Decreased Osteocalcin Levels in Patients With Chronic Obstructive Pulmunary Disease Using Long-Term Inhaled Beclomethasone Dipropionate

Meral Dinc, Yulia Tchugunova, Soykan Dinc, Bahar Cinarli, Tamer Atasever, and Murat Oz

Inhaled corticosteroids have been used in the treatment of chronic obstructive pulmonary disease (COPD) for many years. However the adverse effects of corticosteroids on bone formation may require special consideration in these patients. This study was undertaken to investigate the effects of long-term inhaled beclomethasone dipropionate treatment on the biochemical markers of bone formation. For this purpose, serum osteocalcin, alkaline phosphatase, free calcium, and inorganic phosphate levels were measured in 65 male COPD patients. Patients in the control group (n = 30) had not taken oral or inhaled corticosteroids for at least 1 year. Only those patients using beclomethasone with metered-dose inhalers were included in the treatment group (n = 35). The mean age of the control group was 61.63 ± 1.84 (mean ± SEM). In the treatment group, the mean age was 59.10 ± 2.29 and patients in this group had taken beclomethasone for an average time of 23.94 ± 2.72 months (for at least 12 months) at an average concentration of 1,142.0 ± 79.64 μg/d. The mean serum osteocalcin levels in the control group and treatment group were 7.03 ± 0.19 ng/mL and 3.74 ± 0.12 ng/mL, respectively. Comparison of values between groups indicates that serum osteocalcin levels in patients using beclomethasone were significantly lower than that of patients in the control group. Serum alkaline phosphatase levels were 167.96 ± 1.49 U/L and 168.17 ± 1.60 U/L for the control and treatment groups, respectively. There was no statistically significant difference among these values (Student's *t* test; *P* > .05). The mean values of total serum calcium and inorganic phosphate were not statistically different between the groups (*P* > .05). These results indicate that long-term inhaled beclomethasone treatment in COPD patients may induce significant changes in osteocalcin levels and require close monitoring for osteoporotic changes.

IN RECENT YEARS, the role of inflammatory processes in the pathogenesis of chronic obstructive pulmonary disease (COPD) has been considered as an important phenomenon.[1-4] In fact, approximately 20% to 25% of cases are responsive to treatments with corticosteroids.[5-9] Although the efficacy of corticosteroids in treatment of COPD patients has been questioned,[10-12] several early and recent studies indicate that corticosteroids have beneficial effects on the respiratory status of COPD patients.[13-16]

One of the most important systemic side effects relevant to the use of corticosteroids is osteoporosis.[17-20] The effects of several corticosteroids on the formation of bone structure have been investigated in detail in several earlier studies.[21,22] The osteoporotic effect of corticosteroid use in COPD patients has been especially important since the majority are elderly and particularly vulnerable to debilitating effects of osteoporosis.[23] In order to minimize the systemic toxicities of corticosteroid use in patients with pulmonary diseases, inhaled preparations with enhanced local effectiveness and lesser systemic effects have been used for many years. Although inhaled corticosteroids act locally and have fewer side effects compared to systemically administered drug, current research on this subject indicates that inhaled corticosteroids are not devoid of the toxicities seen with the systemically administered corticosteroids.[24,25] For example, osteoporotic effects of various inhaled corticosteroids such as budesonide and beclomethasone have been demonstrated in earlier investigations.[26] Earlier studies on corticosteroid-induced osteoporosis have suggested that suppression of the function of osteoblasts and thus the inhibition of bone formation is one of the mechanisms mediating this effect on the bone metabolism.[22,27]

Osteocalcin (vitamin K–dependent bone G1a protein) is synthesized by osteoblasts and is an important biochemical indicator for the evaluation of osteoblast activity and bone formation.[28-30] The measurement of serum osteocalcin and alkaline phosphatase levels has been routinely used for this purpose.[31,32]

So far the majority of studies investigating the effects of inhaled corticosteroids on bone formation have been performed on either a population of asthmatic patients[33-35] or on healthy volunteers.[29,36-38] In addition, some of the earlier studies were done in patients using inhaled corticosteroids either in the short term or at higher than routine clinical doses.[34,39] Therefore, we evaluated the effect of inhaled beclomethasone on the levels of osteocalcin, a biochemical marker of bone formation, in COPD patients using long-term corticosteroids as part of their treatment regimen.

MATERIALS AND METHODS

Experimental Subjects

Sixty-five male COPD ambulatory patients with moderate to severe stable airway obstruction were studied in our center. Because serum levels of osteocalcin differ significantly between the sexes and between pre- and postmenopausal ages, only male patients were included. Subjects who had a disease of bone metabolism, malignancy, diabetes mellitus, chronic renal failure, primary or secondary hyperparathyroidism, hyperthyroidism, a history of spontaneous or recently acquired bone fractures, long-term use of oral anticoagulants, or chronic alcoholism were excluded. Since it has been demonstrated that levels of osteocalcin show significant seasonal variations,[40] all of our sample collections and measurements were completed during the fall.

All of the patients had a history of tobacco smoking. The mean duration of tobacco exposure was 28.15 ± 1.84 years (within the range

From the Ataturk Center for Pulmonary Disease and Thoracic Surgery, Kecioren, Ankara, Turkey; Department of Nuclear Medicine, Gazi University, Ankara, Turkey; and the Loeb Research Institute, Ottawa, Ontario, Canada. K1Y 4E9

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Address reprint requests to Murat Oz, MD, PhD, National Institute on Drug Abuse, Intramural Research Program, 5500 Nathan Shock Dr, Baltimore, MD, 21224.

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of 2 to 55 years). On average, the duration of their diagnosed disease was 27.82 ± 1.81 years (in the range of 3 to 45 years). None of the control subjects had received oral or inhaled corticosteroid treatment for at least the last 12 months. Subjects were divided into those using corticosteroids as part of their treatment regimen and those who were not using corticosteroids. Both groups had a similar distribution of age and history of tobacco smoking. In brief, confounding factors such as age, sex, hormonal irregularities, seasonal, and circadian variations were largely eliminated from the present study.

Control group. COPD patients who had not received oral, parenteral, or inhaled corticosteroids during the past year were included in the control group. These patients were also being treated with theophylline, β_2-agonists, and mucolytic agents.

Treatment group. This group included only the COPD patients regularly using inhaled beclomethasone. Patients in this group were receiving corticosteroids at an average dose of 1,142.0 ± 79.64 μg/d for a mean duration of 23.94 ± 2.72 months (in the range of 12 to 72 months). All were using metered-dose inhalation aerosol units without spacer. Patients in this group, as in the control group, were also being treated by theophylline, β_2-agonists, and mucolytic agents. The groups did not differ significantly in age, duration of tobacco smoking, mean forced expiratory volume in second (FEV_1), and average body mass index (Table 1).

Healthy subjects. This group included 20 male, nonsmoking subjects considered healthy based on their general physical examinations and histories. This group did not differ significantly in age and body mass index values from the control and treatment groups (Table 1). The same inclusion criteria described above (presence of systemic disorders, a history of spontaneous or recently acquired bone fractures, chronic alcoholism, etc) were applied to this group.

Each patient participating in the study gave informed written consent. Permission for the study was obtained from the ethics committees of the Ataturk Center for Pulmonary Disease and Thoracic Surgery, and Gazi University, Ankara, Turkey.

Table 1. Summary of the Results for Control, Treatment, and Healthy Subject Groups

	Control	Treatment	Healthy Subjects
No. of subjects	35	30	20
Average age (yr)	61.63 ± 1.84	59.10 ± 2.29	59.92 ± 1.07
FEV_1 (L)	1.12 ± 0.09	1.07 ± 0.08	3.19 ± 0.12†
Body mass index (kg/m^2)	23.6 ± 0.66	22.1 ± 0.69	24.3 ± 0.94
Average tobacco use (yr)	29.04 ± 3.18	26.91 ± 2.78	—
Dose of corticosteroid (μg/d)	—	1,142.0 ± 79.64	—
Duration of corticosteroid use (mo)	—	23.94 ± 2.72	—
Serum Ca^{2+} (mg/dL)	8.63 ± 0.03	8.43 ± 0.03	9.04 ± 0.16
Serum phosphorus (mg/dL)	2.67 ± 0.03	2.95 ± 0.02	3.21 ± 0.12
Alkaline phosphatase (U/L)	167.91 ± 1.49	168.17 ± 1.60	164.81 ± 2.81
Osteocalcin level (ng/mL)	7.03 ± 0.19	3.74 ± 0.12*	11.26 ± 0.42†

NOTE. Values are means ± SEM.

*Significantly different value between control and treatment groups (Student's *t* test; $P < .01$).

†Statistically significant difference between healthy subjects and both control and treatment groups (Student's *t* test; $P < .01$).

Methods

Blood samples were collected by nonvacuum sterile disposable needles (Eczacibasi-Baxter, Istanbul, Turkey) by antecubital puncture. To minimize statistical variations, samples were collected by the same person (M.D.) from each subject. Due to the circadian nature of osteocalcin secretion,[41] blood samples were collected at the same time (2 to 4 PM) of the day. Samples were immediately centrifuged at 3,000 rpm for 3 minutes and stored at -20°C for osteocalcin measurements. Serum levels of the osteocalcin were measured by a Radioimmunoassay technique (Radim SpA, Rome, Italy) using antiserum raised in rabbits to bovine osteocalcin and free ^{125}I-labeled osteocalcin in the laboratory of the Department of Nuclear Medicine, Faculty of Medicine, Gazi University. Coefficients of variation for intra-assay and interassay measurements were 11.8% and 7.8%, respectively. Serum alkaline phosphatase, total calcium, and inorganic phosphorus were measured by standard automated blood chemistry analyzers (Ciba-Corning Express 550, New York, NY) in the Biochemistry laboratory at our center. Statistical data and linear regression analysis were performed using the data analysis software Origin version 5 (Microcal, Northampton, MA). Student's *t* test was performed to compare the differences among the groups. Results were presented as the arithmetic means and SEM of data, and levels of statistical significance were indicated next to each result.

In spirometer recordings, each subject performed at least 3 forced expirations, but not more than 5. FEV was measured for each expiration judged acceptable by the examiner. The average of the best 2 efforts was calculated after correction for body temperature, ambient temperature, and water saturation.

RESULTS

In patients not under corticosteroid treatment (control group), the mean serum level was 7.03 ± 0.19 ng/mL. The average serum level of osteocalcin in patients under long-term corticosteroid treatment (treatment group) was 3.74 ± 0.12 ng/mL. Comparison of mean values indicated that the difference in serum osteocalcin levels between the control group and the treatment group was statistically highly significant (Student's *t* test; $P < .01$)

The average levels of alkaline phosphatase were 167.91 ± 1.49 U/L and 168.17 ± 1.60 U/L for the control group and treatment group, respectively. There was no statistically significant difference between these values ($P > .05$).

The means of the total serum calcium levels were 8.63 ± 0.03 mg/100 mL and 8.43 0.03 mg/100 mL in the control group and treatment group, respectively. These values were not significantly different (Student's *t* test; $P > .05$)

The mean values for inorganic phosphorus levels were 2.67 ± 0.03 mg/100 mL and 2.95 ± 0.02 mg/100 mL for the control group and treatment group, respectively. Although these values were found to be slightly below the normal range, theye were not significantly different (Student's *t* test; $P > .05$). A summary of the results is presented in Table 1.

We have also investigated the possible presence of a linear correlation among daily dosages, duration of treatment, total drug intake, and serum osteocalcin levels. There were no strong correlations between daily dosage ($r = -0.24$) or total intake of beclomethasone ($r = -0.18$) and serum osteocalcin levels. Similarly, no correlation between the duration of treatment and serum osteocalcin levels was found among the patient population using beclomethasone ($r = -0.22$).

In healthy subjects, serum osteocalcin levels were signifi-

cantly higher than both control and treatment groups (Student's t test; $P < .01$). This group did not differ significantly in total alkaline phosphatase, serum calcium, and inorganic phosphorus levels compared to the control and treatment groups (Table 1).

DISCUSSION

The results of our study indicates that the long-term use of the inhaled corticosteroid, beclomethasone, at clinical doses causes a highly significant decrease on the level of osteocalcin in COPD patients.

Since the introduction of inhaled steroids, several studies have investigated their effects on bone formation, but most were performed either in asthmatic patients or in healthy volunteers. Thus, there is a paucity of data on the biochemical indicators of bone formation in COPD patients who are using inhaled corticosteroids in clinically relevant concentrations during long-term treatments.

Earlier studies using short-term (3 months) or long-term oral corticosteroids in asthmatic patients[42,43] demonstrated a decrease of serum osteocalcin levels. In another study, high-dose oral beclomethasone (0.65 mg/h) use in asthmatic patients was investigated,[44] and found to cause a decrease in osteocalcin levels as early as 13 hours after the beginning of treatment.

More recent studies have examined the effect of oral and inhaled corticosteroids on bone formation in either asthmatic patients[35] or in healthy volunteers.[45,46] In almost all of these studies, osteocalcin levels in subjects using oral corticosteroids steroids were found to be lower than the levels found in subjects using inhaled corticosteroids.

As mentioned earlier, the effect of inhaled corticosteroid use on the biochemical markers of bone formation was investigated either in asthmatic patients or in healthy volunteers, and in both cases it was used short-term. This is the first study to examine the effect of long-term inhaled beclamethasone in COPD patients who were taking standard dose. A previous study[39] found that in young and healthy doctors, inhaled beclamethasone (2 mg/d for 28 days) significantly increased the biochemical markers of bone resorption and reduced the serum levels of alkaline phosphatase. Unfortunately, serum osteocalcin levels were not measured. In our study, serum alkaline phosphatase levels were found to be in the normal range in both groups and did not differ between the groups. Although alkaline phosphatase is considered to be one of the biochemical indicators for bone formation, it is not as sensitive and specific as serum osteocalcin.[32] Thus, it is not surprising that studies in which serum osteocalcin levels were found to be decreased also showed serum alkaline phospatase levels to be either increased,[39,47] decreased,[44] or not changed.[29,35,42]

The mean levels of inorganic phosphorus were found to be below normal in both groups, and there was no statistically significant difference between the groups. Total serum calcium levels were slightly below normal values in both corticosteroid-using and nonusing groups.

Interestingly, in our study, COPD patients who are not under corticosteroid treatment were also found to have lower serum osteocalcin levels than the healthy subjects. The average level of osteocalcin measured in 20 healthy, nonsmoking, male subjects with an average age of 59.92 ± 1.07 years was 11.26 ± 0.42 ng/mL (v 7.03 ± 0.19 ng/mL in the control group; $P < .01$, Student's t test). In earlier studies, several factors such as age, sex, and tobacco use have been shown to have significant effects on bone formation and to decrease serum osteocalcin levels.[48,49] Some of these factors in our patient population, such as history of long-term tobacco smoking, may contribute to the lower serum osteocalcin levels in COPD patients who are not receiving inhaled corticosteroids. Since no previous investigation of the levels of osteocalcin in COPD patients versus a healthy control group, we cannot compare this finding with other clinical studies. Nevertheless, in agreement with our findings, tobacco smoking has been demonstrated to be an important factor causing a decrease of serum osteocalcin levels.[49,50] Laroche et al[49] compared 24 volunteers, both tobacco smokers and nonsmokers, and found that osteocalcin levels were significantly lower in smokers. Since all of our COPD patients had a history of tobacco smoking for an average duration of 29 years, lower osteocalcin levels in this patient population who are not using inhaled corticosteroids would also be expected. Because the history of tobacco smoking in asthmatic patients is not as clear a causal factor as it is in COPD patients, these findings may not have been emphasized in earlier investigations focusing on asthmatics. A recent investigation studied the effects of 2 different inhaled corticosteroids, beclomethasone and budesonide, on osteocalcin levels of 33 COPD patients of both sexes and with a wider age range (33 to 75 years). Similar to our results, decreased levels of osteocalcin were reported.[51]

In conclusion, our findings suggest that the use of inhaled corticosteroids decreases serum osteocalcin levels in COPD patients. On the other hand, most of the factors discussed here, including age and history of tobacco smoking, are not necessarily the major causes of lowered serum osteocalcin levels in asthmatic patient populations. Thus, physicians should exercise extreme caution in the treatment of COPD patients with inhaled corticosteroids and should closely monitor patients for the signs and symptoms of osteoporosis.

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REFERENCES

1. Keatings VM, Barnes PJ: Granulocyte activation markers in induced sputum: Comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. Am J Respir Crit Care Med 155:449-453, 1997

2. Confalonieri M, Mainardi E, Della PR, et al: Inhaled corticosteroids reduce neutrophilic bronchial inflammation in patients with chronic obstructive pulmonary disease. Thorax 53:583-585, 1998

3. Pesci A, Balbi B, Cacciani G, et al: Inflammatory cells and mediators in bronchial lavage of patients with chronic obstructive pulmonary disease. Eur Respir J 12:380-386, 1998

4. Pesci A, Majori M, Cuomo A, et al: Neutrophils infiltrating bronchial epithelium in chronic obstructive pulmonary disease. Respir Med 92:863-870, 1998

5. Hudson LD, Monti CM: Rationale and use of corticosteroids in chronic obstructive pulmonary disease. Med Clin North Am 74:661-690, 1990

6. Weir DC, Gove RI, Robertson AA, Burge PS: Corticosteroid trials in non-asthmatic chronic airflow obstruction: A comparison of oral prednisolone and inhaled beclomethasone dipropionate. Thorax 45:112-117, 1990

7. Weir DC, Burge PS: Effects of high dose inhaled beclomethasone dipropionate, 750 micrograms and 1500 micrograms twice daily, and 40 mg per day oral prednisolone on lung function, symptoms, and bronchial hyperresponsiveness in patients with non-asthmatic chronic airflow obstruction. Thorax 48:309-316, 1993

8. Kerstjens HA, Schouten JP, Brand PL, et al: Importance of total serum IgE in airway hyperresponsiveness with inhaled corticosteroids in asthma and COPD. The Dutch CNSLD study group. Am J Respir Crit Care Med 151:360-368, 1995

9. Weiner P, Weiner M, Azgad Y, et al: Inhaled budesonide therapy for patients with stable COPD. Chest 108:1568-1571, 1995

10. Bourbeau J, Rouleau MY, Boucher S: Randomized controlled trial of inhaled corticosteroids in patients with chronic obstructive pulmonary disease. Thorax 53:477-482, 1998

11. Corden Z, Rees PJ: The effect of oral corticosteroids on bronchodilator responses in COPD. Respir Med 92:279-282, 1998

12. Anthonisen NR: Steroids in COPD; The Nearly Eternal Question. Chest 115:3-4, 1999

13. Koyama H, Nishimura K, Mio T, et al: Response to oral corticosteroid in patients with chronic obstructive pulmonary disease. Intern Med 31:1179-1184, 1992

14. Jackevicius CA, Chapmann KR: Prevalence of inhaled corticosteroid use among patients with chronic obstructive pulmonary disease: A survey. Ann Pharmacother 31:160-164, 1997

15. Borron W, deBoisblanc BP: Steroid therapy for chronic obstructive pulmonary disease. Curr Opin Pulm Med 4:61-65, 1998

16. Paggiaro PL, Dahle R, Bakran I, et al: Multicentre randomized placebo-controlled trial of inhaled fluticasone propionate in patients with chronic obstructive pulmonary disease. International COPD Study Group. Lancet 351:773-780, 1998

17. Hodgson SF: Corticosteroid-induced osteoporosis. Endocrinol Metab Clin North Am 19:95-111, 1990

18. Reid IR: Glucocorticoid effects on bone. J Clin Endocrinol Metab 6:1860-1862, 1998

19. Reid IR: Steroid-induced osteoporosis. Osteoporosis Int 3:S213-216, 1997

20. Pearce G, Tabensky DA, Delmas PD, et al: Corticosteroid-induced bone loss in men. J Clin Endocrinol Metab 3:801-806, 1998

21. Toogood JH: Side effects of inhaled corticosteroids. J Allergy Clin Immunol 102:705-713, 1998

22. Rackoff PJ, Rosen CJ: Pathogenesis and treatment of glucocorticoid-induced osteoporosis. Drugs Aging 12:477-484, 1998

23. Toogood JH, Jenning B, Hodsman AB, et al: Effect of dose and dose schedule of inhaled budesonide on bone turnover. J Allergy Clin Immunol 88:572-580, 1991

24. McEvoy CE, Ensrud KE, Bender E, et al: Association between corticosteroid use and vertebral fractures in older men with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 157:704-709, 1998

25. Lipworth BJ: Systemic adverse effects of inhaled corticosteroid therapy: A systematic review and meta-analysis. Arch Intern Med 159:941-955, 1999

26. Efthimiou J, Barnes PJ: Effect of inhaled corticosteroids on bones and growth. Eur Respir J 11:1167-1177, 1998

27. Delany AM, Dong Y, Canalis E: Mechanisms of glucocorticoid action in bone cells. J Cell Biochem 56:295-302, 1994

28. Hauschka PV, Lian JB, Cole DEC, et al: Osteocalcin and matrix G1a protein: Vitamin K-dependent proteins in bone. Physiol Rev 63:990-1047, 1989

29. Peretz A, Praet JP, Bosson D, et al: Serum osteocalcin in the assessment of corticosteroid induced osteoporosis. The effect of long and short term corticosteroids treatment. J Rheumatol 16:363-367, 1989

30. Wolf G: Function of the bone protein osteocalcin: definitive evidence. Nutr Rev 54:332-333, 1996

31. Reid IR: Methods of measurement of bone turnover and clinical evaluation of osteoporosis: Relevance to asthma and corticosteroid therapy. Respir Med 87:9-13, 1993

32. Arnaud CD: Osteoporosis: using bone markers for diagnosis and monitoring. Geriatrics 51:24-30, 1996

33. Puolijoki H, Lippo K, Herrala J, et al: Inhaled beclomethasone decreases serum osteocalcin in postmenopausal asthmatic women. Bone 13:285-288, 1992

34. Boulet LP, Giguere MC, Milot J, et al: Effects of long-term use of high-dose inhaled steroids on bone density and calcium metabolism. J Allergy Clin Immunol 94:796-803, 1994

35. Meeran K, Hattersley A, Burrin J, et al: Oral and inhaled corticosteroids reduce bone formation as shown by plasma osteocalcin levels. Am J Respir Crit Care Med 151:333-336, 1995

36. Pouw EM, Prummel MF, Oosting H, et al: Beclomethasone inhalation decreases serum osteocalcin concentrations. BMJ 302:627-628, 1991

37. Teelucksigh S, Padfield PL, Tibi L, et al: Inhaled corticosteroids, bone formation and osteocalcin. Lancet 338:60-61, 1991

38. Wisniewski AF, Lewis SA, Green DJ, et al: Cross sectional investigation of the effects of inhaled corticosteroids on bone density and bone metabolism in patients with asthma. Thorax 52:853-860, 1997

39. Ali NJ, Capewell S, Ward MJ: Bone turnover during high dose inhaled corticosteroid treatment. Thorax 46:160-164, 1991

40. Douglas AS, Miller MH, Reid DM, et al: Seasonal differences in biochemical parameters of bone remodeling. J Clin Pathol 49:284-289, 1996

41. Nielsen HK, Pedersen B, Brixen K, et al: The effect of single doses of inhaled beclomethasone dipropionate on the circadian rhythm in serum osteocalcin in normals. Acta Endocrinol 128:263-267, 1993

42. Luchert BP, Higgins J C, Stoskopf M: Serum osteocalcin is increased in patients with hyper-thyroidism and decrease in patients receiving glucocorticoids. J Clin Endocrin Metab 35:410-413, 1986

43. Reid IR, Chapman GE, Fraser TR, et al: Low serum osteocalcin levels in glucocorticoid-treated asthmatics. J Clin Endocrin Metab 62:379-383, 1986

44. Ekenstam E, Stalenheim G, Hallgren R: The acute effect of high dose corticosteroid treatment on serum osteocalcin. Metabolism 37: 141-144, 1988

45. Hodsman AB, Toogood JH, Jennings B, et al: Differential effects of inhaled budesonide and oral prednisone on serum osteocalcin. J Clin Endocrinol Metab 72:530-540, 1991

46. Jennings BH, Andersson KE, Johansson SA: Assessment of systemic effects of inhaled glucocorticosteroids: Comparison of the effects of inhaled budesonide and oral prednisolone on adrenal function and markers of bone turnover. Eur J Clin Pharmacol 40:77-82, 1991

47. Morrison D, Ali NJ, Routledge PA, et al: Bone turnover during short course of prednisolone treatment in patient with chronic obstructive airways disease. Thorax 47:418-420, 1992

48. Resch H, Pietschmann P, Kudlacek S, et al: Influence of sex and age on biochemical bone metabolism parameters. Miner Electrolyte Metab 20:117-121, 1994

49. Laroche M, Lasne Y, Felez A, et al: Osteocalcin and smoking. Rev Rhum Ed Fr 61: 433-436, 1994

50. Brot C, Jorgensen NR, Sorensen OH: The influence of smoking on vitamin D status and calcium metabolism. Eur J Clin Nutr 53:920-926, 1999

51. Struijs A, Mulder H: The effects of inhaled glucocorticoids on bone mass and biomedical markers of bone homeostasis: A 1 year study of beclomethasone versus budesonide. Neth J Med 50:233-237, 1997