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Clinical aspects of allergic disease

**Dose-related decrease in bone density among
asthmatic patients treated with
inhaled corticosteroids**

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Background: *Inhaled corticosteroids are being prescribed more commonly and in higher doses than previously in the management of asthma. Although these topically active compounds have less potential for systemic impact than oral steroids, biochemical markers suggest that they are not devoid of systemic side effects. We conducted this study to investigate the effect of commonly prescribed doses of inhaled steroids on bone density.*

Methods: *We studied 36 patients with asthma. Those in group A (n = 18) had been taking inhaled beclomethasone dipropionate or budesonide in a dosage of 800 µg or more per day for at least 1 year. Those in group B (n = 18) had used only bronchodilator therapy. Adrenal function was assessed by morning serum cortisol level and by short adrenocorticotrophic hormone stimulation test. Bone turnover was assessed by measurement of serum osteocalcin, alkaline phosphatase, and urinary pyridinium cross-links. Bone mineral density was measured by dual-energy x-ray absorptiometry with a Hologic QDR-1000 densitometer (Hologic Inc., Waltham, Mass.).*

Results: *Group A, mean age (SD) = 36.6 (8.4) years, had used inhaled corticosteroids at a mean dose of 1323 µg/day (range, 800 to 2000 µg/day) for a median duration of 24 months. Group B, mean age (SD) = 33.4 (8.1) years, had not been taking any form of steroid. Four patients from group A had suppressed morning serum cortisol; three of these had abnormal adrenocorticotrophic hormone stimulation test results. All patients in group B had normal baseline adrenal function and an appropriate response to adrenocorticotrophic hormone. Mean serum osteocalcin level in group A was significantly lower than that in group B (8.8 vs 14.2 ng/ml, $p = 0.0003$). Bone density measurements showed parallel changes: in group A the mean Z score (SD) of the femoral neck was -0.78 (1.02), significantly below predicted normal values ($p = 0.0025$). Mean Z scores of the lumbar spine and of femoral Ward's triangle were not significantly reduced. In group B the mean Z scores of the lumbar spine, femoral neck, and femoral Ward's triangle were all within normal limits. In group A the dose*

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duration of inhaled corticosteroid therapy corrected for body mass index correlated negatively with bone density and adrenal function measurements.

Conclusion: *We conclude that the regular use of conventional doses of inhaled corticosteroids by patients with asthma can suppress adrenal function and decrease bone density in a dose-related fashion. (J ALLERGY CLIN IMMUNOL 1995;96:571-9.)*

Key words: *Asthma, inhaled corticosteroids, bone density, adrenal gland*

Abbreviations used

ACTH:	Adrenocorticotrophic hormone
BDP:	Beclomethasone dipropionate
DXA:	Dual-energy x-ray absorptiometry
SD:	Standard deviation

Antiinflammatory medications are increasingly recommended as initial or early treatment for symptomatic asthma.¹⁻³ The most frequently prescribed antiinflammatory medications are the inhaled corticosteroids. There is evidence from several countries that inhaled corticosteroids are being prescribed more frequently and in higher daily dosages than was previously common.⁴ For most patients such therapy is likely to be long-term, leading to increased concerns regarding systemic absorption and possible systemic side effects.⁵⁻⁸ These effects may include adrenal suppression,⁹ disturbed bone metabolism,¹⁰ decreased growth in children,¹¹ skin thinning,¹² behavioral changes,¹³ and alterations in carbohydrate metabolism.¹⁴

Prolonged oral steroid therapy in patients with asthma is associated with an increased incidence of fractures.¹⁵⁻¹⁷ The detrimental effects of inhaled corticosteroids on bone metabolism are assumed to be much less, although long-term studies of the effect of inhaled steroids on bone density are few and inadequately controlled.¹⁸⁻²³ Short-term changes in biochemical markers of bone turnover have been demonstrated in several studies.^{24, 25} However, the clinical relevance of these biochemical changes is unknown.

We therefore chose to measure bone density and biochemical markers of bone turnover in patients with asthma taking conventional doses of inhaled corticosteroids for more than 1 year and to compare the results with those of age- and sex-matched patients with asthma not receiving steroid medications. We also assessed adrenal function to determine whether any observed changes in bone density would be paralleled by systemic impact in another organ system.

METHODS

Patients

In a cross-sectional controlled study, we examined 36 patients with asthma (12 men and 24 women) recruited from a large tertiary care asthma facility. These patients were divided into two groups. Patients in both groups had been diagnosed with asthma according to the American Thoracic Society criteria²⁶ and were between 18 and 50 years old. Patients in group A had been self-administering inhaled steroids (either beclomethasone dipropionate or budesonide) in a dose of at least 800 µg/day for at least 1 year. Subjects were excluded from the study if they had received more than two rescue courses of systemic steroid treatment at any time in their lives or any course of steroid treatment in the year before the study; a course was defined as any duration of therapy from 1 day to 3 weeks. Dose duration of inhaled steroid therapy for each subject was calculated as the product of the average daily dose in grams and duration of therapy in months. Previous studies examining the systemic effect of inhaled steroids have used daily dose or dose duration. It is likely that the impact of systemically absorbed medication will vary according to body size, and we have therefore expressed the amount of steroid used as (average daily dose × duration) ÷ body mass. Group B comprised patients with asthma who had never required inhaled or oral steroid medication for treatment of asthma.

All women were premenopausal, and none of the patients had a history of osteoporosis, metabolic bone disease, or ingestion of medication known to interfere with bone metabolism. A questionnaire was administered to all patients at the initial encounter, including questions on asthma severity, asthma treatment, and customary levels of physical exercise. As described and validated previously, activity at work and during recreation was quantified as hours of activity per week; activity was defined as physical work that would result in at least a doubling of the basal metabolic rate.^{27, 28} Patients were ranked by the amount of activity per average week: grade 1, less than 1 hour; grade 2, 1 to 3 hours; grade 3, 3 to 6 hours; grade 4, more than 6 hours. Spirometry was performed on all study patients. Written informed consent was obtained from all patients, and approval for the study was given by the local human ethics committee.

Bone turnover

After overnight fasting, serum was collected at 9:00 AM for measurement of serum osteocalcin, calcium, phosphate, and alkaline phosphatase. Serum osteocalcin was measured by radioimmunoassay with a method modified from that of Price and Nishimoto.²⁹ Assay mixtures contained 50 μ l of assay diluent, 50 μ l of sample or standard, 100 μ l of antiserum, and 100 μ l of iodine 125-labeled osteocalcin. The reaction mixtures were incubated at 4° C for 48 hours. Assays were terminated by the addition of 100 μ l of goat antiserum to rabbit γ -globulin and 500 μ l of polyethylene glycol. The sensitivity of the assay was 0.02 ng/tube. During fasting a urine sample from each patient was analyzed for pyridinoline and deoxypyridinoline cross-links. Pyridinoline and deoxypyridinoline cross-links are present in type 1 collagen of bone and are released during its breakdown; they are thus thought to be sensitive markers of bone resorption. Pyridinoline and deoxypyridinoline cross-links were measured by ion-paired reversed-phase high-performance liquid chromatography with a synthetic pyridinoline as an internal standard.³⁰

Adrenal function tests

Adrenal function was assessed by a short cosyntropin test. Subjects were requested to not administer their inhaled steroid on the morning of the adrenal stimulation testing. Serum was sampled for cortisol at 9:00 AM and 30 minutes after intravenous injection of 250 μ g of cosyntropin (a synthetic subunit of adrenocorticotrophic hormone [ACTH]). Reference range for 9:00 AM serum cortisol levels in our biochemistry laboratory is 170 to 660 nmol/L. The stimulation test was considered abnormal if (1) the baseline serum cortisol level was less than 170 nmol/L, (2) the increment after cosyntropin stimulation was less than 200 nmol/L above basal level, or (3) the 30-minute serum cortisol level was less than 500 nmol/L. Serum cortisol was measured by radioimmunoassay with a modification of the competitive binding protein method (Radiochemical Centre, Amersham, Amersham, U.K.).

Bone density measurement

Bone mineral density was measured at the lumbar spine (anteroposterior view L1 through L4) and the left femoral neck and Ward's triangle by dual-energy x-ray absorptiometry (DXA) with a standard single QDR-1000 x-ray densitometer (Hologic Inc., Waltham, Mass.). Previously published in vivo control studies have documented a reproducibility of 0.4% for the lumbar spine and 1.7% for the femoral neck.³¹ In our laboratory we have documented a reproducibility of 2% for both lumbar spine and femoral neck. Z score values for bone density were calculated for each patient, representing the number of standard deviations (SDs) above or below the statistical mean for previously tested age- and sex-matched healthy men and women.

TABLE I. Patient characteristics

	Treatment group (n = 18)	Control group (n = 18)
Gender (F:M)	12:6	12:6
Mean age (yr)	36.55	33.39
SD	8.37	8.10
Median activity grade	3	3
Range	1-4	2-4
Mean FEV ₁ /FVC	76.38	75.11
SD	10.4	10.5
Mean BMI (kg/m ²)	24.62	25.04
SD	3.82	5.16

FEV₁, Forced expiratory volume in 1 second; FVC, forced vital capacity; BMI, body mass index.

Statistical analysis

The continuous data were first tested for normal (Gaussian) distribution by the Shapiro-Wilk test within each of the control and treatment groups.³² No measure was found to deviate significantly from a normal distribution (all $p > 0.05$). Therefore because parametric statistical devices were appropriate for the data, mean values for the two groups were compared by means of the two-group t test, and the relationship between variables within a group was tested with the Pearson product-moment correlation coefficient. Nominal data were compared by means of Fisher's exact probability test. Because we did not expect an increase in bone density with the use of inhaled corticosteroids, one-sided tests were appropriate, and therefore one-tailed p values are reported. The upper limit to the type 1 error (alpha or statistical significance) was set to 0.05. For all statistical analyses, SAS version 6.04 (SAS Institute, Cary, N.C.) was used as computational software.

Power calculations indicate that 18 patients in each group provided the t tests and the correlational analyses with 0.80 statistical power, when alpha (one-tailed) is set to 0.05 and the expected mean population differences between the two groups on a variable are at least 0.84 SD units³³ or the expected population correlation coefficients are at least 0.55.³⁴

RESULTS

Patients

Patients in both groups were comparable regarding age, sex, body mass index, forced expiratory volume in 1 second/forced vital capacity and grade of physical activity (Table I). All patients were white. Ten patients in group A were using beclomethasone dipropionate (BDP) delivered by aerosol inhaler, and eight were using budesonide delivered as dry powder by Turbuhaler (Astra Pharma Inc., Canada). Half the patients taking BDP used an add-on spacer device. All treated patients demonstrated adequate technique in using the inhaler device. Eight of the

TABLE II. Results of biochemical measurements in both groups

	Treatment group	Control group
9:00 AM serum cortisol (nmol/L)	344.61 ± 212.4	429.00 ± 90.8
30-minute serum cortisol (nmol/L)	601.89 ± 230.85*	734.94 ± 105.7
Serum osteocalcin (ng/ml)	8.2 ± 3.94*	14.2 ± 4.08
Urinary pyridinoline (nmol/mmol creatinine)	49.97 ± 28.26	43.41 ± 26.59
Urinary deoxypyridinoline (nmol/mmol creatinine)	7.85 ± 3.23	8.96 ± 1.87
Serum alkaline phosphatase (IU/L)	63.00 ± 13.66	62.76 ± 17.52

All values are expressed as means ± SD.

* $p \leq 0.05$.

patients in group A had never taken systemic steroids; 10 had had one or two short courses of oral steroids (<3 weeks) at some time in the past but not within a year before being enrolled in the study. Inhaled steroids were used in a mean dose (SD) of 1323 (393.8) $\mu\text{g/day}$ (budesonide = 1075 μg , BDP = 1525 μg) for a mean duration (SD) of 29.8 (19.5) months.

Biochemical tests

Serum osteocalcin was significantly lower in group A as compared with group B ($p = 0.0003$). There were no significant differences between the two groups in serum alkaline phosphatase or in urinary pyridinoline and deoxypyridinoline (Table II). Four patients from group A had suppressed 9:00 AM serum cortisol levels and three had subnormal serum cortisol levels after ACTH stimulation, whereas all patients in group B had normal baseline adrenal function and response to ACTH. Baseline 9:00 AM cortisol levels, 30-minute cortisol levels, and rise of cortisol after ACTH correlated negatively with the dose duration of inhaled steroid therapy corrected to the BMI (Fig. 1). Serum osteocalcin levels did not correlate with the dose duration of inhaled steroid therapy.

Bone density

In group A the mean bone density of the femoral neck was significantly below predicted normal values when expressed as Z scores: -0.78 (1.02), $p = 0.0025$. In group B the mean bone density of

the femoral neck was within normal limits: -0.18 (1.48), $p = 0.13$. There was no statistical difference in the mean bone density of the femoral neck between group A and group B (-0.78 vs -0.18 , respectively). The mean bone density measures of the lumbar spine and femoral Ward's triangle, although numerically lower in group A than in group B, were not significantly below normal in either group. For group A values were -0.27 (0.76) and -0.19 (1.12), respectively; for group B values were $+0.04$ (1.22) and $+0.32$ (1.39). Bone density scores of the lumbar spine, femoral neck, and Ward's triangle correlated negatively with the dose duration of inhaled steroid therapy (Fig. 1). In group A only, Z scores of the lumbar spine, femoral neck, and Ward's triangle correlated positively with baseline (9:00 AM) and 30-minute (after ACTH) serum cortisol levels ($p < 0.05$) (Figs. 2 and 3). Serum osteocalcin levels did not correlate with the bone density of the different regions tested.

DISCUSSION

Our data show that the long-term use of inhaled corticosteroids by patients with asthma may be accompanied by a reduction in bone density not seen when patients with asthma are treated with bronchodilators alone. Concurrent reduction in the serum osteocalcin levels of patients with asthma using inhaled steroids suggests that suppressed osteoblast function may play a role in this phenomenon. Patients with asthma who are taking inhaled corticosteroids may also show biochemical signs of adrenal suppression, and biochemical assays may be useful in identifying with bone density loss. In this study we have demonstrated a reduction in bone density measured at the femoral neck by DXA in a group of patients with asthma using inhaled corticosteroids (800 to 2000 $\mu\text{g/day}$) for 29.8 ± 19.5 months when compared with age- and sex-matched healthy control subjects. Although a single report suggested that bone density of patients with asthma not treated with steroids tends to be lower than the values of normal healthy subjects,¹⁸ we could identify no significant reduction in bone density in patients with asthma not treated with steroids.

Our findings are in support of those of Packe et al.¹⁸ who demonstrated an 18% reduction in lumbar spine bone density measured by quantitative computerized tomography in a group of patients with asthma taking high doses of inhaled steroids for a median duration of 3 years. However, the role

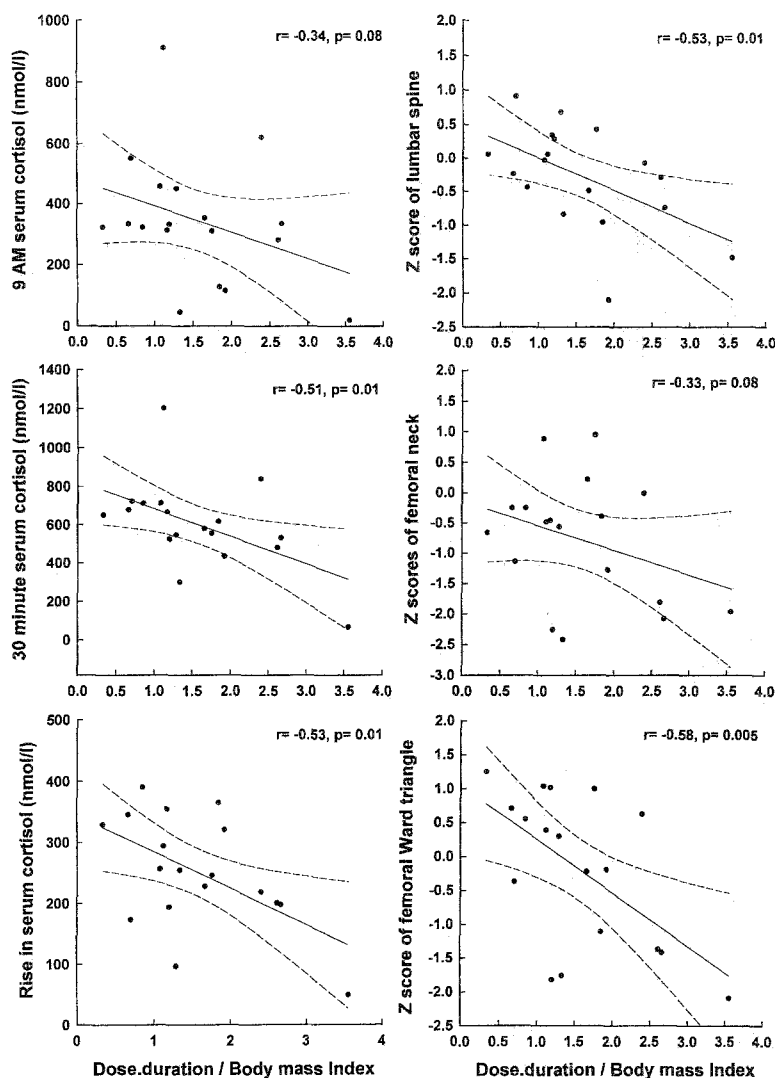


FIG. 1. Correlation between measures of adrenal function and bone density and the dose duration of inhaled corticosteroid therapy divided by the body mass index. The dotted lines represent the 95% confidence limits. The solid line represents the regression line. The coefficients shown represent correlation coefficients.

of inhaled steroids in their cohort of patients ($n = 20$) is unclear; all had received booster courses of oral steroids in the past and half took oral steroids within the year before the study. Reid et al.¹⁹ showed that total body calcium was somewhat reduced (8.8%) in a group of patients receiving only inhaled steroids (BDP) in doses up to 800 $\mu\text{g}/\text{day}$. However, the mean age in their control group was significantly lower than the mean age in the inhaled steroid group, and some of the patients taking inhaled steroids also received booster courses of oral steroids.²⁰ Other studies examining changes in bone mass and density in patients with asthma treated with inhaled steroids are flawed either because of failure to control for previous

oral steroid use²¹ or because the dose of inhaled steroid taken by the patients studied was too low.^{22, 23} In our study none of the patients treated with inhaled steroids had received booster courses of oral steroids within a year of the study, and only 10 had received one or two courses of oral steroids of 21 days or less each in their lives. Although we cannot rule out the possibility that remote doses of oral corticosteroid accounted for the reductions in bone density we observed, we consider it unlikely for two reasons: (1) the reduced serum osteocalcin levels in patients taking only inhaled corticosteroids strongly suggests that osteoblast function is impaired by inhaled corticosteroids alone; and (2) the subjects were rarely exposed to courses of

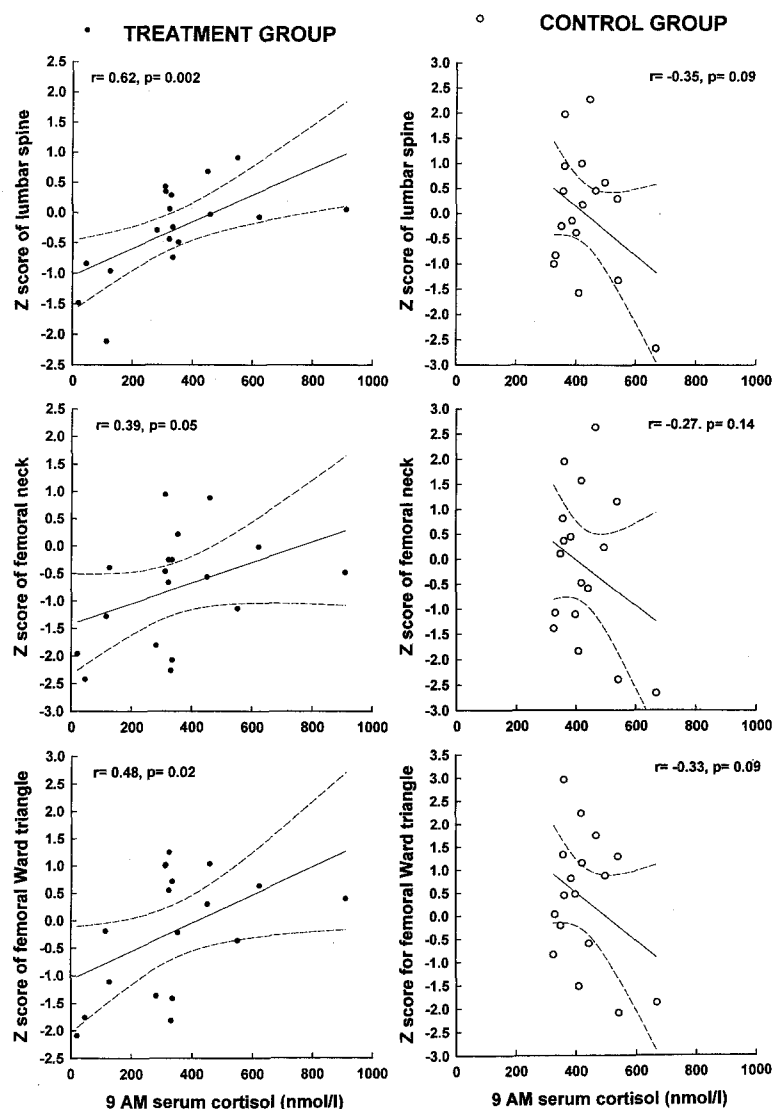


FIG. 2. Correlation between baseline adrenal function (9:00 AM serum cortisol level) and Z scores of lumbar spine, femoral neck, and Ward's triangle in both groups of asthmatic patients. Patients in group A are represented by *filled circles*, and those in group B by *open circles*. *Dotted lines* represent the 95% confidence limits. The *solid line* represents the regression line. The coefficients shown represent correlation coefficients.

systemic steroids, and none had received systemic steroids during the year before entering the study.

DXA is a technique that provides a rapid and accurate measurement of bone density.³⁵ In a recent study bone density of the hip measured by DXA was shown to correlate strongly with the risk of subsequent hip fracture among older women.³⁶ In that study there was a 2.6-fold increased risk for hip fracture for each SD decrease in femoral neck density. Our study is therefore of clinical relevance because we have shown a decrease in femoral neck bone density by a mean value of 0.78 SD in the patients treated with inhaled steroids. Our patient

population did not include postmenopausal women, but our results suggest that a prospective, controlled study of this high-risk group of patients is needed. Although our results show statistically significant differences in bone density at the femoral neck, the clinical significance of a change of less than 1 Z score is not clear. Furthermore, because serial measurements of bone density have not been undertaken in this study, it is not known whether the apparent decrease in bone density is self-limited or progressive. Although steroid-induced bone loss is expected to be more pronounced in trabecular bone-rich sites, such as ribs

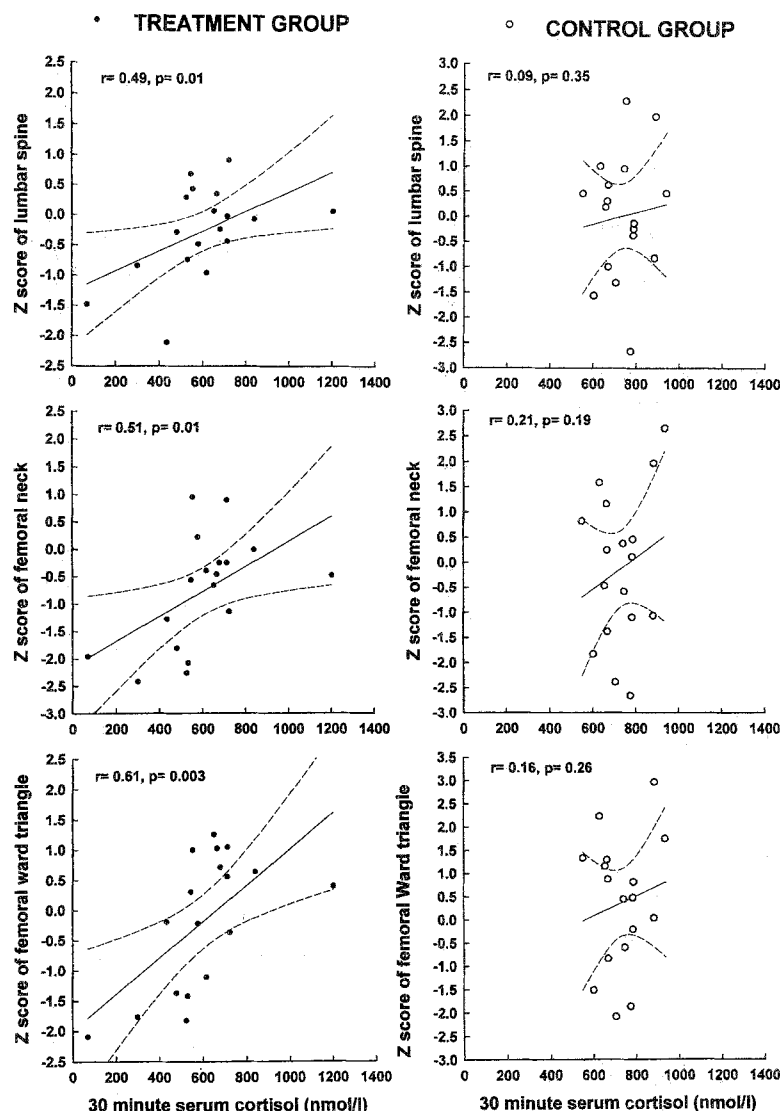


FIG. 3. Correlation between 30-minute serum cortisol levels and Z scores of lumbar spine, femoral neck, and Ward's triangle in both groups of asthmatic patients. Patients in group A are represented by filled circles, and those in group B by open circles. Dotted lines represent the 95% confidence limits. The solid line represents the regression line. The coefficients shown represent correlation coefficients.

and vertebrae, our study supports other reports showing that bone loss caused by steroid treatment can be detected at the hip, as well as at the lumbar spine.^{37, 38} One study suggests that measurement of bone density at the hip may even be preferable to that at the lumbar spine because this measurement is not influenced by osteophytes and aortic calcification, which might artifactually increase lumbar spine bone density estimations.³⁷ However, bone density measurements at the femoral neck, lumbar spine, and Ward's triangle in the group treated with inhaled steroids showed nonstatistically significant trends toward being below that of the group

not treated with inhaled steroids. Studies in larger numbers of patients would be helpful in further assessing the statistical significance of these apparent trends.

Several short-term studies of healthy subjects receiving high doses of inhaled BDP or budesonide have demonstrated a decrease in the osteoblast function represented by a dose-related suppression of serum osteocalcin level.^{24, 39, 40} In one such study serum alkaline phosphatase levels were also suppressed.²⁵ Ali et al.²⁵ examined subjects given BDP in a dose of 2000 µg/day for 1 month and found increased bone resorption reflected by a statisti-

cally significant rise in urinary output of hydroxyproline, a breakdown product of the osteoid matrix, but a comparable dose of budesonide was not associated with increased bone resorption. In this study and in concordance with the previous studies, we found significant decreases in serum osteocalcin in the patients treated with steroids when compared with the asthmatic patients not treated with steroids, suggesting a suppressed osteoblast function. However, we could not demonstrate any changes in the urinary pyridinoline and deoxypyridinoline levels, which are thought to be more sensitive markers of bone resorption than urinary hydroxyproline.⁴¹ This paradox could be explained if bone loss had occurred at the initial period of administration of the inhaled steroid and then stopped while bone formation continued to be suppressed. This possibility is supported by the fact that bone loss is most rapid during the first few months of treatment in patients taking systemic steroids.^{42, 43}

Our results of adrenal function testing confirm many previous observations showing that hypothalamo-pituitary-adrenal axis suppression may occur in some patients receiving high doses of inhaled steroids. The short ACTH stimulation test has been shown to be as useful as urinary free cortisol estimation in testing for hypothalamo-pituitary-adrenal axis suppression in patients with asthma receiving high doses of inhaled steroids.⁴⁴ The correlation between Z score and serum cortisol level suggests that there may be an association between the ability of inhaled steroids to alter serum cortisol and to decrease bone density. Serum cortisol may therefore play a role as an easily measured marker for identifying patients who are susceptible to the systemic effects of inhaled steroids, including osteoporosis. However, the association is weak, and interpatient variability would likely limit the clinical utility of such studies.

The potential for systemic absorption with subsequent effects on various organ systems may vary somewhat with different preparations of inhaled steroids and different delivery systems. The population in this study is not large enough to control for such factors. Future studies must control for type of inhaled steroid, formulation (dry powder or aerosol), presence and type of spacers, and mouth rinsing.

In summary, although inhaled steroids continue to be an effective antiinflammatory maintenance therapy for asthma, long-term use may have potentially serious side effects, such as loss of bone with consequently increased risk of fracture. Pro-

spective studies are needed to confirm the changes we have observed, to determine their time course, to define the characteristics of patients at risk, and to detect any factors that could potentiate or reduce such systemic effects.

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REFERENCES

1. International Consensus Report on Diagnosis and Treatment of Asthma. *Eur Respir J* 1992;5:601-41.
2. British Thoracic Society. Guidelines on the management of asthma. *Thorax* 1993;48(suppl):S1-24.
3. Hargreave F, Dolovich J, Newhouse MT. The assessment and treatment of asthma: a conference report. *J ALLERGY CLIN IMMUNOL* 1990;85:1098-111.
4. Kesten S, Rebuck AS, Chapman KR. Trends in asthma and chronic obstructive pulmonary disease therapy in Canada, 1985 to 1990. *J ALLERGY CLIN IMMUNOL* 1993;92:499-506.
5. Barnes NC. Safety of high-dose inhaled corticosteroids. *Respir Med* 1993;87(suppl A):27-31.
6. Geddes DM. Inhaled corticosteroids: benefits and risks. *Thorax* 1992;47:404-7.
7. Toogood JH. Complications of topical steroid therapy in asthmatics. *Am Rev Respir Dis* 1990;141:S89-96.
8. Barnes PJ, Pedersen S. Efficacy and safety of inhaled corticosteroids in asthma. *Am Rev Respir Dis* 1993;148:S1-S26.
9. Brown PH, Blundell G, Greening AP, Crompton GK. Hypothalamo-pituitary-adrenal axis suppression in asthmatics inhaling high dose corticosteroids. *Respir Med* 1991;85:501-10.
10. Ward MJ. Inhaled corticosteroids - effect on bone? *Respir Med* 1993;87(suppl A):33-6.
11. Wolthers OD, Pedersen S. Growth of asthmatic children during treatment with budesonide: a double blind trial. *BMJ* 1991;303:163-5.
12. Mak VHF, Melchor R, Spiro SG. Easy bruising as a side-effect of inhaled corticosteroids. *Eur Respir J* 1992;5:1068-74.
13. Connett G, Lenney W. Inhaled budesonide and behavioural disturbances. *Lancet* 1991;338:634-5.
14. Kruszynska YT, Greenstone M, Home PD, Cooke NJ. Effect of high dose inhaled beclomethasone dipropionate on carbohydrate and lipid metabolism in normal subjects. *Thorax* 1987;42:881-4.
15. Adinoff AD, Hollister JR. Steroid induced fractures and bone loss in patients with asthma. *N Engl J Med* 1983;309:265-8.
16. Luengo M, Picado C, Del Rio L, Guanabens N, Montserrat JM, Setoain J. Vertebral fractures in steroid dependent asthma and involutional osteoporosis: a comparative study. *Thorax* 1991;46:803-6.
17. Rueggsegger P, Medici TC, Anliker M. Corticosteroid-induced bone loss. A longitudinal study of alternate day therapy in patients with asthma using quantitative computed tomography. *Eur J Clin Pharmacol* 1983;25:615-20.
18. Packe GE, Douglas JG, McDonald AF, Robins SP, Reid DM. Bone density in asthmatic patients taking high dose

- inhaled beclomethasone dipropionate and intermittent systemic corticosteroids. *Thorax* 1992;47:414-7.
19. Reid DM, Nicoll JJ, Smith MA, et al. Corticosteroids and bone mass in asthma: comparisons with rheumatoid arthritis and polymyalgia rheumatica. *BMJ* 1986;293:1463-6.
 20. Crompton GK. Corticosteroids and bone mass in asthma. *BMJ* 1987;294:123.
 21. Stead RJ, Horsman A, Cooke NJ, Belchetz P. Bone mineral density in women taking inhaled corticosteroids. *Thorax* 1990;45:792.
 22. Wolff AH, Adelsberg B, Aloia J, Zitt M. Effect of inhaled corticosteroid on bone density in asthmatic patients: a pilot study. *Ann Allergy* 1991;67:117-21.
 23. Luengo M, Del Rio L, Guanabens N, Picado C. Long-term effect of oral and inhaled glucocorticoids on bone mass in chronic asthma. A two year follow-up study. *Eur Respir J* 1991;(suppl 14):342S.
 24. Toogood JH, Jennings B, Hodsman AB, et al. Effects of dose and dosing schedule of inhaled budesonide on bone turnover. *J ALLERGY CLIN IMMUNOL* 1991;88:572-80.
 25. Ali NJ, Capewell S, Ward MJ. Bone turnover during high dose inhaled corticosteroid treatment. *Thorax* 1991;46:160-4.
 26. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225-44.
 27. Makrides L, Heigenhauser GJ, McCartney N, Jones NL. Maximum short term exercise capacity in healthy subjects aged 15-70 years. *Clin Sci* 1985;69:197-205.
 28. Garfinkel SK, Kesten S, Chapman KR, Rebuck AS. Physiologic and nonphysiologic determinants of aerobic fitness in mild to moderate asthma. *Am Rev Respir Dis* 1992;145:741-5.
 29. Price PA, Nishimoto SK. Radioimmunoassay for the vitamin K-dependent protein of bone and its discovery in plasma. *Proc Natl Acad Sci* 1980;77:2234-8.
 30. Pratt DA, Daniloff Y, Duncan A, Robins SP. Automated analysis of the pyridinium crosslinks of collagen in tissue and in urine using solid-phase extraction and reversed-phase high-performance liquid chromatography. *Anal Biochem* 1992;207:168-75.
 31. Brailion P, Duboeuf F, Meary MF, et al. Mesure du contenu minéral osseux par radiographie digitale quantitative. Premiers résultats au niveau vertébral lombaire. *Presse Med* 1989;18:1062-5.
 32. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika* 1965;52:591-611.
 33. Lachin JM. Introduction to sample size determination and power analysis for clinical trials. *Control Clin Trials* 1981;2:93-113.
 34. Cohen J. Statistical power analysis for the behavioural sciences. Hillsdale, New Jersey: Lawrence Erlbaum Associates, 1988:75-108.
 35. Johnston CC, Slemenda CW, Melton LJ. Clinical use of bone densitometry. *N Engl J Med* 1991;324:1105-9.
 36. Cummings SR, Black DM, Nevitt MC, et al. Bone density at various sites for prediction of hip fractures. *Lancet* 1993;341:72-5.
 37. Reid IR, Evans MC, Wattie DJ, Ames R, Cundy TF. Bone mineral density of the proximal femur and lumbar spine in glucocorticoid-treated asthmatic patients. *Osteoporos Int* 1992;2:103-5.
 38. Sambrook P, Kemper S, Kelly P, et al. Corticosteroid effects on proximal femur bone loss. *J Bone Miner Res* 1990;5:1211-6.
 39. Pouw EM, Prummel MF, Oosting H, Roos CM, Endert E. Beclomethasone inhalation decreases serum osteocalcin concentrations. *BMJ* 1991;302:627-8.
 40. Teelucksingh S, Padfield PL, Tibi L, Gough KJ, Holt PR. Inhaled corticosteroids, bone formation, and osteocalcin. *Lancet* 1991;338:60-1.
 41. Robbins SP, Black D, Paterson CR, Reid DM, Duncan A, Seibel MJ. Evaluation of urinary hydroxypyridinium crosslinks measurement as resorption markers in metabolic bone disease. *Eur J Clin Invest* 1991;21:310-5.
 42. Reid DM. Corticosteroid-induced osteoporosis. In: Smith R, ed. Osteoporosis. London: Royal College of Physicians of London, 1990:99-117.
 43. Gennari C. Glucocorticoids and bone. In: Peck WA, ed. Bone and mineral research/3. Amsterdam: Elsevier Publishers BV, 1985:213-32.
 44. Brown PH, Blundell G, Greening AP, Crompton GK. Screening for hypothalamo-pituitary-adrenal axis suppression in asthmatics taking high dose inhaled corticosteroids. *Respir Med* 1991;85:511-6.

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