A randomised controlled trial of short term growth and collagen turnover in asthmatics treated with inhaled formoterol and budesonide

Carsten Heuck, Lene Heickendorff, Ole D Wolthers

Abstract
Aims—To determine effects on short term growth and collagen turnover of adding formoterol (Eformoterol) to half the glucocorticoid dose in children with asthma, treated with inhaled budesonide (Pulmicort Turbuhaler).

Design—A randomised double blind, placebo controlled crossover study with two six-week periods.

Setting—Outpatient clinic in secondary referral centre.

Subjects—A total of 27 prepubertal children aged 6–13 years.

Interventions—Formoterol 12 µg and dry powder budesonide 100 µg twice daily in one period; placebo and dry powder budesonide 200 µg twice daily in the other.

Outcome measures—Primary outcome measures were lower leg growth rate, and serum and urine markers of type I and type III collagen turnover. Secondary outcome measures were inflammation markers in serum, and parameters of asthma control.

Results—During budesonide 200 µg twice daily treatment, mean lower leg growth rate was 0.14 mm/week (p = 0.02) lower than during the formoterol and budesonide period. Similar statistically significant effects on markers of collagen turnover were found, whereas inflammation markers and asthma control did not vary statistically significantly between the two periods.

Conclusions—In children treated with inhaled glucocorticoids, halving the dose and adding formoterol is associated with faster short term growth and an increase in markers of collagen turnover, with no loss of asthma control.

Keywords: asthma; growth; inhaled glucocorticoids; formoterol; collagen turnover; bronchial inflammation

Prophylactic anti-inflammatory treatment with glucocorticoids in asthmatic children should aim at optimal disease control and make normal development and growth possible. In most schoolchildren this can be achieved with recommended daily doses of 200–400 µg of an inhaled glucocorticoid administered from a metered dose inhaler with a spacer, or with 100–200 µg from a dry powder inhalation device. Children with severe asthma, however, may require higher doses. As the risk of systemic effects of exogenous glucocorticoids depends on dose, systemic adverse effects may be seen in these children. Daily doses of budesonide 800 µg from a metered dose inhaler with a spacer, and dry powder budesonide and beclomethasone dipropionate 400 µg have been found to suppress growth rate and collagen turnover. Although it remains to be properly assessed whether final height may be attained in children during continuous, long term treatment with inhaled glucocorticoids, the need for developing administration regimens which at the same time make disease control possible and alleviate the risk of adverse effects on growth and collagen turnover has been emphasised. Recently, it was reported that combining long acting, inhaled β, agonists, salmeterol xinoate or formoterol fumarate, with an inhaled glucocorticoid, may lead to a greater improvement in control of asthma than treatment with the inhaled glucocorticoid alone. The aim of the present study was to see whether addition of inhaled formoterol to half the glucocorticoid dose in children with asthma requiring high doses of inhaled glucocorticoids, affects growth and collagen turnover.
Inhaled formoterol and budesonide

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the urine samples were stored at −80 °C until the children attended the clinic later that day. Blood samples were taken at the same time (that is, within one hour) between 1300 and 1800. The samples were centrifuged at 4000 rpm for 10 minutes within one hour, after which they and the urine samples were stored at −80°C and batch assayed at completion of the study.

Markers of type I and III collagen turnover
Serum concentrations of the carboxy terminal propeptide of type I procollagen (PICP), the amino terminal propeptide of type I procollagen (PINP), the carboxy terminal pyridinoline cross linked telopeptide of type I collagen (ICTP), and the amino terminal propeptide of type III procollagen (PIIINP) were determined by radioimmunoassays based on human antigen (Orion Diagnostica, Oulunsalo, Finland). Intra- and interassay variations were <5% and <7% for all assays. Normal serum ranges have been published previously.17 20 21 Urinary deoxypyridinoline (DPD) was measured by a solid phase chemiluminescent enzyme immunoassay on an automated instrument (Immuno Pyrilinks-D, Diagnostica Products Corporation, Los Angeles, California). The assay specifically detects non-peptide bond DPD.22 Urinary cross linked N-telopeptides of type I collagen (NTx) were measured by the Osteomark radioimmunoassay (Ostex, Seattle, Washington).23 Sample dilutions were made with urine containing low concentrations of NTx as recommended by the manufacturer. Intra- and interassay variations were 8% and 9%, and 8% and 12%, respectively, for the DPD and NTx assays. Normal ranges of the DPD assay in children are not available, whereas preliminary NTx data standardised for creatinine excretion have been published.24

Markers of airway inflammation
The serum concentration of eosinophil cationic protein (ECP) and eosinophil protein X (EPX) in urine were determined using specific radioimmunoassays (Pharmacia & Upjohn, Sweden).25 26 Intra- and interassay variations were 6% and 7%, and 5% and 9%, respectively. Normal reference values for serum ECP and urine EPX standardised for creatinine are available.25–27

Creatinine in urine was determined by standard laboratory methods.

PULMONARY FUNCTION AND SYMPTOMS
Using a dry bellows spirometer (Vitalograph), pulmonary function was assessed by measuring forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) on the last day of weeks 2 and 6 in the treatment periods. The best of three measurements was recorded. Peak expiratory flow rates, the best of three measurements, were recorded with the Mini-Wright peak flow meter at home, in the morning and in the evening before medication. Day and night asthma symptoms were recorded in diaries according to a four point scale (0 indicating no symptoms; 3 indicating severe symptoms), and the use of rescue terbutaline was recorded.

STATISTICS
Growth rate was calculated as the difference in lower leg length determined at the end of weeks 6 and 2, divided by the time interval between the measurements.15 Data were tested for and found to fulfil conditions for normal distribution. Statistical analysis was performed by Student’s paired t test. For assessment of lung
function data, FEV1/FVC ratios were used. The ratios were tested by one way analysis of variance for repeated measurements. All data were tested for period and carry over effects. The 5% level of significance was used.

**Results**

Twenty four children completed the study according to the protocol. During budesonide–placebo treatment a girl and a boy were withdrawn from the study because of acute exacerbations requiring treatment with systemic glucocorticoids. A boy failed to complete the study because his family moved away. During the first period a girl decided not to have blood samples taken, and three girls failed to collect urine during both periods. Fourteen children were randomised to receive budesonide and formoterol treatment in the first period, 10 children to receive budesonide and placebo first. No period or carry over effects on any of the measured parameters were found.

Compliance with dosage regimen varied from 88% to 100% (mean 97%) and from 90% to 100% (mean 98%) for formoterol and budesonide, respectively. No variations in compliance between the two treatment periods were detected (formoterol: \(p = 0.48, t = -0.7\), 95% CI \(-2.8\%\) to \(1.4\%\); budesonide: \(p = 0.51, t = -0.7\), 95% CI \(-2.4\%\) to \(1.3\%\)).

Figure 1 and table 1 show individual and group mean lower leg growth rates, serum and urine concentrations of collagen markers, and the results of the statistical comparisons.

During budesonide 200 µg twice daily treatment, mean lower leg growth rate was 0.14 mm/week (\(p = 0.02\)) lower than during the formoterol and budesonide period. Serum PICP, PINP, ICTP, and PIIINP, and urine NTx concentrations were reduced by 44 µg/l (\(p = 0.04\)), 64 µg/l (\(p = 0.004\)), 1.1 µg/l (\(p = 0.009\)), 1.2 µg/l (\(p = 0.002\)), and 126 nmol/mmol creatinine (\(p = 0.02\)), respectively, during the budesonide 200 µg twice daily period.

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**Table 1**  Group means of lower leg growth rates, serum PICP, PINP, ICTP, and PIIINP, and urine NTx and DPD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formoterol and budesonide</th>
<th>Placebo and budesonide</th>
<th>(p) and (t) values; 95% confidence interval for the difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower leg growth rate (mm/week)</td>
<td>0.39 (0.05)</td>
<td>0.25 (0.04)</td>
<td>(p = 0.02, t = 2.6, 0.03) to (0.26) mm/week</td>
</tr>
<tr>
<td>PICP (µg/l)</td>
<td>490 (27)</td>
<td>447 (28)</td>
<td>(p = 0.04, t = 2.2, 2) to (85) µg/l</td>
</tr>
<tr>
<td>PINP (µg/l)</td>
<td>582 (40)</td>
<td>517 (39)</td>
<td>(p = 0.004, t = 2.2, 2) to (107) µg/l</td>
</tr>
<tr>
<td>ICTP (µg/l)</td>
<td>11.7 (0.5)</td>
<td>10.5 (0.4)</td>
<td>(p = 0.009, t = 2.9, 0.3) to (1.9) µg/l</td>
</tr>
<tr>
<td>PIIINP (µg/l)</td>
<td>7.7 (0.6)</td>
<td>6.4 (0.4)</td>
<td>(p = 0.002, t = 3.6, 0.5) to (2.0) µg/l</td>
</tr>
<tr>
<td>NTx/creatinine (nmol/mmol)</td>
<td>693 (55)</td>
<td>566 (54)</td>
<td>(p = 0.02, t = 2.4, 18) to (234) nmol/mmol creatinine</td>
</tr>
<tr>
<td>DPD/creatinine (nmol/mmol)</td>
<td>16.6 (1.2)</td>
<td>15.5 (1.0)</td>
<td>(p = 0.25, t = 1.2, -0.9) to (3.1) nmol/mmol creatinine</td>
</tr>
</tbody>
</table>

Serum parameters were measured in 23 asthmatic children (12 boys and 11 girls), and urine parameters in 12 boys and 9 girls during treatment with inhaled formoterol 12 µg and budesonide 100 µg twice daily, and placebo and budesonide 200 µg twice daily.

Results expressed as mean (SEM).

*Formoterol and budesonide versus placebo and budesonide.
Serum ECP and urine EPX showed no significant variations: during treatment with budesonide 200 µg twice daily, serum ECP and urine EPX/creatinine concentrations were 9.7 (SEM 1.6) µg/l and 139 (21) µg/mmol; during formoterol and budesonide treatment concentrations were 9.0 (1.1) µg/l and 142 (18) µg/mmol, respectively (ECP: $p = 0.70$, $t = -0.4$, 95% CI $-4.2$ to $2.9$ µg/l; EPX/creatinine: $p = 0.87$, $t = 0.2$, 95% CI $-30$ to $36$ µg/mmol).

Figure 2 shows group means of peak expiratory flow rates, symptom scores, consumption of short acting $\beta_2$ agonists, and FEV$_1$/FVC ratio. Mean peak expiratory flow rates (morning: $p = 0.33$, $t = 0.99$, 95% CI $-6$ to $16$ l/min; evening: $p = 0.29$, $t = 1.1$, 95% CI $-7$ to $29$ l/min), symptom scores (day: $p = 0.55$, $t = 0.60$, 95% CI $-0.11$ to $0.20$; night: $p = 0.64$, $t = 0.47$, 95% CI $-0.10$ to $0.15$), and consumption of $\beta_2$ agonists ($p = 0.56$, $t = -0.6$, 95% CI $-0.48$ to $0.27$ puffs/day) showed no statistically significant variations. Compared to the budesonide 200 µg twice daily period, mean FEV$_1$ in week 2 was significantly higher ($p = 0.04$, $t = 2.2$, 95% CI $-0.01$ to $0.17$) during the formoterol and budesonide period, whereas no significant difference ($p = 0.32$, $t = 1.0$, 95% CI $-0.02$ to $0.06$) was found in week 6.

Separate analyses of boys and girls revealed no sex differences in any of the outcome measures.

**Discussion**

The principal aim of the present study was to assess whether short term treatment with twice daily inhaled formoterol 12 µg and dry powder budesonide 100 µg reduces the risk of systemic effects on growth and collagen turnover, compared to a double dose of budesonide in children requiring long term treatment with high doses of inhaled glucocorticoid. Primary outcome measures were lower leg growth rates and markers of type I and III collagen turnover.
The sensitivity of knemometry in the assessment of growth suppressive effects of exogenous glucocorticoids depends on a crossover design. 14 The participating children should be enrolled in the study almost simultaneously, and the total study period should be restricted to less than three or four months. Considering both these conditions and the disease severity of the study population, for methodological and ethical reasons run in or wash out periods without any anti-inflammatory treatment could not be included in the present trial. Changes in lower leg growth rates and markers of collagen turnover in response to variations in dose of exogenous glucocorticoid, however, seem to occur well within two weeks. 29–31 Therefore, to avoid carry over effects, the first two weeks of each period were excluded from analysis. The results of the statistical analyses proved that this was achieved, confirming the sensitivity of the study design.

The observed mean growth rate during formoterol 12 µg and dry powder budesonide 100 µg twice daily treatment is in line with growth rates previously observed in children with mild asthma during dry powder budesonide 100 µg twice daily and placebo treatments, and with growth rates in normal children measured during the same time of the year. 32–34 Though no firm conclusions can be drawn as the present trial did not include a placebo period, the finding of a higher growth rate during treatment with formoterol 12 µg and dry powder budesonide 100 µg twice daily compared to budesonide 200 µg twice daily suggests that in children requiring regular inhaled glucocorticoids growth may not be affected when combined treatment is given. The slower growth observed during the twice daily dry powder budesonide 200 µg period is comparable to the results from a crossover knemometry study which applied two week treatment periods. 32

Serum concentrations of PICP and PINP reflect synthesis, and ICTP, urine NTx, and DPD represent degradation of type I collagen, primarily in bone. 17, 22, 23, 35, 36 Serum concentrations of PIIINP correlate with synthesis of type III collagen which is present in connective tissue throughout the body. 37 The findings of suppressed concentrations of PICP, PINP, ICTP, and PIIINP during the twice daily budesonide 200 µg period are consistent with observations in previous studies of equipotent administration of budesonide and beclomethasone dipropionate, indicating suppressive effects on type I (bone) and type III collagen turnover. 4, 6, 7 Studies of the effect of inhaled glucocorticoids on urine DPD have been more conflicting. Some have found suppression, but others have not, in children treated with twice daily 400 µg budesonide from a metered dose inhaler with a spacer. 37 In one study, twice daily dry powder budesonide 400 µg was associated with suppressed DPD concentrations. 38 To some extent the discrepancies may be a result of biological variations in urine DPD. 38 However, recent reports suggest that the sensitivity of urine crosslinks assays for assessment of bone resorption activity may depend on whether total or peptide bound concentrations are measured. Discrepancies in decreases between free, total, and peptide bound crosslinks have been found in several studies of bisphosphonates and after vitamin D and calcium supplementation. 39–42 These observations indicate that changes in bone resorption are reflected with a greater sensitivity with assays that determine peptide bound crosslinks than with free crosslinks, and it has been suggested that the excretion of the markers may be differently influenced. 43 We therefore wanted to include a new marker of type I collagen degradation, NTx, which has been proven sensitive in various conditions of bone resorption. 23, 38 The NTx assay measures only the peptide bound fraction of urine crosslinks. The finding of suppressed urine NTx during the twice daily budesonide 200 µg period which was paralleled by suppressed serum ICTP concentrations suggests that NTx may be more sensitive than DPD for detection of glucocorticoid induced effects on growth in children treated with inhaled glucocorticoids.

Eosinophilic inflammation of the airways is related to disease activity. Activated eosinophils release the cytotoxic granula ECP and EPX which may be detected in serum and urine, respectively, being sensitive markers of eosinophil activation and airway inflammation. 27, 26, 41 Corresponding with an improvement in symptom control, raised concentrations of serum ECP and urine EPX in children with poorly controlled asthma were normalised during treatment with inhaled glucocorticoids. 23, 26, 41 In the present study serum ECP and urine EPX concentrations were almost identical during the formoterol 12 µg and dry powder budesonide 100 µg twice daily, and budesonide 200 µg twice daily periods. This was paralleled by the observation of no statistically significant variations in peak expiratory flow rates, in symptom scores or in the use of rescue terbutaline, indicating a similar control of bronchial inflammation. It may be suggested that these findings could have been caused by overtreatment during treatment with the higher dose of budesonide. However, the observed FEV1 data do not support that. Furthermore, the results are in accord with reports of unaffected bronchial hyperreactivity as measured by provocation tests and improvements in lung function and symptom control when long acting β2 agonists were added to inhaled glucocorticoids in symptomatic patients. 10, 12, 13 Addition of salmeterol to inhaled beclomethasone dipropionate 500 µg provided better symptom control than did doubling the dose of the glucocorticoid. 13 Finally, though formoterol induced effects on acute inflammatory changes cannot be ruled out, regular treatment with long acting β2 agonists do not seem to modify chronic airway inflammation. 15 Therefore, although the present study was not designed or powered to test control of lung function during the two treatment regimens, when the inflammation markers are taken together with symptoms and lung function, the results indicate that disease activity was well controlled during combination therapy with formoterol and budesonide.
Short term knemometry and serum and urine markers of type I and III collagen turnover may be the most sensitive markers of systemic activity of inhaled glucocorticoids available in children. To what extent glucocorticoid induced suppression of the measures may correlate with an increased risk of final height suppression or osteoporosis during long term treatment still remains to be clarified. On the other hand, if an inhaled glucocorticoid is not associated with suppressive effects on growth rates or markers of collagen turnover in short term assessments, deleterious long term effects are most unlikely. Considering that long term combination therapy with formoterol and budesonide effectively controls asthma symptoms, 10 we believe that the present study has provided important new information for the clinical management of children requiring inhaled glucocorticoids. Formoterol 12 µg twice daily can be added to half the dose of the inhaled glucocorticoid; this reduces the risk of suppressive effects on growth and collagen turnover.

Inflammation marker assessments were performed at Research and Development Laboratories, Pharmacia & Upjohn, Diagnostics AB, Uppsala, Sweden, supervised by senior scientist Christian Pettersson. Mini Wright peak flow meters were supplied by Simonsson and Weil AS, Copenhagen, Denmark.