs RD, Body JJ, Barta JM, Heath H III (1986) Secretion and metabolism of monomeric human calcitonin: effects of age, sex and thyroid damage. J Bone Miner Res 1:339–349
19. Prince RL, Dick IM, Price RI (1989) Plasma calcitonin levels are not lower than normal in osteoporotic women. J Clin Endocrinol Metab 68:684–687
20. Parfitt AM (1979) Quantum concept of bone remodelling and turnover: implications for the pathogenesis of osteoporosis. Calcif Tissue Int 28:1–5
21. Overguard K, Riss BJ, Christiansen C, Podenphant J, Johansen JS (1986) Nasal calcitonin for treatment of established osteoporosis. Clin Endocrinol 30:435–442
22. Rizzato G, Tosi G, Schiraldi G, Montemurro L, Zanni D, Sisti S (1988) Bone protection with salmon calcitonin in the long-term steroid therapy of chronic sarcoidosis. Sarcoidosis 5:99–103
23. Luengo M, Picado C, Del Rio L, Guanabens N, Montserrat JM, Setoain J (1990) Treatment of steroid-induced osteopenia with calcitonin in corticosteroid-dependent asthma. A one-year follow-up study. Am Rev Respr Dis 142:104–107
24. Lukert BP, Adams JS (1976) Calcium and phosphorus homeostasis in man: the effect of corticosteroids. Arch Int Med 136: 1249–1253
25. Suzuki Y, Ichikawa Y, Saito E, Homma M (1983) Importance of increased urinary calcium excretion in the development of secondary hyperparathyroidism of patients under glucocorticoid therapy. Metabolism 32:151–156

26. Gennari C, Imbimbo B, Montagnani M, Bernini M, Nardi P, Avioli LV (1984) Effects of prednisolone and deflazacort on mineral metabolism and parathyroid hormone activity in humans. Calcif Tissue Int 36:245–252
27. Seeman E, Kumar R, Hunder GG, Scott M, Heath H, Riggs BL (1980) Production, degradation and circulating levels of 1,25-dihydroxy vitamin D in health and in chronic glucocorticoid excess. J Clin Invest 66:664–669
28. Slovik DM, Neer RM, Ohman JL, Lowell FC, Clark MB, Segre GV, Potts JT Jr (1980) Parathyroid hormone and 25-hydroxy vitamin D levels in glucocorticoid-treated patients. Clin Endocrinol 12:243–248
29. Hahn TJ, Halstead LR, Bran DT (1981) Effects of short-term glucocorticoid administration on intestinal calcium absorption and circulating vitamin D metabolite concentrations in man. J Clin Endocrinol Metab 52:111–115
30. Prummell MF, Wiersinga WM, Lips P, Sanders GTB, Sauerwein HP (1991) The course of biochemical parameters of bone turnover during treatment with corticosteroids. J Clin Endocrinol Metab 72:382–386
31. Need AG, Hartley TF, Philcox JC, Nordin BEC (1986) Calcium metabolism and osteoporosis in corticosteroid-treated postmenopausal women. Aust NZ J Med 16:341–346
32. Packe GE, Douglas JG, McDonald AF, Robins SP, Reid DM (1992) Bone density in asthmatic patients taking high-dose inhaled beclomethasone dipropionate and intermittent systemic corticosteroids. Thorax 47:414–417
33. Teitelbaum SL, Malone JD, Kahn AJ (1981) Glucocorticoid enhancement of bone resorption by rat peritoneal macrophages in vitro. Endocrinology 108:795–799
34. Rodan SB, Fischer MK, Egan JJ, Epstein PM, Rodan GA (1984) The effect of dexamethazone on parathyroid hormone stimulation of adenylate cyclase in ROS 17/28 cells. Endocrinology 115:951–958
35. Zajac JD, Livesey SA, Michelangeli VP, Rodan GA, Martin TJ (1986) Glucocorticoid treatment facilitates cyclic adenosine 3,'5'-monosulphate-dependent protein kinase response in parathyroid hormone-responsive osteogenic sarcoma cells. Endocrinology 118:2059–2064