Cross sectional investigation of the effects of inhaled corticosteroids on bone density and bone metabolism in patients with asthma

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Abstract

Background – Bone mineral density has been reduced in patients with asthma taking inhaled corticosteroids in some cross sectional studies and this could be important if treatment is continued for several decades. The possibility of confounding by age, menopausal status, physical activity and, especially, past oral steroid use has not been excluded in most studies. The present study was designed to assess the magnitude of any reduction in bone mineral density in relation to inhaled steroid use after adjusting for these factors.

Methods – Bone mineral density (BMD), vertebral fractures, and markers of bone metabolism (serum osteocalcin, procollagen peptide I, bone-specific alkaline phosphatase, and urinary deoxypyridinoline cross links) were measured in 81 patients with asthma age 20–40 years; 34 patients (19 men) who had never had inhaled or systemic steroids and 47 (19 men) who had taken inhaled steroids for at least five years with limited exposure to systemic steroids in the past. Data relating to past medication use, physical activity, smoking, and other confounding factors were collected by questionnaire. The relation between inhaled steroid dose and duration and BMD was assessed by linear regression analysis, accounting for potential confounders including weight, exercise, and oral steroid use.

Results – The 47 patients taking an inhaled steroid had a mean current dose of 620 µg/day (range 100–3000 µg), a mean duration of use of 7.8 years, and had had a mean of 0.85 courses of prednisolone in the past. There was no significant difference in mean BMD values between those who were and those who were not on inhaled steroids in men or women. However, on multivariate analysis, cumulative inhaled steroid dose was associated with a reduction in posterior-anterior (P-A) and lateral lumbar spine bone mineral density in women, equivalent to a 0.11 standard deviation reduction in bone density per 1000 µg/day inhaled steroid per year after adjustment for potential confounding factors (95% CI for P-A spine 0.01 to 0.22; for lateral spine 0.02 to 0.21). Previous oral steroid use was not an important confounding factor in these patients. Inhaled steroid use was not related to BMD at the wrist or hip in women or at any skeletal site in men. Women taking an inhaled steroid had lower levels of serum osteocalcin than those not taking them, although this was not dose related. Inhaled steroid use was not associated with differences in other markers of bone metabolism in men or women or with the presence of vertebral fractures.

Conclusions – Although an effect of confounding factors cannot be excluded entirely in a cross sectional study, our findings are in keeping with an effect of inhaled steroid therapy in reducing bone density in the spine in women and provide an estimate of the magnitude of this effect.

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Long term use of oral corticosteroids is associated with decreased bone density and an increased risk of fracture.[1–3] Inhaled corticosteroids have a considerably better safety profile than oral steroids and are used widely and effectively to treat asthma. Recent studies that have looked at the effect of inhaled steroids on bone mineral density (BMD) have yielded contradictory results, with some showing a reduction in BMD in relation to inhaled steroid use[4–6] whilst others have not.[7–13] Studies of biochemical markers of bone metabolism have shown a reduction in serum osteocalcin concentration with the introduction of inhaled steroids in healthy volunteers[14–17] and patients with asthma,[18] whereas other markers, such as alkaline phosphatase[4 7 19 20] and procollagen peptide I (PCP-I),[18] have shown less consistent results or, in the case of urinary pyridinoline and deoxypyridinoline cross links, no relation to inhaled steroid use.[4]

Studying the effects of inhaled steroids on BMD in cross sectional studies is complicated by the possibility of confounding by other factors that affect BMD, such as previous use of oral steroids, age, menopausal status, and physical activity. Most studies have not made adequate allowance for these factors. Past use of oral steroids may have contributed to the reduction in BMD seen with inhaled steroid use in some studies.[1 4 5] Age is also important since studies in children are confounded by differences in growth velocity and hormonal

changes around puberty, and because asthma can delay the onset of puberty.[21] Some studies in adults have included postmenopausal women who may have widely differing rates of bone loss.[179] Although physical activity is important for skeletal remodelling[22 23] and may be reduced in some patients with asthma,[24] it has only been assessed in three of the previous studies.[469]

The present study was designed to reduce the chances of confounding by age, menopausal status, and previous use of systemic steroids and to incorporate these factors, differences in physical activity, and other potential confounding factors into the analysis.

Methods

SUBJECTS

Men and women aged 20–40 years with a documented history of asthma were recruited from our asthma register and from local general practices. They were selected according to predetermined criteria for group 1 if they were using an inhaled β_2 agonist only for their asthma and had never received inhaled, systemic, or topical corticosteroids apart from dermal steroids, and for group 2 if they had used an inhaled steroid for at least five years with no systemic steroids in the past six months and limited use of systemic steroids in the past. Patients were only included in group 2 if their total exposure to oral steroids over the past five years was less than 2000 mg prednisolone or equivalent (plus a maximum daily dose of 60 mg, total duration of treatment <12 weeks, and no more than two courses in one year) and if hydrocortisone by injection did not exceed a total dose of 3200 mg on two occasions. Patients were excluded from both groups if they had been immobilised for long periods, had a history of alcohol or drug abuse, had ever had a metabolic disease which might affect bone density – for example, diabetes, chronic renal or hepatic disease – or if they had used drugs known to affect bone metabolism – for example, calcium supplements, sodium fluoride, >400 mg/day vitamin D, calcitonin, bisphosphonates, hormone replacement therapy, anabolic steroids or thiazide diuretics. All women were premenopausal and said that they were menstruating regularly. The study was approved by the Nottingham City Hospital ethics committee and all patients gave written informed consent.

MEASUREMENTS

Forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were measured by dry bellows spirometer (Vitalograph Ltd, Buckingham, UK) and FEV_1 was expressed as % predicted.[25] Plasma concentrations of calcium, phosphate, total protein and albumin and urinary concentrations of calcium, phosphate, sodium, and creatinine were measured by routine assays. Serum levels of procollagen peptide I (PCP-I), bone specific alkaline phosphatase (ostase), and osteocalcin were assayed by radioimmunoassay (Orion

Diagnostica, Espoo, Finland; Hybridtech Europe, Liege, Belgium; and Nichols Institute, San Jan Capistrano, California, USA, respectively). A 10 ml sample of urine was stored at $-80°C$ before assay for deoxypyridinoline concentration by ELISA (Rowett Research Services, Aberdeen, UK) and values were corrected for urinary creatinine concentration.

Bone mineral density (g/cm^2) of the neck of the non-dominant femur and distal radius, posterior-anterior spine (L2–L4), and lateral spine (body of L3 (B3)) was measured by dual energy x ray absorptiometry (DEXA; Lunar DPX-L scanner, Lunar Corporation, Madison, Wisconsin, USA). All scans were carried out by the same radiographer using "fast mode" at 3000 mA unless adjustment was needed for body mass. Routine calibration checks were carried out in accordance with the manufacturer's specifications including a daily calibration check against a three-chambered phantom containing bone ash of known masses. The coefficients of variation over the period of study for the three chambers were 0.52, 0.51 and 0.43%, respectively. Lateral radiographs focused on thoracic vertebrae T7 and T9 and lumbar vertebrae L2 and L3 were taken. Vertebral fractures were classified from the radiographs and graded according to the method of Eastell *et al*[26] by the same radiologist who was blind to the patients' treatment.

STUDY PROTOCOL

Subjects completed a self-administered questionnaire. A 20 ml sample of venous blood and a first morning void urine sample were obtained between 07.00 and 10.00 hours after a 12 hour fast for routine biochemical screening and markers of bone metabolism. Spirometric tests, bone mineral density measurements, and lateral thoracic and lumbar radiography were then performed. The questionnaire included questions on total pack years of smoking, alcohol consumption (expressed as mean number of units per week over the past five years), number of months milk products were not consumed, and questions relating to the frequency of physical activities (standing, walking and lifting heavy objects, frequency of exercise), and self assessment of fitness and strength. Activity was graded in five categories of increasing relative magnitude (1 = lowest, 5 = highest). Details of systemic steroid use at any time were also obtained. Women were asked about full term pregnancies, length of time breast feeding, and type and duration of contraceptive hormone use from which a life time dose of oestrogen and progesterone was derived.

Patients in the inhaled steroid group were asked about type, dose, and duration of inhaled steroid use. If patients temporarily varied the daily number of puffs during an exacerbation of asthma they were asked to estimate the average number of puffs per day over the episode and this estimate was then used in the calculation of total dose of inhaled steroid. Any change in the formulation or daily number of puffs of inhaled steroid was assessed as a new, discrete episode of inhaled steroid use. Inhaled

Table 1 Demographic data, spirometric values, alcohol consumption, duration of asthma, reproductive history, and contraceptive hormone use by sex and inhaled steroid use

	Group 1 (no inhaled steroids)		Group 2 (inhaled steroids)		p value†
	n	Mean (SD) or *median (range)	n	Mean (SD) or *median (range)	
Men					
Age (years)	19	30.3 (6.4)	19	32.3 (6.2)	NS
Weight (kg)	19	77.3 (13.2)	19	80.5 (11.0)	NS
Height (cm)	19	176 (6.6)	19	176 (6.4)	NS
FVC (litres)	16	5.13 (0.53)	19	4.56 (0.86)	0.029
FEV$_1$ (litres)	16	3.87 (0.59)	19	3.40 (0.85)	NS
Pack years smoking among ever smokers	8	*6.75 (1–25)	10	*5.5 (1–12)	NS‡
Alcohol intake (units/week)	19	*7.0 (1–61)	19	*6 (1–50)	NS‡
Years of asthma	17	*12.0 (2–34)	17	*20 (5–39)	NS‡
Women					
Age (years)	15	25.6 (5.5)	28	32.0 (5.9)	0.001
Weight (kg)	15	68.9 (18.6)	28	65.9 (13.9)	NS
Height (cm)	15	164 (7.5)	28	163 (5.0)	NS
FVC (litres)	14	3.65 (0.55)	28	3.56 (0.61)	NS
FEV$_1$ (litres)	14	3.13 (0.45)	28	2.83 (0.50)	NS
Age at menarche	15	13.5 (1.51)	27	12.5 (1.16)	0.023
Oral oestrogen (µg/month × no. of yrs)	12	159 (87)	15	251 (164)	NS
Oral and depot progestogen (µg/month × no. of yrs)	12	*750 (92–13500)	16	*920 (184–2800)	NS‡
Pregnancies (no. among n women)	4	*1.5 (1–2)	16	*2 (1–4)	NS‡
Breast feeding (no. months among n women)	3	*4 (2–26)	13	*7 (0–24)	NS‡
Pack years smoking among ever smokers	7	*1.25 (0.2–2.5)	9	*4.5 (1–36)	0.01‡
Alcohol intake (units/week)	15	*6 (1–30)	28	*2.5 (0.5–40)	NS‡
Years of asthma	15	*8 (0.5–35)	28	*12.5 (5–37)	0.001‡

Significance derived from one way ANOVA of means † or Mann-Whitney test‡.

budesonide and beclomethasone dipropionate were assumed to be equipotent and no distinction was made between aerosol or powder formulations, method of delivery, or the use of spacer devices. Standardised cumulative inhaled steroid dose was expressed as mg/day. years – for example, 500 µg/day for two years = 1 mg/day.year. Oral steroid use was expressed as the number of courses ever (because precise details of duration were not always available) and parenteral steroids as total life time dose (mg). Drug information was verified from patients' notes when available.

ANALYSIS OF DATA
The findings for men and women were analysed separately. Demographic and biochemical measures were compared between treatment groups by one way analysis of variance after log transformation as appropriate, or Mann-Whitney test. Physical activity grades were compared by the χ^2 test after pooling categories when needed to achieve sufficient numbers for analysis. The independent dose-response effect of three continuous measures of inhaled steroid use (standardised cumulative dose in mg/day.years, total days of use, and average daily dose over the last 12 months) on bone mineral density was assessed using multiple linear regression analysis (SAS version 6, SAS Institute Inc, Cary, North Carolina, USA). Initially, the effect of a number of potential confounding variables was assessed using stepwise and backward selection procedures; these included age, weight, pack years of smoking, units of alcohol per week, and activity grade. FEV$_1$ % predicted was also fitted as a proxy measurement for asthma severity. Age at menarche and life time total dose of oestrogen and progesterone use were fitted for women. A level of $p \leq 0.15$ was assumed in order not to reject variables with a borderline effect on BMD. Prednisolone use was then fitted as life time number of courses and, being the main potential confounder of

interest, was kept in the model irrespective of its statistical significance. The three measures of inhaled steroid dose were then added in turn to the final predictive model for each of the sites at which BMD had been measured. The effects of inhaled steroid use (dose or duration) were considered to be statistically significant when p<0.05. Finally, the potential confounders not so far included in each model were added singly to verify that they had no substantial influence on the estimated effect of inhaled steroid use.

Results
Of the 87 patients who completed the study, six were excluded prior to analysis when they were found to have used prohibited medications; this left 38 men (19 in each group) and 43 women (15 and 28 in groups 1 and 2, respectively) available for evaluation. The demographic data are summarised in table 1. The only significant difference between men in groups 1 and 2 was a higher mean FVC of 0.57 l in group 1 (95% CI 0.09 to 1.05; p <0.03). The significant differences between women in group 2 compared with group 1 were mean age (6.4 years older; 95% CI 2.8 to 10.0, p=0.001), age at menarche (one year earlier; 95% CI −1.82 to −0.18, p <0.03), longer duration of asthma (median 12.5 versus 8 years, p=0.001), and greater number of pack years smoking amongst those who had ever smoked (median 4.5 versus 1.25; p=0.01). The differences between the two groups in life time total dose of contraceptive oestrogens and progesterone, number of full term pregnancies, and total number of months breast feeding were not statistically significant.

PREVIOUS STEROID USE AND PHYSICAL ACTIVITY
None of the patients in group 1 had ever received inhaled or systemic steroids. Inhaled and oral steroid use for patients in group 2 is

Table 2 Estimated inhaled and oral steroid use in men and women in group 2

	Men (n=19) Median (range)	Women (n=28) Median (range)
Inhaled steroid		
Total dose (g)	1.37 (0.6–15.6)	0.94 (0.4–5.0)
Duration of use (years)	9.00 (4.4–17.1)	6.29 (4.5–15.2)
Total dose (mg/day.years)*	3.75 (1.7–42.7)	2.57 (1.1–13.8)
Daily dose over last 12 months (μg)	500 (100–3000)	450 (200–1600)
	Men (n=6)	Women (n=14)
Oral steroid		
Mean (range) number of courses amongst those who received it	1.33 (1–2)	2.46 (1–6)

* Estimated life time dose in mg/365 (total dose equivalent to number of years of having 1 mg/day).

summarised in table 2. Forty one of the 47 patients taking an inhaled steroid were using beclomethasone dipropionate (BDP), one having taken budesonide in the past; the other six were currently taking budesonide all having taken BDP in the past. Of the 47 patients, 36 were using a metered dose inhaler and 11 a dry powder inhaler (nine BDP, two budesonide). Six of 19 men and 14 of 28 women had received oral steroids, invariably prednisolone, in the past with a mean of 1.33 and 2.46 courses, respectively (giving a mean of 0.42 and 1.14 courses for all men and all women on inhaled steroids). None of the patients had received intravenous steroids, one man had had intramuscular steroids for hayfever on two occasions in the last five years, and two patients (one man) in group 1 and six (three men) in group 2 were currently using dermal steroids (past users numbered one (man) and 11 (four men), respectively).

Frequency of physical activity and grading of self assessed fitness and strength were similar for most measures in the two groups except that more women in group 2 were in the lower categories for walking frequency (grades 1 + 2 + 3; 40% versus 71% for groups 1 and 2, respectively, difference 31% (95% CI 1 to 62), p <0.05). None of the patients had avoided milk products in the past.

BIOCHEMICAL MARKERS

Of the biochemical markers assayed (table 3) the only significant differences were that women in group 2 had lower serum levels of osteocalcin (4.5 versus 6.3 μg/l; difference 1.8 μg/l (95% CI 0.17 to 3.43); p = 0.04) and a lower mean plasma phosphate concentration (0.90 versus 1.01 mmol/l; difference −0.11 (95% CI −0.21 to −0.01); p = 0.04) whereas the urinary sodium concentration was higher in men taking inhaled steroids than in non-users (144 versus 106 mmol/l; difference 38 (95% CI 5.6 to 70); p <0.03). However, in linear regression there was no significant dose-response effect of cumulative or current daily dose of inhaled steroids on the serum concentration of osteocalcin.

BONE MINERAL DENSITY

There were no significant differences in mean bone density values between the two treatment groups for either men or women (table 3). Amongst patients in group 2 there was no significant difference in BMD between those who had received oral steroids in the past and those who had not, apart from BMD of the radius which was higher in women who were past oral steroid users (difference 0.039 g/cm² (95% CI 0.002 to 0.076); p = 0.04). Before adjustment for potential confounders there was a significant negative dose-response relationship between bone density and cumulative dose of inhaled steroid at the two spine sites in women (L2–L4 (n=43): estimate of slope −0.016 (95% CI −0.004 to −0.03), p = 0.01; lateral B3 (n=42): estimate of slope −0.023 (95% CI 0.009 to −0.030), p = 0.002; fig 1).

MULTIVARIATE ANALYSIS

In women, multivariate linear regression showed that the negative association between standardised cumulative inhaled steroid dose and bone mineral density at the spine was independent of the number of prednisolone

Table 3 Concentrations of serum and urinary bone markers and bone mineral density measurements (BMD) by sex and inhaled steroid use

	Men						Women					
	Group 1 (no inhaled steroids, n=19)		Group 2 (inhaled steroids, n=19)		p value		Group 1 (no inhaled steroids, n=15)		Group 2 (inhaled steroids, n=28)		p value	
	Mean (SD)	Range	Mean (SD)	Range			Mean (SD)	Range	Mean (SD)	Range		
Bone marker												
Serum bone specific alkaline phosphatase (μg/l)	16.8[G]	6.7–42.0	14.8[G]	9.0–29.5	NS†		11.4[G]	3.5–22.0	12.1[G]	7.7–30.5	NS†	
Serum procollagen peptide I (μg/l)	147[G]	94–235	147[G]	93–234	NS†		118[G]	96–161	118[G]	83–212	NS†	
Serum osteocalcin (μg/l)	7.3 (3.5)	1.9–13.5	6.2 (2.5)	1.9–12.5	NS*		6.3 (3.3)	2.0–13.0	4.5 (2.2)	1.8–9.1	0.04*	
Urinary deoxypyridinoline:creatinine ratio	3.9 (1.2)	3.1–7.8	4.6 (1.5)	1.9–7.6	NS*		5.3 (1.1)	3.6–7.6	5.1 (1.5)	3.3–11.0	NS*	
	Mean (SD)	t score	Mean (SD)	t score			Mean (SD)	t score	Mean (SD)	t score		
BMD (g/cm²)												
L2–L4 spine	1.21 (0.17)	−0.21	1.28 (0.13)	0.33	NS*		1.25 (0.12)	0.40	1.205 (0.13)	0.04	NS*	
B3 spine	0.98 (0.23)	NA	1.03 (0.15)	NA	NS*		0.87¶ (0.14)	1.24	0.78 (0.15)	0.53	NS*	
Femoral neck	1.09 (0.14)	0.20	1.17 (0.18)	0.85	NS*		1.10 (0.14)	1.02	1.04 (0.14)	0.49	NS*	
Distal radius	0.44 (0.08)	0.38	0.45 (0.06)	0.72	NS*		0.34¶ (0.07)	−1.18	0.36‡ (0.05)	−0.43	NS*	

Significance derived from one way ANOVA of means * or of [G] geometric means †; t score = number of SDs away from mean of young adult (age 20–40 years) reference population (LUNAR data on file); NA = reference data not available.
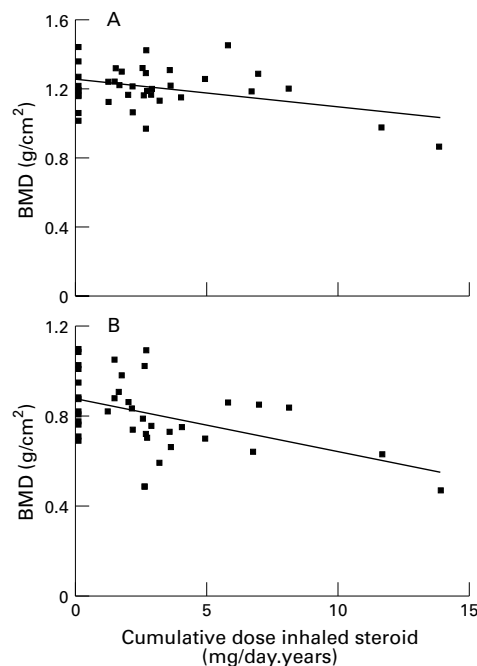¶ n = 14; ‡ n = 27.

Figure 1 Univariate regressions of bone mineral density (BMD) of (A) L2–L4 (n=43) and (B) B3 (n=42) on cumulative dose of inhaled steroids in women.

courses and the variables shown in table 4. These variables were included in the model as potential confounders because they had a significant effect (p<0.15) on the total variance of the model when added or removed during the stepwise and backward selection procedure. For each mg/day.year of inhaled steroid use there was a decrease in bone density of 0.015 (95% CI 0.002 to 0.028) and 0.017 (95% CI 0.002 to 0.031) g/cm^2 at the L2–L4 and B3 sites, respectively (p=0.03 for both). When expressed as standard deviations both equate to a reduction in BMD of 0.11 SD per mg/day.year inhaled steroid use (L2–L4: 95% CI 0.01 to 0.22 SD; B3: 95% CI 0.02 to 0.21 SD). Bone density at the B3 site was independently related to life time progesterone dose such that a two fold increase in progesterone dose would result in a 6×10^{-6} g/cm^2 increase in BMD (p <0.03). None of the other measures of inhaled steroid use was significantly related to bone density in the spine and none was related to

bone density at the femoral neck or distal radius. Bone density at the femoral neck was related to weight (0.003 g/cm^2 per kg increase in weight (95% CI 0.0004 to 0.0056); p <0.04) and bone density at the radius was positively associated with smoking (0.003 g/cm^2 per pack year (95% CI 0.0007 to 0.0055); p <0.02).

Having completed our primary analysis we carried out two further analyses. Firstly, when the analysis was recomputed excluding the two women on the highest cumulative dose of inhaled steroid the estimates of the slopes for the two spine sites (L2–L4 and B3) were 0.0001 (95% CI −0.018 to 0.018) g/cm^2, p=0.99 and −0.0098 (95% CI −0.031 to 0.012) g/cm^2, p=0.36, respectively. Secondly, when we repeated the multivariate analysis of the two spine sites for women excluding the 15 patients who had not had inhaled steroids, the estimates for the slopes did not change markedly but were no longer statistically significant (estimates of slopes for L2–L4 and B3 were −0.012 (95% CI −0.026 to 0.0012) g/cm^2; p=0.09 and −0.016 (95% CI −0.033 to 0.002) g/cm^2; p= 0.10, respectively, for each mg/day.year inhaled steroid).

In men, none of the measures of inhaled steroid use showed a significant relation to bone density at any of the skeletal sites. Bone density at the L2–L4 and B3 spine and distal radius were significantly associated with weight (estimates of 0.0044 (95% CI 0.0001 to 0.0087) g/cm^2, p=0.04; 0.0095 (95% CI 0.0051 to 0.0139) g/cm^2, p=0.001; and 0.0034 (95% CI 0.0014 to 0.0054) g/cm^2, p=0.001, respectively, per kg increase in body weight). Self assessed fitness was significantly associated with bone density at the two spine sites with estimates for the slope of 0.0622 (95% CI 0.0039 to 0.1205) g/cm^2, p <0.04, and 0.0745 (95% CI 0.0106 to 0.1384) g/cm^2, p <0.03, per category of fitness. No variable was significantly associated with bone density at the femoral neck in men.

No fractures were detected in women on vertebral morphometry of spine radiographs. Six men in group 1 and two in group 2 had a total of 13 vertebral fractures; these were not related to bone density or inhaled steroid dose.

Discussion

In this study there was no difference in mean BMD values for men or women who were and

Table 4 Results of the L2–L4 and B3 spine linear regression analysis in women

Explanatory variable	Estimate of slope (g/cm^2 BMD per unit increase)	95% CI for estimate of slope	p value for estimate of slope
L2–L4 P-A spine (n=43)			
Frequency of physical activity (grade)	0.029	−0.001 to 0.059	0.06
Age (year)	0.006	−0.0002 to 0.009	0.07
Weight (kg)	0.001	−0.001 to 0.003	0.35
FEV$_1$ % predicted (%)	−0.001	−0.004 to 0.001	0.39
Prednisolone (course)	−0.004	−0.031 to 0.023	0.76
Inhaled steroid (mg/day.year)	−0.015	−0.028 to −0.002	0.03
Body of L3 lateral spine (n=42)			
Strength (grade)	0.1	−0.006 to 0.021	0.07
Progesterone [log$_{10}$(cumulative dose in μg)]	2.0×10^{-5}	4.0×10^{-7} to 3.9×10^{-5}	0.03
Smoking (pack year)	0.005	−0.001 to 0.011	0.12
Prednisolone (course)	−0.009	−0.039 to 0.021	0.58
Inhaled steroid (mg/day.year)	−0.017	−0.031 to −0.002	0.03

Explanatory variables resulting in a significant (p<0.15) change in residual variance during stepwise or backward selection modelling are shown with estimate, 95% confidence intervals (CI) and p value for the slope.

were not using inhaled steroids. There was, however, a statistically significant reduction in BMD in women with increasing dose of inhaled steroid, equivalent to a 0.11 SD reduction in BMD of the lumbar spine for every year's use of 1000 µg/day inhaled steroid after correction for confounding factors. Women using inhaled steroids also had significantly lower serum osteocalcin concentrations.

Cross sectional group comparisons are susceptible to confounding by differences in age, menopausal status, physical activity, and previous drug use including, in the case of asthma, systemic steroid use. Less well characterised are the effects of alcohol, smoking, diet, parity, and past use of contraceptive hormones.[27] In the present study we reduced the effects of age and menopause by only including patients aged 20–40 years when bone density is reasonably stable,[28] and by excluding women who were not menstruating regularly. Other factors allowed for in the analysis were physical activity, weight, smoking, alcohol consumption, oral contraceptive use, and parity.

Past use of systemic steroids is probably the most important potential confounder in this type of study. It would have been ideal to have excluded any patients with previous exposure to corticosteroids but this was difficult in practice. (Over 1000 patients were screened to obtain the current numbers.) We therefore allowed patients with limited prior exposure to systemic steroids into the study. Less than half the patients had had oral steroids previously with no courses exceeding two weeks in length or doses above 40 mg prednisolone/day. We also allowed for previous steroid use in the regression analysis. Studies in patients treated for Cushing's syndrome[29] or following cessation of long term oral steroids for sarcoidosis[30] suggest that, once treatment stops, bone density returns towards predicted levels, at least in patients under 45 years of age. Only one female patient had had oral steroids in the six months before the study. Previous use of nasal steroids was an exclusion criterion. Dermatological steroids were allowed since systemic effects have only been seen when topical steroids are applied to large areas of broken skin with occlusion[31 32] which was not the case with our subjects. The long term effects of skin steroids on bone density are not known.

We also considered whether confounding by asthma severity might result in reduced BMD among users of inhaled steroids by inhibiting physical activity or through delayed onset of puberty. The range and frequency of physical activities between the two groups were similar, except that women in the inhaled steroid group tended to walk for less time than those in the non-steroid group. Whether this is relevant is uncertain since bone density is stimulated to a greater extent by exercises that involve skeletal loading or a high impact component such as jumping.[33] Delayed puberty was not relevant since women in the inhaled steroid group had an earlier menarche. Finally, when we included FEV_1 % predicted as a measure of asthma severity in the regression model it was not a significant independent predictor of BMD at any site. These data suggest that asthma severity was not an important determinant of BMD in our patients.

Women in the inhaled steroid group were less likely to smoke than those in the non-steroid group, although those who did smoked more cigarettes per day. The positive relationship between smoking and wrist BMD in women was surprising and may be a chance finding as smoking has been associated with lower values of BMD, at least in postmenopausal women.[34]

Although the relation between BMD and inhaled steroid use was statistically significant, it was influenced by two women who had received high cumulative doses of inhaled steroids (fig 1). One of the women had never received oral steroids whilst the other had received a total of six courses (life time dose 1200 mg prednisolone) which would be taken into account in the multivariate analysis. Statistical significance was lost when the multivariate regressions for the two spine sites was recomputed excluding the data from these women. We also tested whether the estimate for the slope for the relationship between inhaled steroid dose and bone density was altered by excluding the patients who had never had corticosteroids. The estimates were largely unaltered although they were no longer statistically significant due to the smaller numbers. Although our findings could be spurious we think this is very unlikely since BMD was our primary end point, and the fact that it was the lumbar spine in women that was affected is biologically plausible in the light of oral steroid studies. Our findings need to be confirmed in studies that include more patients on high doses of inhaled steroids.

The finding of lower osteocalcin concentrations among patients using inhaled steroids is consistent with other studies.[6 18] Osteocalcin is considered to be a marker of osteoblast function although its exact role in bone metabolism is unclear.[35] There was no correlation between osteocalcin concentration and bone density, which may be because osteocalcin levels reflect recent changes in bone turnover.

An association between inhaled steroid use and BMD was only seen in women. This may be a reflection of the relatively small numbers, although a similar sex difference was reported in another study.[5] Inhaled steroids may alter bone metabolism through an effect on endogenous sex hormones, in particular, oestrogen. Serum oestrone concentrations were lower in postmenopausal women using systemic corticosteroids than in those not using them in a study by Marshall *et al.*[36] Oestrogen is thought to reduce bone resorption,[37] although concentrations of urinary deoxypyridinoline, a marker of bone resorption, were unchanged in our study.

All the vertebral fractures were seen in men and there was no relationship between the vertebral fractures and inhaled steroid use or bone density. This was surprising but may reflect a greater involvement by young men in occupations or leisure activities which carry a higher risk of fractures.

This study is the first to try to assess the magnitude of change in BMD associated with inhaled steroid use in patients with asthma. Our findings of a reduction of 0.11 SD per 1000 µg inhaled steroid use/day per year had wide confidence intervals and larger studies are needed to provide a tighter estimate of the slope of the association. There will almost certainly be differences between different inhaled steroids and delivery devices but our study did not have sufficient power to look at this. (The majority of patients had taken beclomethasone by metered dose inhaler for most of the time.)

If the reduction in BMD is of the order of 0.1 SD per 1000 µg/day inhaled steroid per year, there are two important implications. The first is that the effect over one year is small and hence prospective studies will need to be long or very large, or both, if they are to detect any difference in bone density with inhaled steroid use; for example, a prospective study comparing a dose of 1000 µg/day with placebo would need 2500 patients in each limb for 95% power to detect a difference in bone mineral density of 0.1 SD. A cross sectional study involving more patients may be more practical. The second point is that, although 0.1 SD a year is unimportant in the short term, it may be very important with long term treatment. If, for example, the relationship between inhaled steroid use and BMD was linear, the use of an inhaled steroid at 1000 µg/day for 30 years could cause a reduction in BMD of 3 SD. Since several studies have shown that the risk of vertebral fracture doubles for each standard deviation reduction in BMD,[38] this could lead to an eight fold increase in the risk of vertebral fracture. These figures are hypothetical and are based on several assumptions, particularly that the relationship is linear for which there is conflicting evidence.[39 40] This study provides a first attempt to estimate the reduction in BMD with inhaled steroid use, and hence the likely risk of osteoporosis, and it also provides a basis for designing future studies to obtain tighter estimates of this relationship.

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