

Osteocalcin, Cortisol Levels, and Bone Mineral Density in Prepubertal Children with Asthma Treated with Long-Term Fluticasone Propionate

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Key Words

Asthma · Bone mineral density · Cortisol · Osteocalcin · Children · Fluticasone propionate

Abstract

Aims: The objective of this study is to determine the effects of the long-term treatment with inhaled fluticasone propionate on osteocalcin, cortisol levels, and bone mineral status in children with asthma. **Methods:** This cross-sectional study examined 230 prepubertal children with asthma (aged 6–11) who had intermittently used inhaled fluticasone propionate for at least 5 years at a mean daily dose of 200 µg (range: 200–350 µg). Serum osteocalcin, cortisol, and bone mineral density (BMD) of the lumbar spine were obtained from each participant. The control group consisted of gender- and age-matched children (n = 170) who were newly diagnosed with asthma and who were not being treated with corticosteroid. **Results:** The average age (±SEM) was 8.9 ± 0.7 years, their mean (±SEM) daily steroid dose was 180.3 ± 55.0 µg, with 236.5 ± 17.2 g total steroid use during treatment. Between the study and the control groups, no significant differences were observed in cortisol, osteocalcin levels, and BMD (p > 0.05). **Conclusion:** Long-term treatment with inhaled fluticasone propionate (100 µg twice daily) revealed no negative effects on serum osteocalcin, cortisol levels, and BMD in children with asthma.

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Introduction

Asthma is the most common chronic childhood disease and has shown an apparent increase in recent years [1]. National Guidelines (USA) recommend that adults and children with asthma receive daily inhaled corticosteroids (ICSs) as first-line treatment [2, 3]. The systemic bioavailability of an ICS is determined by the amount of drug delivered and subsequently absorbed by the lungs and the amount of drug absorbed from the gastrointestinal tract [4]. In contrast to intranasal steroids, ICSs cause a high degree of deposition in the oropharynx and nasal cavity, followed by mucociliary clearance to the throat and, eventually, to the gastrointestinal tract, and absorption from the mucosal surface can contribute up to 50% systemic bioavailability of the ICS [5]. The low frequency of side effect observed after long-term use of ICS shows the safety of ICS; however, systemic side effects of ICS should be carefully monitored, especially in patients such as children with asthma who are undergoing long-term treatment [6]. Glucocorticoid-induced osteoporosis or reduced bone mineral density (BMD) has been reported with low doses (5–10 mg/day) of corticosteroids taken orally as well as with high doses that are inhaled [7]. The number of studies concerning the effects of inhaled fluticasone propionate (FP) on the mineral status of bone is limited and results are conflicting [8, 9]. Although large

studies have reported no substantial risk of ICS on bone metabolism in children with asthma [10–12], there are no new data available as to long-term intermittent FP use and its effects on children's bone metabolism and its associated biomarkers.

The present study assessed the BMD and its associated parameters in children with asthma who had been treated for at least 5 years with intermittently inhaled FP. The findings were compared with those of children with asthma who had never received treatment with corticosteroids. The following questions will be addressed by our study: Are children with asthma who are using FP at risk for decreased BMD? Are they experiencing adverse effects on associated parameters such as osteocalcin and cortisol? If so, how large is the risk compared with subjects not using this drug, and does the risk vary according to the dose or duration of FP use?

Subjects and Methods

Study Population

Participants had come to the authors' Pediatric Allergy-Pulmonology Outpatient Clinic and were enrolled between May 2010 and December 2011. Eligible subjects had a documented history of reversibility of $\geq 12\%$ in FEV₁ or of $\geq 15\%$ in peak expiratory flow within 15–30 min after inhaling salbutamol as tested with dynamic spirometry (Vitalograph, G.W. Berg, UK). Subjects were 230 children (aged 6–11) who had documented diagnoses of mild to moderate asthma and had been using inhaled FP intermittently for 5 or more years. Diagnosis and severity of asthma was defined according to American Thoracic Society guidelines [13]. At the time of the study, the children had been seen at our outpatient Pediatric Allergy-Pulmonology Clinic at least every 3–4 months for 5 years. Informed consent was given by the families of the patients after an explanation of the study.

Study Design

The present prospective case-control study investigates serum osteocalcin, cortisol, and BMD of a total of 270 children who had received inhaled FP intermittently for 5 or more years. The following information was recorded at each visit: participant's age, height, weight, number of hospital admissions due to acute asthma during the previous 3 months, use of concurrent medicine(s), inhalation device, and dose of inhaled FP. Between clinic visits, changes in FP or other allergic medications were made under the supervision of the clinic and transient changes in treatment during periods of increased asthma symptoms were recorded. These recordings made it possible to accurately calculate the average dose of exogenous corticosteroid during the previous 5 years as well as the accumulated dose of FP. In order to treat each child with the minimal effective dose, adjustments of the inhaled FP dose were made based upon the assessment of clinical control of the disease.

Patient compliance was checked at each visit by first asking the child and family about their compliance and then by checking

inhalation skills and medication levels. Whenever inhaler strength was changed, at the clinic the child was given another pressurized metered dose inhaler (pMDI). In such situations, the child was scheduled to return to the clinic for another visit 2–3 months later and to bring the inhaler at that visit. This allowed assessment of compliance by determining the number of doses taken, either by weighing the canister (Flixotide inhaler® 50 µg, or 125 µg, pMDI, GlaxoSmithKline, UK) or by counting the number of doses left (Flixotide Diskus® 100 µg or 250 µg, GlaxoSmithKline, UK).

We identified all prescriptions for FP that had been filled by patients in the prior 5 years, and studied the risk of current extra exposure to FP. To investigate the exposure to FP according to dose, we calculated the average daily dose of FP by dividing the total quantity (in micrograms) by the days of supply for that prescription. Participants had not received specific immunotherapy, and none had allergic rhinitis requiring chronic use of nasal or systemic steroids. All of the study group patients had been using only one type of inhaled steroid, FP.

In order to obtain a sufficient number of patients for comparison, we added to the control group 170 children newly diagnosed with mild to moderate asthma. None of them had received oral, inhaled, or nasal corticosteroids for over 2 weeks, and none of them had any chronic systemic diseases. Age, sex, body height and weight, body mass index, the presence of bronchial asthma, daily and total FP, duration of treatment, history of any incidents (trauma) or fractures, time of diagnosis, family history of atopy, Tanner stage results and skin test results were recorded for all study group participants. To avoid the confounding influence of some covariates, the following exclusion criteria were used in the present study: patients who had a Tanner stage level $\geq II$, >14 days treatment with systemic corticosteroids ever (both groups of children), a history of topical corticosteroids ever applied to 25% of the body surface (both groups), additional nonatopic systemic disease (e.g., disorders of calcium metabolism, spine demineralization, osteoarthritis, metabolic bone disease, anorexia, obesity, or rheumatologic and autoimmune disorders) or injuries to the spine with risk of bone density loss (e.g., prior fracture/immobilization), and chronic use of some supplements (e.g., anticonvulsants, nondietary vitamin D, ketoconazole, hormone replacement therapy).

Measures

BMD (g/cm²) was measured using dual-energy X-ray absorptiometry (DEXA, GE Lunar DPX Duo Bone Densitometer, Absolute Medical Equipment, Inc., Monsey, N.Y., USA). The densitometer was calibrated daily, 30 min after turning on the apparatus. Quality control was performed using a calibration standard and the QC phantom. BMD scans of the AP lumbar spine/pediatric (L₁–L₄) vertebrae were analyzed using the World Health Organization criteria for bone mass [14]. The femoral neck was not examined for BMD. Data are expressed as z-scores, the number of standard deviations (SD) above or below the mean value of an age- and sex-matched reference population. Reference data for healthy Turkish children are available for the bone densitometer. This reference population consists of age- and sex-specific BMD reference values from more than 300 Caucasian children in Turkey [15]. Because BMD in DEXA is dependent on bone size, and larger bone size may artificially inflate BMD, correcting z-scores for body height is necessary to prevent incorrect diagnosis of osteo-

Table 1. Patient characteristics of the study and control groups of children with asthma

	Fluticasone group (n = 230)	Control group (n = 170)	p value ^a
Mean age \pm SD, years	8.2 \pm 0.7	8.4 \pm 0.5	0.460 ^a
Male, n (%)	148 (64)	105 (61)	0.440 ^b
Height SDS, mean	-0.1 \pm 1.1	-0.2 \pm 0.90	0.370 ^a
Weight SDS, mean	0.41 \pm 0.86	0.480 \pm 0.77	0.430 ^a
Patients with skin prick test positivity to at least 1 allergen, n	230	170	
IgE, kU/l ^c	400.7 \pm 44	439.6 \pm 21.5	0.452 ^a
Mean symptom duration \pm SD, years	4.5 \pm 0.7	3.7 \pm 0.4	0.160 ^a
BMI SDS, mean	0.295 \pm 0.32	0.320 \pm 0.45	0.460 ^a
Mean FEV ₁ \pm SD, % predicted	85.70 \pm 13.2	84.55 \pm 15.3	0.260 ^a
Sports activities, times per week	1.25	1.40	0.470 ^a
Fracture incidence (any time), n	18	12	0.540 ^a

^a Comparison made by independent samples t test.

^b Comparison made by Pearson's χ^2 test.

^c Total IgE level is expressed as median value.

porosis in children with small body size for their age [16]. Children with asthma can have small body height as a result of chronic inflammation, and we corrected all analyses for body height (age-adjusted).

Morning (8 a.m.) venous blood samples were taken to assess osteocalcin, alkaline phosphatase (ALP), calcium, phosphorus, and cortisol levels (before steroid dose). ALP, calcium, and phosphorus were analyzed spectrophotometrically by using a Mega Automatic Analyzer (Merck, Tempe, Ariz., USA). Osteocalcin, intact parathyroid hormone (PTH), and cortisol samples were analyzed using an Immulite chemoluminescence immunoassay (Diagnostic Products Corp., Inc., Los Angeles, Calif., USA).

Ethical Approval

The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice and was approved by the local Ethics Committee.

Statistical Analysis

SPSS program (v11.5, SPSS Inc., Chicago, Ill., USA) was used for all statistical analyses. Signal intensities are given in arbitrary units with mean, SD, or standard error of the mean. General characteristics were analyzed by an independent-sample t test or by Pearson's χ^2 test for category-wise data. To investigate the relative importance of the variables associated with bone metabolism in relation to dependent factors and in cases of any confounding between them, they were fitted together using a multivariate linear regression model to control confounding factors and to determine which characteristics were independent of the total steroid dose of the children with asthma. A p value <0.05 or OR with a 95% confidence interval that did not include 1.00 was considered statistically significant.

Results

A total of 400 children were studied: 230 in the FP group and 170 in the control group. Their characteristics are shown in table 1. The two groups were comparable with respect to age, height, and weight, as well as the average number of times per week they participated in sports activities (1.25 for the study group and 1.40 for the control group). Study patients (148 males) ranged in age from 6 to 11 (8.2 \pm 0.7) years. Follow-up time ranged from 43 to 70 (60.67 \pm 7.86) months. Average height and weight was 134.0 \pm 3.5 cm and 29.8 \pm 1.2 kg, respectively. Children in the study group used a mean FP dose of 188.3 \pm 47.0 μ g per day. Their mean total accumulated FP dose was 230.5 \pm 15.2 g of steroid(s). The mean FEV₁ (predictive %) result was 85.70 \pm 13.20. The average IgE titer was 400.7 \pm 44 kU/l (normal: 0–52 kU/l). All patients had positive skin test results for at least one allergy (table 1).

The 170 control patients (105 males) ranged in age from 6 to 11 (8.4 \pm 0.5) years. Their average height and weight were 133.7 \pm 3.4 cm and 28.7 \pm 1.2 kg, respectively. The mean FEV₁ (predictive %) result was 84.55 \pm 15.30. The average IgE concentration was 439.6 \pm 21.5 kU/l. All patients had positive skin test results.

BMD Parameters and Fluticasone Treatment

Despite a tendency towards lower levels for mean basal serum cortisol and osteocalcin and higher levels for

Fig. 1. a Comparison of unadjusted lumbar-spine (LS) BMD between PF and control group. **b** Comparison of height-adjusted LS BMD (z-score) between groups.

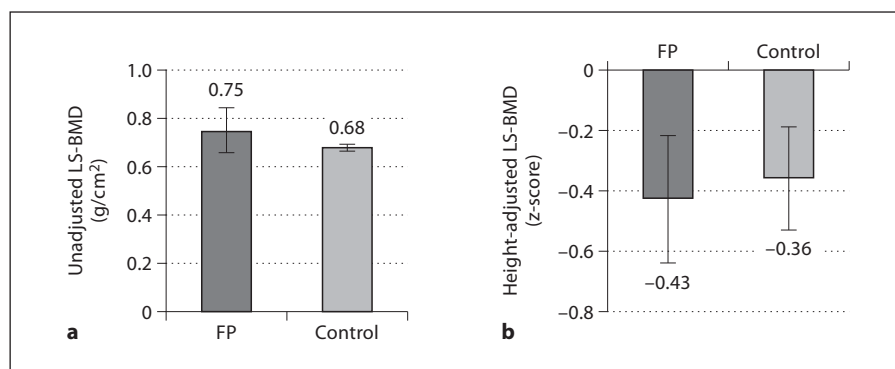


Table 2. Comparison of some bone metabolism-associated parameters between two groups

Parameters	Fluticasone group (n = 230)	Control group (n = 170)	p value
Mean BMD \pm SD, g/cm ²	0.75 \pm 0.092	0.68 \pm 0.012	0.32 ^a
Mean BMD Z-score \pm SD	-0.43 \pm 0.21	-0.36 \pm 0.17	0.23 ^b
Calcium, mg/dl	8.9 \pm 0.6	9.2 \pm 0.40	0.56 ^a
Phosphorus, mg/dl	4.4 \pm 0.4	4.6 \pm 0.5	0.60 ^a
ALP, IU/l	470 \pm 165.5	426.4 \pm 148.7	0.39 ^a
PTH, pg/ml	18.3 \pm 0.5	18.7 \pm 0.3	0.47 ^a
Osteocalcin, ng/ml	73.6 \pm 19.8	76.5 \pm 27.5	0.32 ^a
Cortisol, μ g/dl (min.–max.)	9.2 \pm 1.64 (8.5–11.3)	9.4 \pm 1.70 (8.9–12.1)	0.40 ^a

Values are given as mean \pm standard error of the mean.

^a Comparison made by independent-samples t test.

^b Comparison made by Pearson's χ^2 test.

Table 3. Estimates of total steroid dose effect of variables associated with bone metabolism status of the children with asthma through logistic regression (n = 230)

	OR	95% CI
BMD	1.4	0.80–3.40
ALP	1.1	0.70–2.60
PTH	1.6	0.97–3.71
Osteocalcin	1.6	0.95–3.82
Cortisol	0.8	0.70–2.76

phosphorus, ALP, and PTH in the study group, there were no statistically significant differences between the two groups ($p > 0.05$). The BMD z-scores (adjusted for height) of the two groups were also similar and comparable with that of the control group (table 2; fig. 1). Use of

inhaled FP was not associated with a decrease in lumbar spine z-score. The authors also assessed the body height, weight, and body mass index-associated standard deviation scores separately for the two groups. FP-treated children tended to have lower scores for all parameters, but no statistically significant difference was found when compared with the control group (table 1). Finally, the possible association of BMD results and FP treatment was analyzed, but no correlation was found between the BMD scores and associated parameters and the total dose of FP (table 3).

Discussion

The assessment of possible systemic side effects of long-term ICS treatment is a central issue in pediatrics. Corticosteroids are prescribed to more patients with

asthma and for longer periods of time than ever before [17]. Currently available ICSs include beclamethasone dipropionate (BDP), budesonide (BUD), flunisolide, FP, mometasone furoate, and ciclesonide. These differ from each other in terms of their systemic absorption, volume of distribution, half-life, and the extent of systemic bioavailability [4, 5]. Systemic bioavailability of ICSs is determined by the amount of the drug delivered and subsequently absorbed in the lungs and gastrointestinal tract. FP has a high degree of deposition in the nasal cavity, followed by mucociliary clearance to the throat and, eventually, in the gastrointestinal tract. Absorption from the mucosal surface can contribute up to 50% to systemic bioavailability [18]. A meta-analysis of comparative clinical trials demonstrated that half the dose of FP (as compared to BUD and BDP) was numerically superior in four of them when compared with BUD and BDP. Despite difficulties with standardization, trials suggest that when using pMDI, FP is more effective than BDP and BUD [19]. Studies using oral dosing of labeled and unlabeled drug have demonstrated that the oral systemic bioavailability of FP is negligible (<1%) – primarily due to incomplete absorption and presystemic metabolism in the gut and liver – but that systemic availability of FP will equal lung deposition [18, 19]. FP is said not to be metabolized locally in the lung. The drug is cleared rapidly by liver metabolism, with a total blood clearance equivalent to hepatic blood flow; therefore, the fraction of drug inhaled contributes substantially to systemic availability [20]. Lower bioavailability and more extensive metabolism of FP than other corticosteroids may explain partly the lack of bone-associated side effects in our study.

Results of the present study corroborate the results reported in previously published research of smaller groups of less well-characterized children who have used FP for shorter periods of time [8, 21]. Current studies show no evidence that long-term treatment of children using FP with low to medium doses is associated with reduction of BMD or with increased risk of osteoporosis [22]. On the other hand, changes have recently been documented as to total bone mineral content in children treated for 12 months with high doses of BDP, BUD, and FP [23].

The effects of exogenous corticosteroids on bone can be evaluated by biochemical markers of bone metabolism, BMD, or frequency of fractures [24, 25]. The lumbar spine is the ideal site for DEXA scans because of its high content of metabolically active trabecular bone, its propensity for fractures as well as its sensitivity, and changes in the mineral density and bone size in growing children [15, 26]. Results from the study group showed no signifi-

cant differences when compared with the control group as to the effects of BMD after 5 years of intermittent treatment with FP spray.

When designing the present study, the authors tried to avoid some of the problems of interpreting potential findings in the FP-treated children. This was achieved by restricting the study to carefully selected patients who had received no systemic corticosteroids for over 14 days, and by including a control group of children with asthma who had never received exogenous corticosteroids. To minimize possible bias, technicians who took BMD measurements were blinded with respect to the treatment given. BMD results from this study corroborate those of Kemp et al. [27], i.e. that even the most commonly bioavailable formulation of FP at a relatively high dose does not cause reduced BMD in the lumbar spine, the most appropriate and sensitive marker for the skeletal effects of systemic corticosteroid therapy.

Studies of biochemical markers of bone metabolism such as serum osteocalcin may have potential as indicators of the long-term effects of corticosteroids, but they are less indicative than DEXA regarding cumulative effects over a 5-year period. In this study, serum osteocalcin values were not statistically different between the two groups. The serum osteocalcin values were highly variable and may not be reliable or predictive of other systemic effects of ICSs [28, 29]. It is unclear whether individual markers of systemic effects of corticosteroids correlate with each other, nor is it clear as to which is the most sensitive and predictive of problems in other organ systems. We can, however, say that serum osteocalcin levels in our study were not predictive of changes in skeletal BMD measurements in children with asthma who had used FP intermittently for 5 years.

Suppression of the hypothalamic-pituitary-adrenal (HPA) axis is one way to determine if exogenous steroids have potentially negative effects. Determining cortisol in the total urine output of a 24-hour period of time is a reliable index of HPA axis function since it is not affected by circadian variations in cortisol secretion [30]. This study, on the other hand, checked serum levels of cortisol in the morning (before dose), a method that provides a momentary value only. Conclusions as to cortisol patterns throughout the day cannot be drawn. Still, as the values were gathered at the same time in both of our groups, they are comparable. Endogenous cortisol production was active and the children using FP did not show statistically different values from the reference values of the controls. It can be concluded that treatment with low or moderate doses ($\leq 400 \mu\text{g}$ in children) of ICS

is usually not associated with suppression of the HPA axis in children with asthma [31].

The authors found no significant changes in other biochemical markers of bone metabolism such as serum calcium, phosphorus, ALP, or PTH. Regrettably, because of a lack of measurement technique, they could not evaluate serum procollagen peptide I levels, another important marker for bone metabolism. Dietary calcium intake is another important point to be considered. Some authors believe that decreased BMD may be partly related to low calcium intake, while vitamin D is thought to be helpful to control the negative side effects of the ICS on bone turnover [32–34]. A recent study showed that serum 25-hydroxy vitamin D levels were inversely associated with asthma, with a direct and significant relationship between vitamin D levels and pulmonary function test outcomes in asthmatic children [35]. Our study showed that long-term FP treatment was not associated with increased total fracture risk. The 230 participants in the FP group had a combined history of 18 fractures compared to 12 fractures in the 170 children in the control group. The prevalence of total fractures between the two groups was not statistically significant. Ma and Jones [36] found that BMD may be less important than clinical risk factors for total fracture risk in prepubertal children.

Our study has several limitations. The levels of plasma vitamin D may be associated with the severity of asthma and respond to therapy, but our aim was not primarily to examine the association of disease severity and bone

markers. For this reason, we did not collect data on calcium intake or vitamin D status which may be associated with BMD and associated markers. While we thus cannot assume that these asthmatic children represented a selected group, socioeconomic factors may affect calcium intake and vitamin D more than asthma. In addition, limited financial resources precluded us from assessing other markers of bone reabsorption such as hydroxyproline, pyridinoline, and calcium excretion.

Conclusion

This study has shown that in prepubertal children with asthma, long-term usage of inhaled FP at an average daily dose of 200 µg for up to 5 years has no statistically significant negative effects on bone metabolism biomarkers such as osteocalcin, cortisol, and on BMD at the lumbar spine. The markers of bone formation and resorption failed to reveal differences in age- and disease-matched controls. The results of this study add to the body of evidence supporting the safety of long-term FP use in children with asthma.

Disclosure Statement

The authors have no financial or personal relationships with other people or organizations that could pose a conflict of interest in connection with the present work.

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