Osteocalcin, growth, and inhaled corticosteroids: a prospective study

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Abstract

Objectives—To determine the relationship between biochemical markers of bone metabolism and statural growth, and their suitability as surrogate markers of inhaled corticosteroid induced growth suppression.

Design—Randomised, double blind, placebo controlled comparison of inhaled beclomethasone dipropionate 200 µg twice daily as dry powder for six months.

Setting—Southampton.

Outcome measures—Serum osteocalcin, urinary deoxyypyridinoline, and statural growth.

Subjects—7 to 9 year old children with recurrent wheeze.

Results—There were no significant differences in serum osteocalcin between the beclomethasone dipropionate and placebo group measured at baseline or after three and six months’ treatment, while deoxypyridinoline was significantly higher in the placebo treated children after three months. Growth was significantly decreased in the beclomethasone dipropionate group over the course of the study. Growth over the six months, both in those receiving beclomethasone dipropionate and those receiving placebo, was significantly correlated with serum osteocalcin measured at three months and six months.

Conclusion—Although serum osteocalcin shows excellent correlation with growth, it is a poor marker for decreased growth associated with use of inhaled corticosteroids.

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Keywords: osteocalcin, growth, inhaled corticosteroids.

Systemic corticosteroids are an effective treatment of asthma, but in childhood result in impaired statural growth1 and in adults in trabecular bone mineral loss of up to 7% per year, with an increased risk of fractures.2 Inhaled corticosteroids have fewer side effects than oral corticosteroids, but may nevertheless impair growth in childhood3 and in adults adversely affect bone turnover manifest as decreased serum osteocalcin concentrations.4–11

Osteocalcin is the most abundant non-collagenous protein found in bone accounting for 1–2% of the total protein. It is a 49 amino acid residue peptide, two or three of which are the vitamin K dependent amino acid, carboxyglutamic acid. It is almost exclusively synthe-
sised by osteoblasts and is incorporated into the bone matrix and dentine where it binds to hydroxyapatite.12 A small amount of the newly formed osteocalcin is released into the circulation, and as it is not normally released during the resorption of bone protein, it is therefore a specific marker for osteoblastic activity. Cross sectional studies have shown that serum osteocalcin concentrations in childhood parallel growth velocity.13–15 Deoxypyridinoline is found mainly in type I collagen of bone, and is a marker of collagen breakdown. It is formed during the post-translational extracellular maturation of fibrillar collagen, and is released in to the circulation as bone is resorbed, being excreted via the kidneys. Unlike hydroxyproline, deoxypyridinoline is neither metabolised in the body nor influenced by dietary intake of collagen. In adults deoxypyridinoline excretion shows good correlation with radioisotopic16 and histomorphometric17 assessment of bone turnover.

The aim of this study was to prospectively establish the relationship between biochemical markers of bone metabolism and statural growth, and if inhaled corticosteroids did adversely affect growth or bone metabolism, to establish whether biochemical indices of bone metabolism might act as surrogate markers for growth suppression.

Methods

SUBJECTS

The subjects took part in a community based study to assess the effect of inhaled beclomethasone dipropionate administered as dry powder on wheezing episodes in schoolchildren as previously described.18 Children suitable for the longitudinal study were selected on the basis of either five or more wheezing episodes in the preceding year or an episode of wheezing lasting for three days or more in the preceding year. Exclusion criteria included the use of inhaled or oral corticosteroids, or coexistent respiratory disease such as cystic fibrosis. Altogether 104 children fulfilled the enrolment criteria and agreed to enter the study.

Before treatment randomisation the children were asked to give a sample of blood and collect an overnight sample of urine. Blood was taken using topical anaesthetic (EMLA, Astra Pharmaceuticals), centrifuged and the serum stored at -20°C within three hours of collection. Blood samples were always collected after school, usually at the exact same time of day for each child. A timed overnight urine sample was collected on the same day. Urine volumes were
measurements were normalised within the whole group. A procedure was pressed for, using deoxypyridinoline/creatinine ratio. Overnight urine samples varied from 10.3% to 9.55. (mean 99.4). There were no significant differences in concomitant medication between the two groups. In addition two children receiving beclomethasone dipropionate and three receiving placebo were given oral corticosteroids by their general practitioner during the study. Mean compliance with treatment was similar in the two groups: 75.2% in the beclomethasone dipropionate treated group compared with 75.9% in the placebo group (95% CI diff 8.15 to 9.55).

OSTEocalcin
Mean serum osteocalcin in the beclomethasone dipropionate and placebo groups over the six months are shown in fig 1. There was no significant difference in serum osteocalcin between the beclomethasone dipropionate and placebo groups at baseline or at either of the two measurements while on treatment, and thus for further analysis the two groups are combined. Mean (SD) serum osteocalcin for all the children decreased from 20.3 (4.90) ng/ml at baseline to 19.1 (4.66) after three months and 16.5 (4.81) ng/ml after six months, with a significant decrease between three months and six months (95% CI diff 0.96

Table 1 Regular concomitant medication taken by children in the beclomethasone dipropionate and placebo groups

<table>
<thead>
<tr>
<th>Regular medication</th>
<th>Beclomethasone dipropionate</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium cromoglycate</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Nasal beclomethasone dipropionate</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Theophylline</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Topical corticosteroids for ecema</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>1</td>
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Results
Of the 104 children who entered the study 52 received inhaled beclomethasone dipropionate (37 boys, 15 girls) and 52 placebo (36 boys, 16 girls). Ninety four children completed the study of whom 50 received beclomethasone dipropionate and 44 placebo. Before randomisation there were no significant differences between the beclomethasone dipropionate and placebo treated groups in mean age at entry in to the study (100.3 months ± 99.4 months, range 86 – 119 ± 86 – 119) or in height SD scores (mean 0.53 ± 0.33, p=0.27). Ten children were removed from the analysis due to confounding factors: six children were entering puberty, three had been started on regular inhaled corticosteroids by their general practitioners, and one child had injured his back affecting his growth. Seventeen children were receiving concomitant medication (see table 1). There were no significant differences in concomitant medication between the two groups.

Mathematical analysis was performed using SPSS-PC. Growth over the study was calculated by linear regression of the multiple height measurements on treatment for each child, and expressed as mm/week. Urinary deoxypyridinoline/creatinine ratio was log transformed to normalise the distribution. Comparison of use of concomitant medication used the x² test. Paired Student’s t test was used for changes within the whole group over time, and unpaired Student’s t test to compare differences between the two groups at different time points. Association between continuous variables was analysed by correlation and multiple linear regression. Results are presented as means and the 95% confidence interval of the difference (95% CI diff), and for the log transformed data ratio of means and 95% CI diff of the ratio of means.

MEASUREMENTS OF GROWTH
The children were assessed every two to four weeks and their height recorded as previously described. At each visit the child’s height was measured by a single observer (ID) using a Raven Minimeter (Raven Equipment Ltd). The Minimeter is portable, and as accurate as a fixed stadiometer. The standard deviation of a single height measurement calculated as per Voss et al was 0.10 cm. The child was measured barefoot with the Minimeter being positioned on the child’s head ‘blindly’ before being read. Three measurements were taken in this manner with no reference to the previous measurements, and the mean calculated. If one measurement was very discrepant the entire procedure was repeated. Weight was measured using Seca scales. The pubertal stage of the child was assessed at the end of the study. Compliance with treatment was assessed by counting the used Diskhaler blisters.

BIOCHEMICAL MEASUREMENTS
All measurements were performed in duplicate. Osteocalcin was measured by radioimmunoassay (Diagnostic Systems Laboratories) using antiserum raised in rabbits to bovine osteocalcin and free [125I]labelled osteocalcin. Urinary deoxypyridinoline was measured by competitive immunoassay (Metra Biosystems) using a monoclonal antibody to deoxypyridinoline. Urinary creatinine was measured by standard automated methods. Overnight urinary deoxypyridinoline excretion was expressed as deoxypyridinoline/creatinine ratio. Interassay coefficient of variation was 11.2% for osteocalcin and 10.3% for deoxypyridinoline.

Permission for the study was obtained from the ethics committee of the Southampton University Teaching Hospitals. The children gave informed assent and the parents informed written consent.

DATA ANALYSES
Statistical analysis was performed using SPSS-PC. Growth over the study was calculated by linear regression of the multiple height measurements on treatment for each child, and expressed as mm/week. Urinary deoxypyridinoline/creatinine ratio was log transformed to normalise the distribution. Comparison of use of concomitant medication used the x² test. Paired Student’s t test was used for changes within the whole group over time, and
to 5.3, p=0.006). There was no significant difference in serum osteocalcin between girls and boys at any of the three time points.

**URINARY DEOXYPYRIDINOLINE/CREATININE RATIO**

Geometric mean urinary deoxypyridinoline/creatinine ratio in the beclomethasone dipropionate and placebo groups over the six months are shown in fig 2. There were no significant differences in urinary deoxypyridinoline concentrations between the beclomethasone dipropionate and placebo treated group at baseline (20.2 ± 18.4 nmol/mmol creatinine, ratio 0.911, 95% CI 0.75 to 2.95) or at six months (16.5 ± 18.0 nmol/mmol creatinine, ratio 1.19, 95% CI 0.94 to 1.51), but urinary deoxypyridinoline was significantly higher in the placebo treated group compared with the beclomethasone dipropionate treated group after three months (17.6 ± 22.64 nmol/mmol creatinine, ratio 1.29, 95% CI 1.01 to 1.65). Geometric mean (SD) urinary deoxypyridinoline for the children as a whole decreased over the course of the study from 19.4 (1.58) nmol/mmol creatinine at baseline to 19.8 (1.66) nmol/mmol creatinine after three months and 16.4 (1.55) nmol/mmol creatinine after six months, with a significant decrease from three months to six months (ratio 1.35, 95% CI 1.15 to 1.6, p=0.01). There was no significant difference in urinary deoxypyridinoline/creatinine between girls and boys at any of the three time points.

**GROWTH**

There was no significant difference in height at baseline between the beclomethasone dipropionate and placebo treated groups (130.1 v 128.4 cm, p=0.216). As previously reported, the mean growth was significantly decreased in the beclomethasone dipropionate treated group compared with placebo (0.79 v 1.14 mm/week, 95% CI diff 0.25 to 0.46, p < 0.0001).

Mean growth over the six months of the study was significantly correlated with serum osteocalcin measured at three (fig 3) and six months (fig 4), both in those children receiving beclomethasone dipropionate and in those children receiving placebo (r=0.44, p=0.01 and r=0.57, p=0.005 respectively at three months; and r=0.5, p=0.01 and r=0.52, p=0.05 respectively at six months), but was unrelated to serum osteocalcin measured at baseline (r=0.1, p=0.6 and r=0.05, p=0.8).

Mean growth over the six months was significantly correlated with urinary deoxypyridinoline/creatinine ratio at three months in only the beclomethasone dipropionate treated group (r=0.4, p=0.02), but was unrelated to any other measurements.

**Discussion**

Over a six month period, compared with placebo, regular inhaled beclomethasone dipropionate 200 μg twice daily had no significant effect on either serum osteocalcin, a marker of bone formation, nor urinary deoxypyridinoline, a marker of bone resorption. Of
note was that serum osteocalcin measured after three and six months was significantly related to statural growth over the six month period.

These findings may at first appear at odds with our previously reported finding in this group of children of decreased growth in those receiving beclomethasone dipropionate. Although serum osteocalcin showed significant correlation with growth over the six month period, and we have previously demonstrated decreased growth in those children randomised to receive beclomethasone dipropionate, beclomethasone dipropionate did not have any discernible effect on serum osteocalcin. However as the correlation between growth and serum osteocalcin was 0.5, only 25% of the variation in growth would be dependent on the serum osteocalcin. Three other possibilities warrant consideration. Firstly it may be that beclomethasone dipropionate at a dose of 200 µg twice a day has little effect on bone turnover in children, and although it clearly has an effect on growth, this is not mediated through an effect on bone. Secondly beclomethasone dipropionate may have an effect on bone turnover that results in decreased growth, but that neither serum osteocalcin nor urinary deoxypyridinoline are sufficiently sensitive to detect this effect. A third possibility is that beclomethasone dipropionate’s effect on bone turnover is short term, and that bone turnover had returned to normal by the time we measured it after three months’ treatment. We feel that the first possibility is the most likely. Osteocalcin has been demonstrated to be a sensitive measure of the effect of low dose prednisolone in childhood, and our findings clearly demonstrate osteocalcin’s relationship to growth.

Cross sectional studies where serum osteocalcin has been measured report broadly similar findings with serum osteocalcin concentrations in childhood paralleling growth velocity. The only prospective study to date of growth and serum osteocalcin reported no significant relationship between growth in the first 9 months of age and serum osteocalcin measured at 0, 6, and 9 months of age. In this age group serum osteocalcin might however reflect vitamin K status rather than osteoblastic activity. Serum osteocalcin has been proposed as a predictive marker of response to treatment in children being started on growth hormone replacement treatment, although the published evidence is conflicting. To our knowledge ours is the first prospective study demonstrating a significant relationship between serum osteocalcin and growth in normal children.

Both serum osteocalcin and urinary deoxypyridinoline for the group of children as a whole decreased over the course of the study. Furthermore growth over the six months of our study was significantly related to serum osteocalcin measured at the three month midpoint and after six months, but was unrelated to the baseline measurement. Both statural growth and serum osteocalcin demonstrate seasonal variation, with both growth and osteocalcin being higher in the summer. It may be that the initial baseline sample reflected growth over the preceding summer. It is interesting that the urinary deoxypyridinoline increased after three months in the placebo treated group, the opposite of what would have been expected if the beclomethasone dipropionate had a significant effect on bone turnover. We are unable to explain this finding, and it may be a statistical artefact.

Most of the information on the effects of corticosteroids on biochemical markers of bone turnover in asthmatics has been obtained in adults. In non-asthmatic adult volunteers a mean daily dose of beclomethasone dipropionate 400 µg to 2000 µg/day or budesonide 800-3200 µg/day significantly decreased serum osteocalcin within one week. In a prospective study of newly diagnosed asthmatics started on beclomethasone dipropionate increasing from 400 to 2000 µg/day, serum osteocalcin significantly decreased by nine weeks.

There is a paucity of data on the effect of corticosteroids on bone turnover in asthmatic children. König et al related bone markers and bone mineral content in three groups of children: a group of children receiving inhaled corticosteroids, a group of control asthmatic children not requiring inhaled corticosteroids, and a group of normal children. They concluded that beclomethasone dipropionate up to 800 µg/day did not reduce bone mineralisation or increase resorption. Of note however was that both groups of asthmatic children had significantly lower serum osteocalcin concentrations than the normal controls, suggesting that asthma per se can reduce osteocalcin independent of the effect of corticosteroids. Wolthers et al described the short term (two week) effect of oral prednisolone and inhaled budesonide in prepubertal asthmatic children. Oral prednisolone 2.5 mg and 5 mg/day demonstrated a dose related reduction in serum osteocalcin, but budesonide at a dose of 800 µg/day caused no significant effect. Similarly in an open follow up of 23 children started on inhaled budesonide or inhaled budesonide 400 µg/m²/day, Sorva et al reported no significant change in serum osteocalcin.

Thus the limited reports on the effect of corticosteroids on bone turnover in asthmatic children suggest that oral steroids have a significant effect on biochemical markers of osteoblastic function and bone resorption, but that inhaled corticosteroids up to a dose of 800 µg/day have little effect on biochemical markers or bone mineralisation. Our findings are in accordance with these conclusions, but further suggest that biochemical indices of bone turnover are poor markers for the growth suppressive actions of inhaled corticosteroids.
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1 Spock A. Growth patterns in 200 children with bronchial asthma. Am Allergy 1965; 23: 608-15.