

Effects of inhaled budesonide on serum markers of bone metabolism in children with asthma

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The effects of inhaled glucocorticoids on serum markers of bone formation were evaluated in asthmatic children. Serum total alkaline phosphatase (AP), bone alkaline phosphatase (BAP), osteocalcin, and the novel marker of bone formation, carboxypropeptide of type I procollagen (PICP), were measured. In the cross-sectional part, long-term glucocorticoid users were compared with sodium cromoglycate (SCG) users. In the boys (n = 16), but not in the girls (n = 11), PICP was significantly lower in the glucocorticoid users than in the SCG users. PICP correlated positively with BAP (n = 54; groups combined, $r = 0.29$, $p < 0.05$). In the longitudinal part, the effects of inhaled budesonide or SCG, both used for the first time, were evaluated before and after 1 and 5 months of treatment. The budesonide dose was 800 $\mu\text{g}/\text{m}^2/\text{day}$ for 1 month and thereafter half of that. The SCG dose was 30 mg/day throughout the study. Only during budesonide use did osteocalcin and PICP decrease, the median osteocalcin by 8% at 1 month ($p < 0.05$) and by 6% at 5 months ($n = 15$), and PICP by 5% at 1 month ($p < 0.05$) and by 28% at 5 months ($n = 7$, $p < 0.01$). AP and BAP did not change significantly. Decreased PICP suggests decreased bone formation rate. PICP might be clinically useful as a marker of early adverse effects of glucocorticoids on bone. (J ALLERGY CLIN IMMUNOL 1992;90:808-15.)

Key words: Asthma, bone metabolism, calcium, children, glucocorticoids, growth, osteocalcin, procollagens

Inhaled glucocorticoids have been advocated as primary treatment for adults even in patients with newly detected mild asthma.¹ However, growth deceleration and bone loss are among the most common side effects of oral glucocorticoids.²⁻⁴ Reduced total body calcium in adults and deceleration of growth in children have been reported even with inhaled glucocorticoids.^{5, 6} Therefore their more liberal than conventional use may be anticipated also for children.

Bone formation rates have usually been evaluated by measuring serum alkaline phosphatase (AP), bone alkaline phosphatase (BAP) in particular.⁷ More recently the serum level of osteocalcin, the most abun-

Abbreviations used

AP:	Alkaline phosphatase
BAP:	Bone alkaline phosphatase
Ca:	Serum total calcium
Cai:	Serum ionized calcium
CV:	Coefficient of variation
FEV ₁ %:	Ratio of forced expiratory volume in 1 second to forced vital capacity
IGF-I:	Insulin-like growth factor I
P:	Serum phosphate
PICP:	Carboxypropeptide of type I procollagen
SCG:	Sodium cromoglycate
SDS:	Standard deviation score

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dant noncollagen protein of bone, has been used.⁷⁻⁹ Furthermore, osteocalcin might serve as a serum marker of actual growth velocity.^{7, 10}

Type I collagen accounts for more than 90% of the organic matrix of bone and is the only collagen type found in mineralized bone. During collagen synthesis, carboxypropeptide of type I procollagen (PICP) is cleaved from type I procollagen and released into

blood. The serum PICP level correlates with type I collagen synthesis and bone formation rates.¹¹⁻¹³

The main aim of the present study was to investigate the effects of inhaled glucocorticoids on PICP and osteocalcin in comparison with AP and BAP and on serum insulin-like growth factor I (IGF-I). The effects of long-term glucocorticoid use were evaluated in a cross-sectional study, and the effects of the very first period of glucocorticoid treatment were evaluated in a longitudinal study. Little is known of the effects of inhaled glucocorticoids on the serum levels of ionized calcium (Cai), the "true" calcemic parameter, in children. Thus serum total calcium (Ca), Cai, and serum phosphate (P) were also measured.

PATIENTS

The children were treated for their asthma as outpatients at the Department of Allergic Diseases of Helsinki University Central Hospital in 1989 to 1991. They were free of other diseases and medications known to affect calcium or bone metabolism.

Cross-sectional study

The patients were 54 children who had been treated with inhaled budesonide or sodium chromoglycate (SCG) for a considerable time. The patients on budesonide (steroid group) were matched by age and sex with patients on SCG (SCG group). There were 16 boys and 11 girls in both groups. The median age (range) was 9.5 (6 to 14) years in the steroid group and 9.6 (5 to 14) years in the SCG group. It was 9.5 years (5.7 to 12.8) for the boys and 10.4 years (6.5 to 13.9) for the girls in the steroid group, and 9.6 years (5.2 to 12.9) for the boys and 10.5 years (6.3 to 13.6) for the girls in the SCG group.

The patients in the steroid group had been using inhaled budesonide for a median duration (range) of 2.0 (0.5 to 5.9) years. The actual dose was 400 $\mu\text{g}/\text{m}^2/\text{day}$ for 20 patients, 200 $\mu\text{g}/\text{m}^2/\text{day}$ for four, and 600 $\mu\text{g}/\text{m}^2/\text{day}$ for three. Seven out of the 27 patients in the steroid group had previously used inhaled beclomethasone, 12 had had one or several intermittent oral prednisolone courses, 11 used nasal steroids regularly, and three used them intermittently, 100 μg twice daily. Oral theophylline was used by 11 patients regularly and by six intermittently. All patients used inhaled beta-adrenergic agonists as needed.

The patients in the SCG group ($n = 27$) had never used inhaled, nasal, or systemic glucocorticoids. The patients in this group had been using inhaled SCG for a median duration (range) of 2.0 (0.5 to 5.8) years. The actual dose was 30 mg/day for 21 children, 20 mg/day for four, and 60 mg/day for two. Two were regular users and five were intermittent users of oral theophylline. All the patients used inhaled beta-adrenergic agonists as needed.

Longitudinal study

The patients in this section of the study were 23 children who were first started on inhaled budesonide ($n = 15$) or

SCG ($n = 8$) (no previous steroid use in any form) in 1989 to 1990. Both drugs were administered as an aerosol with a valved spacer device (Nebuhaler, Draco AB, Lund, Sweden). The daily dose of budesonide was 800 $\mu\text{g}/\text{m}^2$ for the first month, and 400 $\mu\text{g}/\text{m}^2$ for the next 4 months, both divided into morning and evening doses. SCG was given in three daily doses of 10 mg each. One patient took oral theophylline regularly. The patients were permitted to use inhaled beta-adrenergic agonists as needed. The median ages (range) were 8.3 (5.4 to 12.2) years for the budesonide group and 8.6 (5.8 to 11.8) years for the SCG group. Eleven boys and four girls were in the budesonide group, and four boys and four girls were in the SCG group.

METHODS

The height and weight of the patients were recorded at each visit. Height was expressed in standard deviation scores (SDS), as compared with the height of Finnish children of the same age and sex.¹⁴ Weight was expressed in percentages (%) of the median weight of Finnish children of the same height and sex.¹⁴ In the cross-sectional study, growth velocity (centimeters/year, cm/yr) was calculated from the actual height data and those gathered 3 to 8 months previously.

The ratio of forced expiratory volume in 1 second to forced vital capacity (FEV₁%) was used in the evaluation of pulmonary function. Pulmonary function was assessed by pneumotachograph (Vitalograph Compact, Vitalograph Ltd., Buckingham, U.K.) whenever the patients were able to use it correctly. In the cross-sectional study, pulmonary function was assessed in 21 of 27 patients on budesonide and in 26 of 27 patients on SCG. In the longitudinal study, pulmonary function was assessed at entry and after 5 months of treatment in 13 of 15 patients on budesonide and in all eight patients using SCG.

The venous blood and the capillary blood (for Cai) were drawn in the morning after 12 hours of fasting. In the longitudinal study, blood samples were taken before and after 1 and 5 months of treatment. Deep-frozen (-20°C) serum was used for determination of osteocalcin, IGF-I, and PICP. Pretreatment and posttreatment samples were determined simultaneously. In the longitudinal study no frozen sera were available from the eight patients who were started on budesonide in 1989. Thus IGF-I and PICP were determined only in the seven patients (6 boys, 1 girl) started on budesonide in 1990 and all the eight patients (4 boys, 4 girls) taking SCG.

Ca and P were determined by automated methods. Cai at pH 7.4 was determined by a Radiometer ICA1 (Radiometer, Copenhagen, Denmark). BAP was determined after separation by lectin precipitation (Boehringer Mannheim, Mannheim GMBH, Germany), with an intraassay coefficient of variation (CV) of 4.1% and an interassay CV of 4.8%.¹⁵ Osteocalcin was determined by competitive RIA (CIS, Gif-sur-Yvette, France) with an interassay CV of 5%. PICP was determined by competitive RIA (Farnos Diagnostica, Finland) with an intraassay CV of 2.9% to 3.5% and an interassay CV of 5.0%. IGF-I was first purified by

TABLE I. Cross-sectional study: median (range) serum concentrations of calcemic variables and markers of bone formation

		Steroid group	SCG group
Ca, mmol/L		2.45 (2.20-2.60)	2.40 (2.20-2.55)
Cai, mmol/L		1.27 (1.17-1.35)	1.29 (1.23-1.39)
P, mmol/L		1.40 (0.96-1.65)	1.43 (1.10-1.74)
AP, IU/L		506 (255-946)	578 (220-876)
BAP, IU/L	Boys	434 (230-686)	508 (275-778)
	Girls	477 (331-899)	495 (155-832)
	Both	440 (230-899)	495 (155-832)
OC, µg/L	Boys	27.3 (19.0-34.4)	29.0 (20.9-42.3)
	Girls	29.2 (24.0-39.5)	26.0 (12.1-33.0)
	Both	28.1 (19.0-39.5)	28.3 (12.1-42.3)
PCIP, µg/L	Boys	371 (128-462)	419 (230-624)**
	Girls	420 (190-492)	308 (100-618)
	Both	390 (128-492)	368 (100-624)
IGF-I, nmol/L		18.5 (10-38)	18.6 (6-33)
Height, SDS		-0.5 (-1.9 - +1.3)	-0.3 (-1.7 - +2.9)
Weight, %		+1 (-16 - +36)	+1 (-16 - +26)
Growth velocity		5.8 (0.6 - 11.2)	6.4 (1.8 - 9.6)

OC, Osteocalcin.

TABLE II. Longitudinal study: median (range) serum concentrations of calcemic variables and markers of bone formation in children with inhaled budesonide (*n* = 15, for PICP and IGF-I *n* = 7)

	At entry	At 1 mo	At 5 mo
Ca, mmol/L	2.43 (2.30-2.55)	2.45 (2.30-2.50)	2.43 (2.20-2.55)
Cai, mmol/L	1.27 (1.15-1.37)	1.29 (1.21-1.39)	1.29 (1.18-1.38)
P, mmol/L	1.48 (1.26-1.70)	1.39 (1.25-1.64)	1.40 (1.20-1.60)
AP, IU/L	532 (312-885)	488 (313-682)	502 (297-763)
BAP, IU/L	435 (207-840)	405 (245-645)	425 (235-610)
OC, µg/L	23.6 (16.8-36.0)	21.8** (16.3-35.3)	22.1 (16.0-29.8)
PICP, µg/L	354 (228-647)	336* (206-478)	254*** (174-452)
IGF-I, nmol/L	15 (12-17)	18 (10-21)	17 (11-22)
Height, SDS	-0.2 (-1.7 - +1.5)		-0.3 (-1.9 - +1.3)
Weight, %	+2 (-13 - +24)		+1 (-19 - +34)

p* < 0.05; *p* < 0.02; ****p* < 0.01; as compared with the levels at entry.

chromatography, and then determined by RIA (INC, Stillwater, Minn.) with an interassay CV of 8.2%.

The patients and their parents gave their informed consent. The study protocol was approved by the Ethical Committee of the Department of Allergic Diseases of the Helsinki University Central Hospital.

Statistics

The distributions of the main data (AP, BAP, osteocalcin, PICP) were not Gaussian, and therefore the results are expressed as medians and ranges. In the cross-sectional study, the paired data were compared with use of the Wilcoxon test. In the longitudinal study the parameters at entry and

after 1 and 5 months were compared with use of the Friedman test (nonparametric analysis of variance [ANOVA]). When there were only two measurements (pulmonary function, height and weight), the Wilcoxon test was used. The correlations were calculated with Spearman's rank correlation test, and the between-group comparisons of correlation coefficients were made after *z* transformation.¹⁶

RESULTS Pulmonary function

In the cross-sectional study the median FEV₁% (range) was 82% (56% to 94%) in 21 patients on budesonide and 77% (64% to 99%) in 26 patients on

TABLE III. Longitudinal study: median (range) serum concentrations of calcemic variables and markers of bone formation in children with inhaled SCG (*n* = 8)

	At entry	At 1 mo	At 5 mo
Ca, mmol/L	2.38 (2.10-2.55)	2.38 (2.25-2.55)	2.38 (2.30-2.60)
Cai, mmol/L	1.31 (1.18-1.44)	1.29 (1.26-1.32)	1.29 (1.22-1.35)
P, mmol/L	1.42 (1.22-1.64)	1.46 (1.24-1.54)	1.49 (1.40-1.78)
AP, IU/L	596 (397-868)	577 (435-1001)	550 (435-902)
BAP, IU/L	521 (318-780)	485 (265-900)	480 (350-810)
OC, µg/L	26.3 (21.9-32.4)	25.6 (21.0-30.4)	24.0 (20.3-33.9)
PICP, µg/L	334 (288-442)	332 (298-412)	357 (214-542)
IGF-I, nmol/L	16 (7-28)	13 (6-29)	13 (5-35)
Height, SDS	-0.6 (-2.1 - +0.3)		-0.5 (-1.8 - +0.3)
Weight, %	+6 (-14 - +38)		+6 (-16 - +42)

TABLE IV. Correlation matrix for BAP, osteocalcin, PICP, and growth velocity in the cross-sectional study; correlation coefficients are given for the steroid group (*n* = 16 boys and 11 girls)/for the SCG group (*n* = 16 boys and 11 girls)/and for both groups combined (*n* = 54)

	Osteocalcin	PICP	Growth velocity
BAP	0.365/0.330/0.331**	0.137/0.396*/0.294*	0.295/0.472**/0.425**
Osteocalcin—		0.059/0.308/0.190	0.314/0.113/0.197
PICP—			
Boys and girls combined			0.231/0.471**/0.333**
Boys			-0.035/0.474/0.259
Girls			0.428/0.460/0.411*

p* < 0.05; *p* < 0.02; ****p* < 0.01. Differences in correlation coefficients between steroid and SCG groups, and between boys and girls, not statistically significant.

SCG. In the longitudinal study FEV₁% increased significantly (*n* = 13, *p* < 0.01) during budesonide use, from 75% (63% to 99%) to 82% (74% to 93%), whereas it was not altered during SCG use (*n* = 8): 85% (77% to 92%) before and 85% (68% to 90%) at 5 months.

Growth

In the cross-sectional study height, weight, and growth velocity did not differ significantly between the steroid and the SCG groups (Table I). In the longitudinal study neither height nor weight changed significantly (Tables II and III).

IGF-I

In the cross-sectional study no differences in IGF-I was found between the groups. IGF-I was associated with age (*r* = 0.452, *p* < 0.01, both groups combined; *r* = 0.564, *p* < 0.01, for the steroid group, and *r* = 0.333, for the SCG group; the between-group difference in *r* was not statistically significant).

In the longitudinal study, IGF-I was unchanged in both groups (Tables II and III).

Calcium homeostasis

In the cross-sectional study, Ca, Cai, and P did not differ between groups (Table I). Nor did Ca, Cai, or P change significantly during budesonide or SCG use in the longitudinal study (Tables II and III).

Alkaline phosphatase

In the cross-sectional study, no significant differences were observed in AP and BAP in the steroid or SCG group (Table I). BAP correlated with growth velocity when both groups were combined and in the SCG group (Table IV).

No significant changes were observed in AP and BAP in either group in the longitudinal study (Tables II and III). However, BAP tended to decrease during budesonide use (*p* = 0.074 for overall change, *p* = 0.086 for the change between the levels at entry and at 1 month).

Osteocalcin

In the cross-sectional study osteocalcin did not differ significantly between groups (Table I). There was a positive correlation between osteocalcin and AP

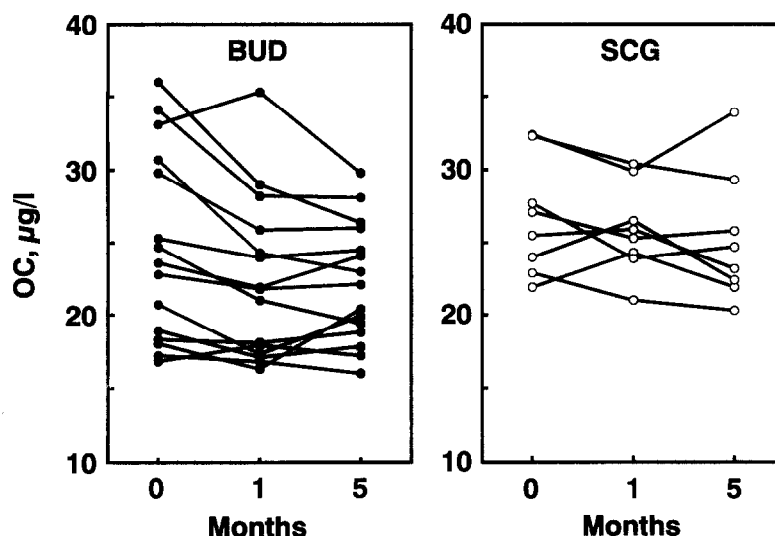


FIG. 1. Serum osteocalcin (OC) at entry (0) and at 1 and at 5 months of treatment with inhaled budesonide (BUD) (*left*, $p = 0.015$ for overall change) and SCG (*right*, $p = 0.206$ for overall change). Higher dose of BUD ($800 \mu\text{g}/\text{m}^2/\text{day}$) was given during the first month ($p = 0.018$) and lower dose ($400 \mu\text{g}/\text{m}^2/\text{day}$) during the next 4 months ($p = 0.086$ for the entry versus 5 months comparison).

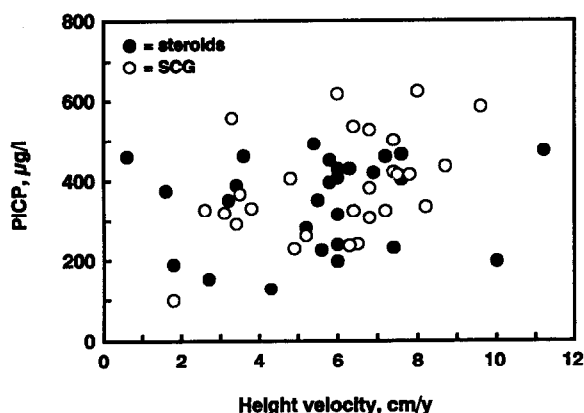


FIG. 2. The cross-sectional study: PICP versus height velocity in children treated with steroids and with SCG. $r = 0.333$, both groups combined ($n = 54$, $p < 0.02$; $r = 0.231$ for the steroid ($n = 27$) and 0.471 for the SCG group ($n = 27$, $p < 0.02$).

($r = 0.309$, $p < 0.05$ both groups combined, $r = 0.374$ for the steroid and $r = 0.282$ for the SCG group), as well as between osteocalcin and BAP (Table IV).

In the longitudinal study osteocalcin decreased significantly during budesonide use ($p = 0.015$, Fig. 1, *left*). From the level at entry, the decrease was 8% ($p = 0.019$) at 1 month (higher dose of budesonide for 1 month) and 6% ($p = 0.086$) at 5 months (lower dose of budesonide for 4 months) (Table II). No sig-

nificant change occurred in osteocalcin during SCG ($p = 0.206$, Fig. 1, *right*, Table III).

Carboxypropeptide of type I procollagen

In the cross-sectional study, PICP was not significantly different between the steroid and the SCG groups (Table I), but when the boys ($n = 16$) and the girls ($n = 11$) were analyzed separately, it was found that the boys in the steroid groups had significantly lower levels of PICP than the boys in the SCG group ($p = 0.003$, Table I). The same was not true for the girls ($p = 0.174$). Neither was any sex-difference found in the other parameters of the present study.

There was a significant positive correlation between PICP and growth velocity (Fig. 2, Table IV) and between PICP and BAP (Table IV) in the SCG group and when the groups were combined.

In the longitudinal study PICP decreased during budesonide use ($p = 0.004$ for overall change, Fig. 3, *left*). The decrease was 5% ($p = 0.049$) at 1 month (higher dose of budesonide for 1 month) and 28% ($p = 0.004$) at 5 months (lower dose of budesonide for 4 months) (Table II). No significant change occurred in PICP during SCG ($p = 0.607$, Fig. 3, *right*, Table III).

DISCUSSION

Serum osteocalcin levels are known to increase during normal growth.¹⁷⁻¹⁹ In the cross-sectional part of

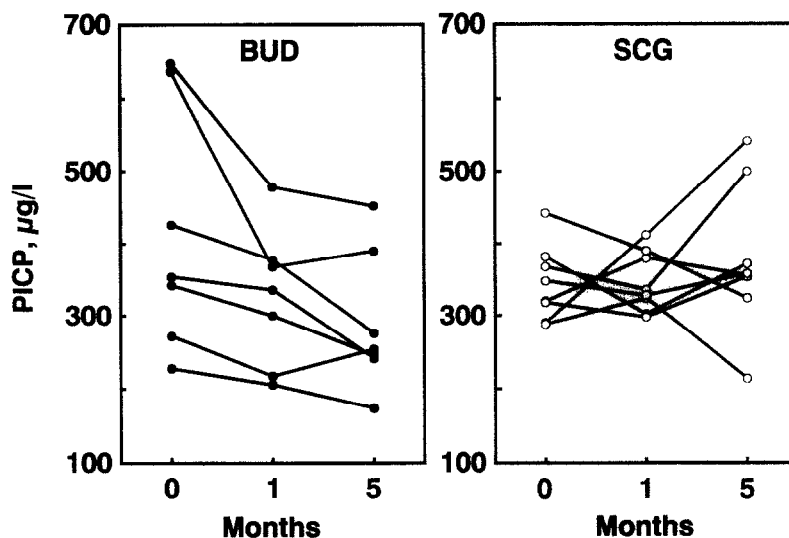


FIG. 3. PICP at entry (0) and at 1 and 5 months of treatment with inhaled BUD (left, $p = 0.004$ for overall change) and SCG (right, $p = 0.607$ for overall change). Higher dose of BUD ($800 \mu\text{g}/\text{m}^2/\text{day}$) was given during the first month ($p = 0.049$) and lower dose ($400 \mu\text{g}/\text{m}^2/\text{day}$) during the next 4 months ($p = 0.004$ for the entry versus 5 month comparison).

the present study no significant differences in osteocalcin could be demonstrated between the steroid and SCG groups. In the longitudinal part a small but significant decrease (median, -8% at 1 month) was found in osteocalcin when the dose of inhaled budesonide was $800 \mu\text{g}/\text{m}^2/\text{day}$. After the next 4 months when the budesonide dose was $400 \mu\text{g}/\text{m}^2/\text{day}$, osteocalcin levels still tended to be lower than at entry ($p < 0.10$). No significant longitudinal changes were observed in the SCG group.

A dose-related decrease in osteocalcin during treatment with inhaled glucocorticoids has been demonstrated in short-term studies on healthy adults.²⁰⁻²³ Although a positive correlation exists between osteocalcin and AP in healthy children and adults,²⁴ and although a similar relationship was found in the present study, the changes of osteocalcin and AP have not always been consistent.²⁴⁻²⁷ AP has been reported to decrease after inhaled beclomethasone,²⁸ whereas during treatment with inhaled budesonide a decrease in osteocalcin was not accompanied by a decrease in AP in healthy adults.²⁰ Similarly, in the present study no change in AP was observed, and the decrease in BAP was not significant. Thus osteocalcin seems to be a more sensitive marker of bone formation than AP or BAP.

According to the few preliminary reports available, oral glucocorticoid treatment reduces PICP in adults.²⁹ In the present study budesonide, even in inhaled doses, decreased PICP. In contrast to osteocalcin, PICP levels were still decreased at 5 months, after the bu-

desonide dose for 4 months had been half of that during the first month. The median PICP level had decreased by 5% at 1 month and by 28% at 5 months. It could be hypothesized that the decrement in PICP is more influenced by the cumulative dose and/or the duration of inhaled glucocorticoid treatment than by the size of the daily dose.

In the cross-sectional study osteocalcin levels were not lower in the steroid group than in the SCG group. However, the boys on glucocorticoids had lower PICP than the boys on SCG. No differences were found in any other parameters or in any parameters in the girls. In the longitudinal study the numbers of boys and girls separately were too small to allow evaluation of the influence of sex on the parameters, and further studies are needed to confirm the sex difference in the effects of glucocorticoids on PICP.

PICP correlates not only with bone formation but also with growth velocity.³⁰⁻³² Treatment with budesonide for as short a period as 2.5 weeks (dose $200 \mu\text{g}/\text{day}$) has been reported to reduce mean growth velocity of the lower legs, as assessed by knemometry.³³ In the present study PICP correlated with growth velocity in the children treated with SCG and in both groups combined. This relationship as well as that between PICP and BAP, and that between growth velocity and BAP, were weaker in the steroid group. We found no significant changes in height and weight growth, measured conventionally, during the 5-month period of medication with inhaled glucocorticoids. The study may have been too short in duration and

the methods too insensitive to detect glucocorticoid-induced growth deceleration.

Serum IGF-I levels were also measured because the circulating IGF-I may affect bone formation.³⁴ However, the decreases in osteocalcin and PICP were not associated with decreases in IGF-I levels in our patients.

As demonstrated by a significant improvement of FEV₁% during budesonide in the present longitudinal study, inhaled glucocorticoids are an effective treatment in asthma. However, the present study suggests that even small inhaled doses of budesonide may decrease bone formation rate. PICP seems to be a most sensitive marker, and appears to be affected by small inhaled glucocorticoid doses that do not affect, for example, adrenal function or lipid and carbohydrate metabolism.³⁵ Together with potential future markers of bone resorption,³⁶ it might provide clinicians with a useful marker of net turnover. At present, PICP appears to be a promising clinical tool for early detection of the adverse effects of glucocorticoids on bone.

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Passive cutaneous anaphylaxis in mouse skin is associated with local accumulation of interleukin-6 mRNA and immunoreactive interleukin-6 protein

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We used a BALB/c model of passive cutaneous anaphylaxis (PCA), an IgE-mediated, mast cell-dependent reaction, to demonstrate the early production of the proinflammatory cytokine interleukin-6 (IL-6) mRNA and protein product. Northern blot analysis detects IL-6 mRNA 1, and 2 hours after antigen challenge (dinitrophenyl₃₀₋₄₀ human serum albumin [DNP₃₀₋₄₀-HSA]) and in situ hybridization reveals that it is primarily cells with round-to-oval nuclei within the dermis (1 to 3 per high-power field) expressing IL-6 mRNA. Immunohistochemistry revealed perinuclear and cytoplasmic staining for immunoreactive IL-6 in mononuclear dermal cells and also cells within the basal keratinocyte layer. Injection of recombinant murine IL-6 (rmIL-6) either systemically or locally during antidinitrophenyl IgE skin sensitization resulted in increased vasopermeability at the PCA site after DNP₃₀₋₄₀-HSA. However, this increased permeability was not associated with a change in the character of the cellular infiltrate at the PCA site 8 hours later. Although the specific role of IL-6 in the generation of the allergic response remains unknown, its detection during PCA unequivocally demonstrates that IL-6 be considered one of the mediators identified in inflammation that follows allergic reactions. (J ALLERGY CLIN IMMUNOL 1992;90:815-24.)

Key words: Mast cell, IgE, murine, skin, IL-6, allergy, immediate hypersensitivity

Mast cells have been shown to produce mRNA for a number of proinflammatory cytokines including macrophage inflammatory protein 1 α and 1 β (MIP1-

α , MIP1- β), JE, T-cell activation gene 3 (TCA-3), tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), and IL-6 after Fc ϵ RI mediated activation.¹⁻⁴ Studies of bone marrow cultured mast cells (BMCMCs) as well as murine mast cell lines have shown that mRNA for these proinflammatory cytokines appears at high levels within 30 minutes after activation.¹⁻⁴ In terms of biologic product, both bioactive TNF- α and IL-6 have been shown to be produced by BMCMCs and mast cell lines.^{2, 4} TNF- α has also been shown to be released as both preformed granule-associated bioactivity and as newly synthesized product after Fc ϵ RI activation.⁴ Among the proinflammatory cytokines

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