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Effects of ciclesonide and fluticasone on cortisol secretion in patients with persistent asthma

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ABSTRACT: We compared the systemic and clinical effects of ciclesonide (CIC) and fluticasone propionate (FP) administered, in addition to CIC 160 μg·day^{-1} and salmeterol 50 μg twice daily, in 32 patients with persistent asthma using a randomised double-blind, placebo-controlled, double-dummy, five-period crossover design.

All patients exhibited a provocative concentration leading to a 20% decrease in forced expiratory volume in 1 s (PC_{20}) methacholine <8 mg·mL^{-1} and a PC_{20} adenosine <60 mg·mL^{-1}. Primary outcome was 24-h serum cortisol suppression after 7 days. Secondary outcomes were changes in PC_{20} methacholine and adenosine after 9 days.

FP 500 μg·day^{-1} and 1,000 μg·day^{-1} significantly suppressed cortisol secretion *versus* placebo by -46.2 (95% confidence interval (CI) -83.8– -8.5) nmol·L^{-1} and by -76.1 (95% CI -112.9– -39.3) nmol·L^{-1}, respectively. Neither dose of CIC (320 nor 640 μg·day^{-1}) had a significant suppressive effect (-28.2 (95% CI -65.5–9.2) nmol·L^{-1} and -37.3 (95% CI -74.7–0.0) nmol·L^{-1}, respectively). Differences between FP 1,000 μg·day^{-1} and both CIC treatments were statistically significant (CIC 320 μg·day^{-1}: -48.0 (95% CI -84.8– -11.1) nmol·L^{-1}; CIC 640 μg·day^{-1}: -38.8 (95% CI -75.7– -1.9) nmol·L^{-1}). Compared with placebo, the increase in PC_{20} adenosine after the four treatments was small, but significant. Greater improvements in PC_{20} adenosine were seen with FP 500 μg·day^{-1} (1.8 (95% CI 1.0–2.6) doubling concentrations) compared with CIC 320 μg·day^{-1} (0.9 (95% CI 0.1–1.7) doubling concentrations). No significant difference was seen between CIC 640 μg·day^{-1} and FP 1,000 μg·day^{-1}.

For a similar decrease in hyperresponsiveness, cortisol secretion was suppressed significantly with moderate-to-high doses of fluticasone propionate, but not with ciclesonide.

KEYWORDS: Aerosol therapy, anti-asthmatic agent, asthma, bronchial hyperreactivity, cortisol, inhaled corticosteroids

Inhaled corticosteroids (ICS) are the most effective controller medications currently available to treat asthma. They reduce airway inflammation and hyperresponsiveness, improve symptoms, pulmonary function and quality of life [1, 2], and decrease hospitalisations [3] and mortality rate [4]. Thus, ICS are the guideline-recommended first-line treatment for all patients with persistent forms of the disease [5]. Although the vital role of ICS in the management of asthma is generally recognised and ICS are well tolerated at low-to-medium doses, it has been claimed that the long-term administration of high doses of ICS has a potential for systemic adverse events (AEs), such as growth inhibition, osteoporosis, suppression of hypothalamic–pituitary–adrenal (HPA)-axis function [6], or even adrenal crisis [7]. This potential for AEs with ICS is a concern for patients and physicians, and may contribute to intentional nonadherence [8] and sub-optimal prescribing [9].

Ciclesonide (CIC) is a novel, airways-targeted ICS that is delivered as an inactive compound and converted by esterases to the active metabolite (desisobutyryl-ciclesonide) in the airways, where it elicits its anti-asthmatic effect [10–13]. Several trials have shown that doses ⩽1,280 μg·day^{-1} of CIC do not produce clinically relevant HPA-axis suppression in both healthy volunteers and asthma patients [13–18]. Fluticasone propionate (FP) is an established ICS, which, however, has been associated with pronounced suppression of HPA-axis function in healthy volunteers [19] and, to a lesser extent, in asthma patients [14, 15, 20–22]. Thus, the current study was designed to assess the safety of CIC

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and FP in patients with persistent asthma chronically treated with ICS. More specifically, we wanted to address the following. 1) Whether moderate-to-high doses of inhaled CIC suppress 24-h serum and urinary cortisol levels and biochemical markers of bone formation in patients with moderate, persistent asthma and how these effects compare with those of moderate-to-high doses of FP. 2) To what extent do moderate-to-high doses of CIC reduce airway responsiveness to adenosine and methacholine, and how these effects compare with those of moderate-to-high doses of FP. 3) Whether one of the two investigated formulations is superior in terms of the ratio between clinical effect and systemic effect.

METHODS

Patients

Male and female patients (aged 18–65 yrs) known to have persistent asthma for >6 months, as defined by the Global Initiative for Asthma, were allowed to participate in the study. Patients were included if their current treatment consisted of a constant dose of a moderate-to-high daily dosage of ICS alone (beclomethasone dipropionate $\leqslant$1,000 μg·day^{-1} or equivalent) or a combination of low doses of ICS with long-acting β_2-agonists (LABA; beclomethasone 200 μg *b.i.d.* or equivalent plus salmeterol 50 μg *b.i.d.* or formoterol $\leqslant$12 μg *b.i.d.*) for >4 weeks. Patients with severe persistent asthma were excluded to avoid drop-outs. Patients had to demonstrate a forced expiratory volume in 1 s (FEV_1) of >60% predicted at the start of the study and at randomisation. All the patients exhibited a provocative concentration leading to a 20% decrease in post-saline FEV_1 (PC_{20}) methacholine <8 mg·mL^{-1} and a PC_{20} adenosine <60 mg·mL^{-1}. Patients were also required to have normal HPA-axis function (serum cortisol concentration at 08:00 h ($\pm$30 min) >5 μg·dL^{-1} (>138 nmol·L^{-1})) and not to have experienced an asthma exacerbation or respiratory tract infection within 8 weeks prior to the start of the study.

Patients were excluded if they: had used systemic steroids within 4 weeks of the start of the study or more than three times during the last 6 months; had chronic obstructive pulmonary disease and/or other pulmonary diseases; had a history of other medical conditions known to affect cortisol levels (*e.g.* Cushing's Syndrome); or were receiving drugs known to affect endogenous cortisol production (*e.g.* anabolic steroids or androgens). Females were excluded if they were pregnant, breastfeeding or not using safe contraception, were of childbearing potential (*i.e.* might become pregnant) or were <1 yr post-menopausal.

This study was conducted in accordance with the rules of the International Conference on Harmonisation Good Clinical Practice and the ethical principles of the Declaration of Helsinki. Written consent was obtained from the patients before the start of the study, and the protocol was reviewed and approved by the appropriate Independent Ethics Committee (Ghent University Hospital, Ghent, Belgium or University of Liège, CHU Sart-Tilman, Liège, Belgium) or Institutional Review Boards.

Study design

This randomised, double-blind, double-dummy, placebo-controlled, five–period crossover study was conducted at two centres in Belgium (fig. 1). During a 4–6 week run-in period, patients were administered CIC 160 μg in the evening plus salmeterol 50 μg *b.i.d.* This treatment was continued throughout the entire study. CIC was chosen because previous studies have shown that daily doses of $\leqslant$1,280 μg CIC had no clinically relevant effect on cortisol secretion [14, 16, 17]. Following the run-in period, patients were randomly assigned to one of 10 treatment sequences, occurring in a Latin square and its mirror, for which a computer generated randomisation list was used (table 1). These 10 sequences were uniform on the periods (each treatment was applied with the same frequency in each period) and on the subjects (each treatment was applied with the same frequency within each subject), and balanced with respect to a first-order carry-over effect (each treatment preceded every other treatment the same number of times).

Each treatment sequence consisted of five-period treatments which contained one of the following study medications (all administered *via* hydrofluoroalkane-metered dose inhaler), which were administered in addition to the maintenance dose of CIC 160 μg·day^{-1}. The treatment sequences were: CIC 160 μg *b.i.d.* (ex-actuator); CIC 320 μg *b.i.d.* (ex-actuator); FP 250 μg *b.i.d.* (ex-valve; 220 μg *b.i.d.* ex-actuator); FP 500 μg *b.i.d.* (ex-valve; 440 μg *b.i.d.* ex-actuator); or placebo. Due to the code labelling, neither the investigator nor anyone at the study centre knew which drug or dosage was administered. The FP doses were based on previous observations, showing the equivalence of CIC 320 μg·day^{-1} with FP 500 μg·day^{-1} in terms of bronchial responsiveness to methacholine [15, 23]. The study medication was inhaled at 08:00 h and 20:00 h ($\pm$30 min), starting at the evening of each period. The last inhalation took place 30–60 min before the methacholine provocation on the ninth day of treatment. Each treatment period was separated by a 4–12-week washout period, to allow for all previously administered study drug to be cleared from the system and to allow PC_{20} adenosine [14] and serum cortisol [20, 24] to return to baseline values.

Spirometry and measurement of airway hyperresponsiveness

Spirometry was performed at the start of the study (at 08:00–10:00 h) and repeated at the beginning and end of each 9-day treatment period at approximately the same time-point. The highest value from three acceptable tests was recorded for FEV_1. Rescue medication had to be withheld for $\geqslant$8 h and LABAs for $\geqslant$24 h prior to each lung function measurement.

Challenge tests were performed at the start of the study and at the end of each treatment period (visits T_2, T_5, T_8, T_{11} and T_{14}; fig. 1) 30–60 min after the last dose of study medication, according to a protocol that has been described elsewhere [14, 25]. Methacholine solutions were nebulised with a Wiesbadener Doppel inhalator (Wiesbadener Inhalatoren-Vertrieb, Wiesbaden, Germany), driven at an airflow of 6 L·min^{-1} and generating an output of 0.1 mL·min^{-1} [25]. Median mass particle size of the aerosol was 3.5 μm. Each patient used the same nebuliser for the whole study. The aerosol was inhaled during 2 min of quiet breathing with the outlet of the nebuliser in the mouth and the nose occluded with a clip. Three baseline readings were followed by inhalation of aerosolised saline. If the FEV_1 had not fallen by >10%,

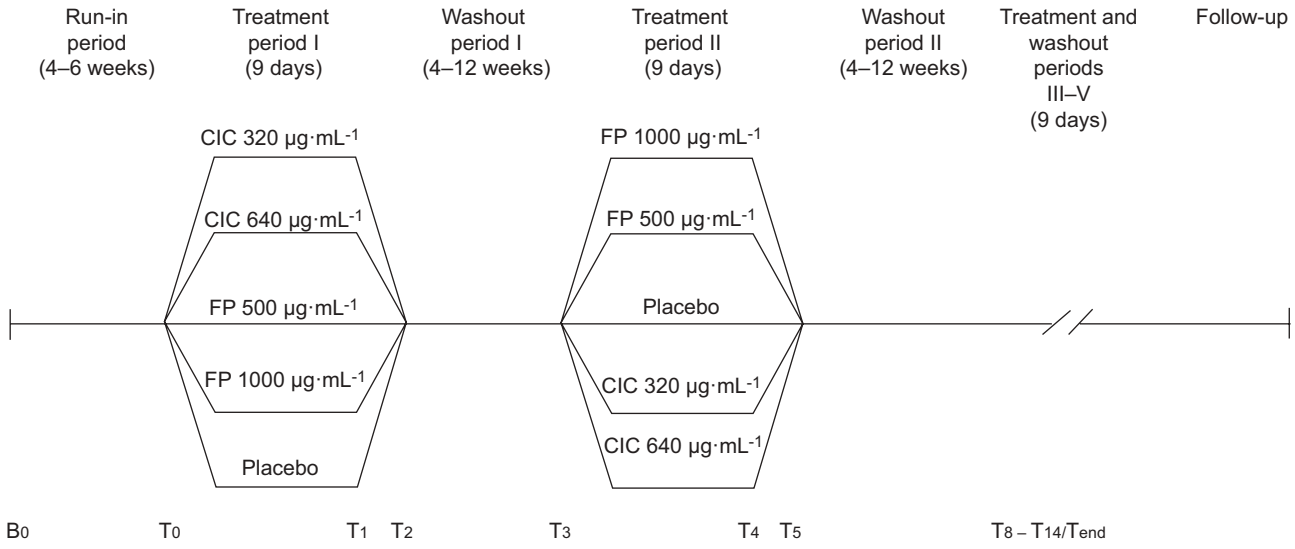

FIGURE 1. Study design. Treatment visits (T) T_0–T_1, T_3–T_4, T_6–T_7, T_9–T_{10} and T_{12}–T_{13} were separated by 7±0 days. Visits T_0–T_2, T_3–T_5, T_6–T_8, T_9–T_{11} and T_{12}–T_{14} were separated by 9 (-2/+3) days. CIC: ciclesonide; FP: fluticasone propionate.

aerosolised methacholine was administered with the initial concentration being 0.031 mg·mL^{-1}. The concentration was doubled after each step. Spirometric measurements were performed 1 and 3 min after each concentration; the lowest out of these two was retained for analysis. The time interval between each step was 5 min. The procedure was terminated once FEV_1 had decreased by ⩾20% or when the maximum methacholine concentration (32 mg·mL^{-1}) had been reached. The PC_{20} methacholine was calculated *via* linear interpolation on a logarithmic dose–response curve. If the FEV_1 had not fallen by ⩾20% at the maximum methacholine concentration of 32 mg·mL^{-1}, the value was substituted by 64 mg·mL^{-1}.

Following methacholine challenge, the patient was allowed to recover for 2–4 h (without use of rescue medication). If FEV_1 had returned to >90% of the pre-challenge value, an adenosine challenge was performed in the same manner as detailed above (doubling concentrations ranging 1.563–410 mg·mL^{-1} diluted in 0.9% saline) and PC_{20} was recorded. If the FEV_1 had not fallen by ⩾20% at the maximum adenosine concentration of 410 mg·mL^{-1}, that value was substituted by 820 mg·mL^{-1}. Patients unable to complete an adenosine challenge on the same day as the methacholine challenge returned on the following day.

TABLE 1 List of the 10 permutations of order used for the five treatments

Sequence	TI	TII	TIII	TIV	TV
1	CIC 320	CIC 640	PLAC	FP 500	FP 1000
2	CIC 320	PLAC	CIC 640	FP 1000	FP 500
3	CIC 640	CIC 320	FP 500	PLAC	FP 1000
4	CIC 640	FP 500	CIC 320	FP 1000	PLAC
5	FP 500	CIC 640	FP 1000	CIC 320	PLAC
6	FP 500	FP 1000	CIC 640	PLAC	CIC 320
7	FP 1000	FP 500	PLAC	CIC 640	CIC 320
8	FP 1000	PLAC	FP 500	CIC 320	CIC 640
9	PLAC	CIC 320	FP 1000	CIC 640	FP 500
10	PLAC	FP 1000	CIC 320	FP 500	CIC 640

T: treatment period; CIC: ciclesonide; FP: fluticasone propionate; PLAC: placebo.

Cortisol assessments

After 7 days of each treatment, 24-h hour serum profiles were obtained from all patients (visits T_1, T_4, T_7, T_{10} and T_{13}; fig. 1). At these visits, patients stayed at the study site for 24 h and 5 mL of blood was drawn at 2-h intervals starting at 20:00 h (±10 min) until 20:00 h (±10 min) the following day. Urine was collected over 24 h at the same visits. Creatinine was also measured in the samples.

Bioanalytical methods

Blood samples for cortisol analysis were collected in tubes without anticoagulant. After collection the tubes were mixed gently and incubated for a minimum of 10 min and a maximum of 2 h before centrifugation for 15 min at 1,600 × *g* at room temperature. The serum was then transferred to new tubes and stored at -20°C until analysis. Urine was collected for ~24 h, the total volume recorded and one teaspoon of sodium-azide per 2.5-L container added as a preservative. Well-mixed aliquots were stored at -20°C. Serum and urinary cortisol were measured using the GammaCoat (^{125}I) Cortisol Radioimmunoassay Kit (Diasorin, Saluggia, Italy), which is based on the competitive binding principles of radioimmunoassay. Urine was extracted before radioimmunoassay of cortisol after addition of a titrated cortisol internal standard for recovery monitoring. The limit of quantification was 0.5 µg·dL^{-1} with an intra-batch coefficient of variation of 3% and an inter-batch coefficient of variation between 5.5–7.1%. For a given patient, all samples were assayed for cortisol within the same assay run. Possible interference of the trial medication with the cortisol assay antibody was assessed and no interference was found. Urinary creatinine was measured

with a kinetic colorimetric assay using a Hitachi MODULAR-P analyzer (Roche, Tokyo, Japan).

Assessment of bone formation makers

Blood samples to determine serum biochemical markers of bone formation were obtained on the second day of visits (T_1, T_4, T_7, T_{10} and T_{13}) at 08:00 h (±10 min) after 8 h of fasting. All samples from a given patient were assayed in a single assay run using commercial immunoassays for bone alkaline phosphatase (ACCESS Immunoassay Systems; Beckman Coulter Inc., Galway, Ireland), serum osteocalcin (Osteometer Biotech A/S, Copenhagen, Denmark) and serum N-terminal propeptide of type 1 procollagen (P_1NP; Orion Diagnostica, Espoo, Finland).

Adverse events

Safety was assessed throughout the study by neutral questioning.

Statistical analysis

The primary variable was the 24-h serum cortisol mesor, calculated by means of the area under the curve of the 24-h serum cortisol profile ($AUC_{0–24h}$) divided by the respective time interval (20:00 h until 20:00 h the following day) using the trapezoidal rule. Replacement of missing values or of outliers was not performed. A second important variable was 24-h free urine cortisol adjusted for creatinine.

To address the multiplicity issue, a strategy with *a priori* ordered hypotheses was applied which preserves the family wise error of the procedure at $\alpha=0.025$ (one-side). Consequently, superiority hypotheses for 24-h serum cortisol mesor and 24-h free urine cortisol adjusted for creatinine were one-sided at a significance of $\alpha=0.025$. Only if the previous null hypothesis could be rejected, the subsequent superiority test would be carried out in the following order: superiority of CIC 640 $\mu g \cdot day^{-1}$ to FP 1,000 $\mu g \cdot day^{-1}$ for difference in serum cortisol mesor; superiority of CIC 640 $\mu g \cdot day^{-1}$ to FP 1,000 $\mu g \cdot day^{-1}$ for change in 24-h urine cortisol adjusted for creatinine; superiority of CIC 320 $\mu g \cdot day^{-1}$ to FP 500 $\mu g \cdot day^{-1}$ for difference in serum cortisol mesor; superiority of CIC 320 $\mu g \cdot day^{-1}$ to FP 1,000 $\mu g \cdot day^{-1}$ for change in 24-h urine cortisol adjusted for creatinine.

All statistical analyses were carried out with SAS (release 9.1; SAS Institute Inc., Cary, NC, USA). Serum cortisol mesor, urine cortisol variables, bone formation markers, log-transformed PC_{20} and lung variables were analysed by means of an ANCOVA or ANOVA [26] with treatment, period, sequence, patient within sequence and sex as factors. For computation of the ANOVA and ANCOVA analyses the SAS procedure PROC MIXED was utilised, using the baseline value as continuous covariate, the patient within sequence effect as random nested factor and all other factors as fixed effects. Asthma pre-treatment and centre as factors were added for specific end-points or analyses. T-tests of difference between the treatment least square means are given as two-sided, with an α-level of 5%. The sample size was estimated based on findings from a previous study [14]. In the case of normally distributed difference in time-averaged cortisol levels $AUC_{0–24h}$, a sample size of 30 randomised patients was estimated to ensure a power of 80% to correctly conclude a difference in mean values of 49 $nmol \cdot L^{-1}$ under assumption of a common SD of 66.6 $nmol \cdot L^{-1}$. The sample size estimation was based on a two-independent group t-test which provides a conservative acceptable approximation of the t-test for comparing least-square means utilised in the PROC MIXED procedure.

TABLE 2 Baseline demographics and characteristics

Subjects	32
Age yrs	27 (18–59)
Weight kg	70±16
Height cm	171±9
Sex	
Male	12
Female	20
Race	
Caucasian	31
Black	1
ICS pre-treatment	
ICS	12
ICS/LABA	20
Smoking status	
Nonsmokers	19
Ex-smokers	11
Current smokers	2
FEV_1 % predicted#	84.9±13.2
Mean PC_{20} AMP $mg \cdot mL^{-1}$	16.7 (2.0–60)
Mean PC_{20} MCh $mg \cdot mL^{-1}$	2.0 (0.1–8.0)

Data are presented as n, median (range) or mean±SD. ICS: inhaled corticosteroid; LABA: long-acting β_2-agonist; FEV_1: forced expiratory volume in 1 s; PC_{20}: provocative concentration leading to a 20% decrease in FEV_1; AMP: adenosine 5-monophosphate; MCh: methacholine. #: taken at randomisation.

RESULTS

Patient characteristics

A total of 83 patients were screened. Of these, 51 were not eligible because of a negative methacholine or negative adenosine challenge test. The remaining 32 patients (20 females) were randomised (table 2, fig. 2). The first patient was included on May 27, 2003, and the last patient left the study on April 10, 2006. The characteristics of the 32 patients included in the study are summarised in table 2. Median age was 27 yrs. Most patients were pre-treated with a combination of a LABA and an ICS. Mean PC_{20} methacholine was 2.0 $mg \cdot mL^{-1}$ and mean PC_{20} adenosine was 16.7 $mg \cdot mL^{-1}$. Washout period was 4 weeks on most occasions, and did not exceed 8 weeks. There were no dropouts due to asthma exacerbations. Two patients ended the study prematurely for nonmedical reasons and were excluded from the safety analysis. One further patient was excluded from all analysis for erroneously using his previous ICS (FP Diskus; GlaxoSmithKline, Ware, UK) during the study.

Cortisol assessments

Serum cortisol mesor

The serum cortisol mesor data are presented in table 3. Both FP doses significantly suppressed cortisol secretion *versus* placebo, serum cortisol reaching 323.0±22.6 $nmol \cdot L^{-1}$ after

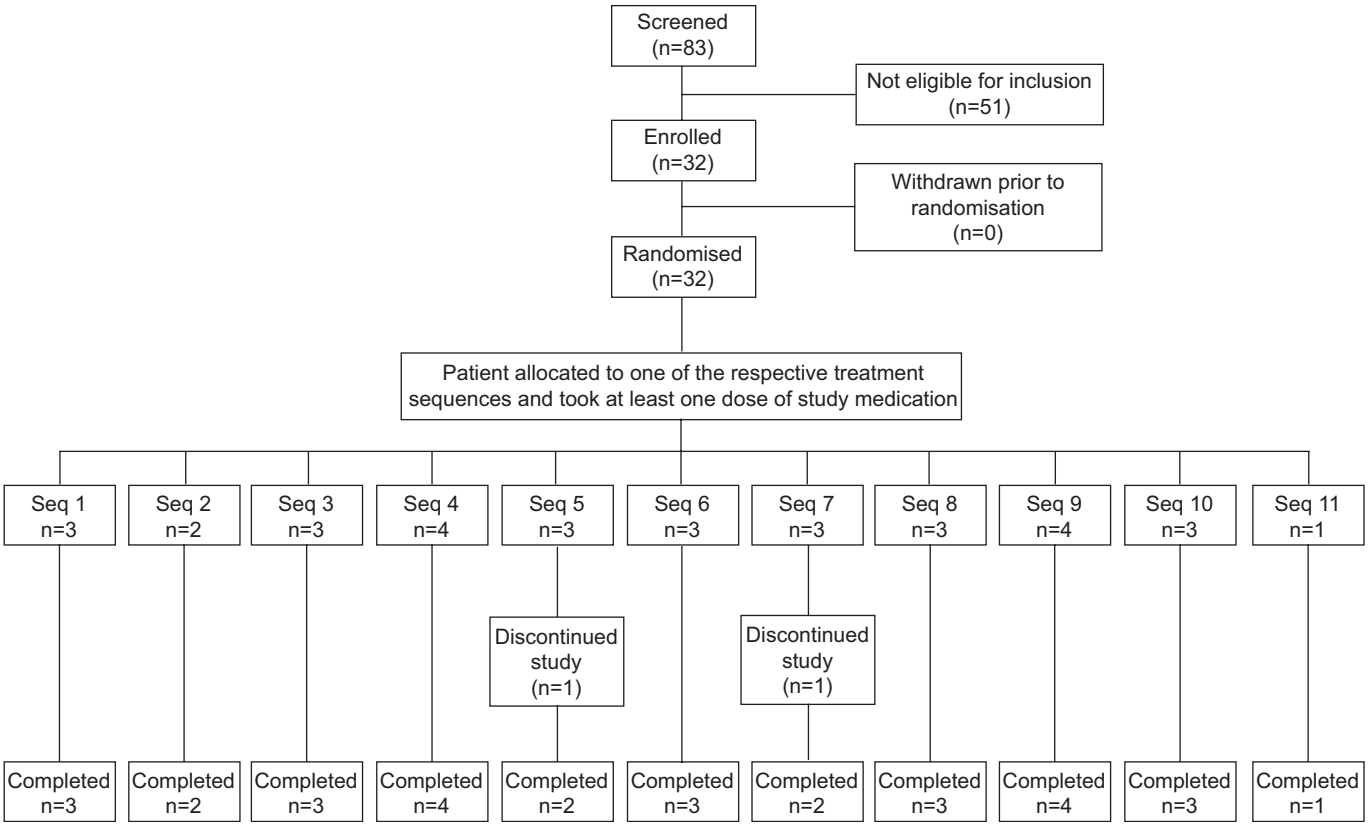

FIGURE 2. Consort diagram showing the flow of the patients. In total, 83 patients were screened and 32 patients received study medication according to one of 10 sequences (seq). Two patients discontinued the study for nonmedical reasons. The sequence of the patient who erroneously continued to use his FP Diskus (GlaxoSmithKline, Ware, UK) on top of his study medication, was *a posteriori* called sequence 11, since that treatment did not correspond with one of 10 sequences originally scheduled before the start of the study

FP 500 μg·day^{-1} (-46.2 (95% confidence interval (CI) -83.8– 18.5) nmol·L^{-1} or -10.3%) and 293.0±22.3 nmol·L^{-1} after FP 1,000 μg·day^{-1} (-76.1 (95% CI -112.9– -39.3) nmol·L^{-1} or -19.8%). Differences in supression between FP 1,000 μg·day^{-1} and the CIC 320 μg·day^{-1} (-48.0 (95% CI -11.1– -84.8) nmol·L^{-1}) and CIC 640 μg·day^{-1} (-38.8 (95% CI -1.9– 175.7) nmol·L^{-1}) treatments also reached statistical significance. Neither dose of CIC had a significant suppressive effect (table 3, fig. 3).

24-h urine cortisol adjusted for creatinine

Data for 24-h urine cortisol adjusted for creatinine are presented in table 3. Urinary cortisol excretion over 24 h adjusted for creatinine was significantly suppressed by both FP doses as compared with placebo. Neither dose of CIC demonstrated a significant effect on 24-h urinary cortisol adjusted for creatinine compared with placebo.

Assessments of bone formation markers

No significant differences were noted after either CIC treatment compared with placebo for any bone formation marker assessed (table 4). However, FP 1,000 μg·day^{-1} caused significant decreases in P1NP ($p=0.0126$) and serum osteocalcin levels ($p=0.0054$) compared with placebo (table 4).

Pulmonary function measures

FEV_1 remained stable over time, with 90 mL being the largest difference between the highest and the lowest value. Changes from baseline in FEV_1% pred (least square means) were small for all treatments (CIC 320 μg·day^{-1}: -0.2%; CIC 640 μg·day^{-1}: -0.3%; FP 500 μg·day^{-1}: 1.4%; FP 1,000 μg·day^{-1}: 3.3%; placebo: -3.1%).

Methacholine and adenosine 5-monophosphate challenge

Mean PC_{20} methacholine, which was 2.0 mg·mL^{-1} at inclusion increased during the study by one doubling concentration (DC), reaching 5.6 mg·mL^{-1} under placebo conditions. In this case, placebo means that patients remained under an evening dose of CIC 160 μg throughout the study. Further improvements in airway hyperresponsiveness with the active treatments were small compared with placebo and were less than one DC (fig. 4, table 5). Thus, PC_{20} methacholine after the two FP treatments increased by 0.6 and 0.7 DC compared with placebo ($p \leqslant 0.0228$), whereas the changes in hyperresponsiveness (0.3 and 0.5 DC) after CIC did not reach statistical significance (table 5). Statistically significant differences between the CIC and FP treatments for PC_{20} methacholine challenge were not observed.

Mean PC_{20} adenosine, which was 16.7 mg·mL^{-1} at inclusion, increased during the study by almost two DC, reaching 51.3 mg·mL^{-1} under placebo conditions. The further increase in PC_{20} adenosine with all four treatments was statistically significant compared with placebo, ranging between one and two DC ($p<0.05$; fig. 5, table 5). Differences between the lower and the higher dose of CIC did not reach statistical

TABLE 3 Effects of treatments on serum cortisol mesor and urine cortisol, adjusted for creatinine

	Placebo#	CIC 320 μg·day^{-1}	CIC 640 μg·day^{-1}	FP 500 μg·day^{-1}	FP 1000 μg·day^{-1}
Serum cortisol mesor nmol·L^{-1}					
Subjects n	27	27	27	27	29
Mean±SD	381.2±98.5	352.8±110.4	341.6±95.9	332.9±94.2	304.4±150.6
LS mean±SEM	369.2±22.6	341.0±22.7	331.8±22.7	323.0±22.6	293.0±22.3
Difference *versus* placebo					
LS mean±SEM		-28.2±18.8	-37.3±18.8	-46.2±19.0	-76.1±18.6
95% CI		-65.5–9.2	-74.7–0.0	-83.8– -8.5	-112.9– -39.3
p-value		0.0251	0.0687	0.0084	<0.0001
Difference *versus* FP 1000 μg·day^{-1}					
LS mean±SEM	76.1±18.6	48.0±18.6	38.8±18.6	30.0±18.7	
95% CI	39.3–112.9	11.1–84.8	1.9–75.7	-7.2–67.1	
p-value	0.0001	0.0057	0.0197	0.0563	
Change in serum cortisol to placebo %					
Subjects n		26	26	25	27
Mean±SD		-6.1±26.1	-7.9±18.5	-10.3±20.9	-19.8±28.0
24-h urine cortisol adjusted for creatine nmol·mmol^{-1}					
Subjects n	25	26	27	27	28
Mean±SD	25.74±17.24	22.95±10.17	23.72±10.75	20.49±7.49	20.74±10.93
LS mean±SEM after treatment	25.04±2.44	22.12±2.43	23.32±2.39	20.06±2.39	19.80±2.37
Difference *versus* placebo LS mean±SEM		-2.92±2.38	-1.72±2.35	-4.98±2.36	-5.24±2.32
95% CI		-7.64–1.80	-6.38–2.94	-9.66– -0.30	-9.84– -0.64
p-value		0.1111	0.2326	0.0186	0.0130

For all safety parameters: the data provided are for the restricted safety analysis, excluding one patient. CIC: ciclesonide; FP: fluticasone propionate; LS: least squares; CI: confidence interval. #: maintenance dose of CIC 160 μg·day^{-1}.

significance. Likewise, the differences between the two FP doses were not statistically significant. FP 500 μg·day^{-1} resulted in significantly greater improvements in PC_{20} adenosine (one DC) compared with CIC 320 μg·day^{-1} (p=0.0238). No significant difference was seen between CIC 640 μg·day^{-1} and FP 1,000 μg·day^{-1}, or between other doses.

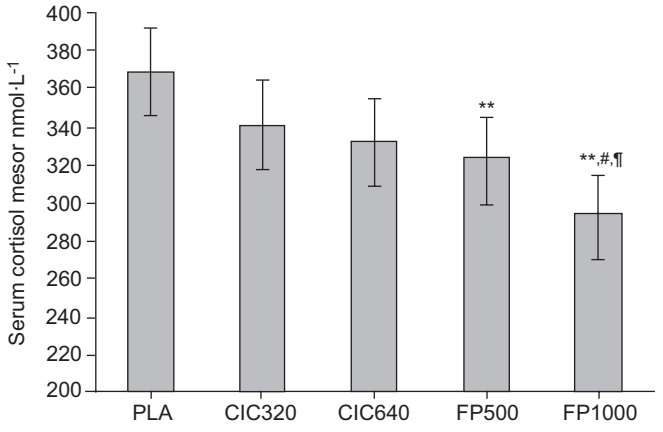

FIGURE 3. Mean serum cortisol mesor following placebo (PLA), ciclesonide (CIC) 320 μg·day^{-1} (CIC320), CIC 640 μg·day^{-1} (CIC640), fluticasone propionate (FP) 500 μg·day^{-1} (FP500) or FP 1,000 μg·day^{-1} (FP1000). All treatment groups were administered CIC 160 μg once daily in the evening plus salmeterol 50 μg twice daily. Data are presented as at least squares mean±SD. **: p<0.01 *versus* PLA; #: p⩽0.0057 *versus* CIC 320 μg·day^{-1}; ¶: p=0.0197 *versus* CIC 640 μg·day^{-1}.

In total, 22 patients experienced 56 AEs during the treatment period. The percentage of patients experiencing AEs was comparable across all treatment groups (CIC 320 μg·day^{-1}, 33.3%; CIC 640 μg·day^{-1}, 26.7%; FP 500 μg·day^{-1}, 31.3%; FP 1,000 μg·day^{-1}, 22.6%; placebo, 33.3%). The majority of AEs were mild or moderate in intensity and none was assessed as definitely related to study medication. One patient in the placebo group reported two serious AEs (face oedema and laryngeal oedema), which were caused by allergy to concomitant use of antibiotics and resolved completely.

DISCUSSION

The present study is the first placebo-controlled, crossover study assessing simultaneously the effects of ICS on cortisol secretion, bone markers and bronchial hyperresponsiveness in ICS-dependent asthma patients. The results indicate that daily doses of CIC 320 μg and 640 μg, administered in addition to a low maintenance dose of CIC 160 μg·day^{-1}, did not appear to exert significant systemic effects, whereas daily doses of FP 500 μg and 1,000 μg significantly suppressed adrenal function and bone formation markers. All active treatments improved airway responsiveness, but clinically relevant differences between the treatments were not observed.

The magnitude of the suppression of serum cortisol mesor, the primary variable, reached 10% with FP 500 μg·day^{-1} and

TABLE 4 Least squares (LS) mean changes in bone formation markers compared with placebo[#]

	CIC 320 μg·day^{-1}	CIC 640 μg·day^{-1}	FP 500 μg·day^{-1}	FP 1000 μg·day^{-1}
Subjects n	27	27	27	27
P1NP μg·L^{-1}				
LS mean±SEM	-2.7±4.1	0.8±4.1	-3.3±4.2	-10.4±4.1
95% CI	-10.9–5.5	-7.4–9.0	-11.6–4.9	-18.4– -2.3
p-value	0.5156	0.8376	0.4258	0.0126
Serum osteocalcin ng·mL^{-1}				
LS mean±SEM	0.7±1.2	0.0±1.2	-1.8±1.2	-3.3±1.2
95% CI	-1.7–3.0	-2.4–2.3	-4.2–0.6	-5.6– -1.0
p-value	0.5814	0.9799	0.1312	0.0054
Bone specific AP μg·L^{-1}				
LS mean±SEM	0.4±0.4	-0.1±0.4	0.3±0.4	0.0±0.4
95% CI	-0.4–1.2	-0.9–0.7	-0.5–1.1	-0.7–0.8
p-value	0.3304	0.8710	0.4473	0.9067

CIC: ciclesonide; FP: fluticasone propionate; P1NP: N-terminal propetide of type 1 pro-collagen; CI: confidence interval; AP: alkaline phosphatase. The p-values are set *versus* placebo. [#]: maintenance dose of CIC 160 μg·day^{-1}.

almost 20% with FP 1,000 μg·day^{-1}, administered in addition to a low maintenance dose of CIC 160 μg·day^{-1}. Likewise, 24-h urinary cortisol excretion (adjusted for creatinine) was lower with FP than with placebo treatment. Substantial suppression of adrenal function after inhalation of FP has been previously reported in healthy volunteers [19] and asthmatic patients [14, 20–22]. The presently observed degree of adrenal suppression with FP 1,000 μg·day^{-1} is somewhat smaller than the 29–34% suppression reported previously [14, 17, 20], possibly because it was given on top of a low maintenance dose of inhaled CIC. The duration of the treatment cannot explain the difference between the currently and previously reported decreases in suppression, as adrenal suppression with inhaled FP is close to maximum after 7 days [27]. Possibly, the alterations in pulmonary function and airway inflammation in patients with more severe asthma resulted in a less distal lung deposition of FP, leading to a reduced pulmonary absorption, a decreased systemic bioavailability and a less pronounced adrenal suppression [22, 28–30].

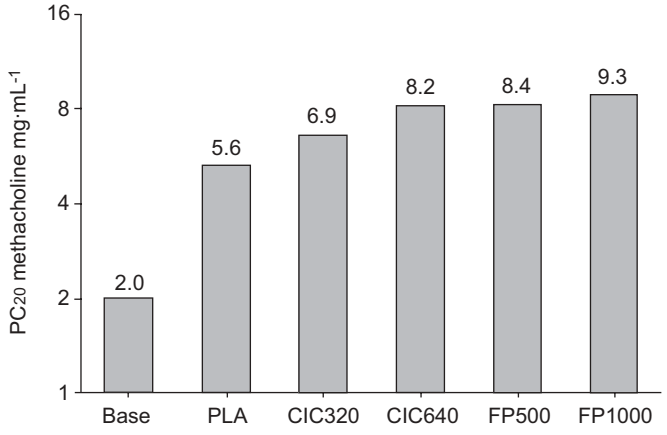

FIGURE 4. Provocative concentration leading to a 20% decrease in forced expiratory volume in 1 s (PC_{20}) methacholine at intake (base) and after placebo (PLA), ciclesonide (CIC) and fluticasone propionate (FP). All treatment groups were administered CIC 160 μg once daily in the evening plus salmeterol 50 μg twice daily. CIC320: CIC 320 μg·day^{-1}; CIC640: CIC 640 μg·day^{-1}; FP500: 500 μg·day^{-1}; FP1000: FP 1,000 μg·day^{-1}.

In contrast to FP, CIC 320 μg·day^{-1} and 640 μg·day^{-1}, even when administered in addition to a low maintenance dose of CIC 160 μg·day^{-1}, did not significantly alter cortisol production. Indeed, mean change in serum cortisol was -6.1% for CIC 320 μg·day^{-1} and -7.9% for CIC 640 μg·day^{-1}, which is in complete agreement with changes reported in previous studies [14, 17]. An important finding was that differences in serum cortisol mesor between FP 1,000 μg·day^{-1} and the two CIC treatments reached statistical significance. Similar observations have been reported in other studies, in which the systemic effects of CIC and FP in healthy volunteers or patients with mild asthma have been assessed [14–17], albeit at higher doses. Thus, it appears that the effects on the 24-h cortisol profile induced by FP are an intrinsic characteristic of this molecule and occur in both healthy subjects and patients with intermittent and persistent asthma. Interestingly, such effects have not been reported with CIC, even in doses as high as 1,280 μg·day^{-1} [14, 17].

Differences in pharmacokinetic properties between FP and CIC may largely explain the more beneficial profile of CIC [31, 32]. Although the clinical relevance and long-term consequences of mild adrenal suppression remain to be elucidated, the potential clinical relevance of this finding should not be underestimated. Moreover, the wide confidence intervals for serum cortisol for all comparisons indicate that the individual variability of the response of the HPA-axis and the potential occurrence of measurable systemic effects towards different doses of different inhaled steroids cannot be neglected. Indeed, a substantial number of patients with moderate or severe asthma are treated with high doses of FP, *i.e.* ⩾1,000 μg·day^{-1}, in order to reach asthma control [5, 33].

Admittedly, a carry-over effect could have been missed since this study was powered for the primary outcome. However, it

TABLE 5 Change in PC_{20} methacholine and PC_{20} adenosine (doubling doses) compared with placebo#

	CIC 320 μg·day^{-1}	CIC 640 μg·day^{-1}	FP 500 μg·day^{-1}	FP 1000 μg·day^{-1}
Subjects n	29	29	30	30
Methacholine				
LS mean±SEM	0.3±0.3	0.5±0.3	0.6±0.3	0.7±0.3
95% CI	-0.3–0.8	0.0–1.1	0.1–1.2	0.1–1.3
p-value	0.3356	0.0645	0.0228	0.0145
Adenosine				
LS mean±SEM	0.9±0.4	1.6±0.4	1.8±0.4	1.4±0.4
95% CI	0.1–1.7	0.6–2.4	1.0–2.6	0.6–2.2
p-value	0.0218	⩽0.0001	⩽0.0001	0.0007

PC_{20}: provocative concentration leading to a 20% decrease in forced expiratory volume in 1 s; CIC: ciclesonide; FP: fluticasone propionate; LS: least squares; CI: confidence interval. #: maintenance dose of CIC 160 μg·day^{-1}.

is unlikely that such a carry-over effect may have occurred, since des-ciclesonide has a half-life of just over 3 h [32] and fluticasone has a half-life of 7–14 h [31], whereas washout in the present study was at least 4 weeks. Moreover, cortisol secretion recovers completely 24 h after a single inhalation of 1,000 μg fluticasone [24], while PC_{20} adenosine normalises completely 4 weeks after discontinuation of treatment with inhaled steroids [14, 34].

Looking at markers of bone metabolism is a different way of assessing systemic effects of ICS. The current study suggests that FP 1,000 μg·day^{-1} significantly decreased P1NP and serum osteocalcin, whereas the lower dose of FP and both doses of ICS did not. Long-term studies with FP, in which doses of 400–750 μg·day^{-1} were administered for 1–2 yrs, demonstrated no clinically relevant effect on markers of bone formation compared with baseline [35, 36]. To the best of our knowledge, no studies with higher doses of FP have been performed to date. The clinical relevance of our findings remains to be determined, although some evidence exists that long-term ICS use affects bone mineral density and increases the risk of fractures [6].

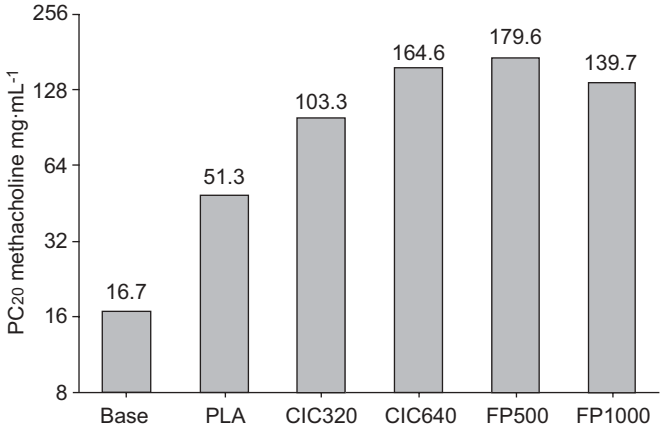

FIGURE 5. Provocative concentration leading to a 20% decrease in forced expiratory volume in 1 s (PC_{20}) adenosine at intake (base) and after placebo (PLA), ciclesonide (CIC) and fluticasone (FP). All treatment groups were administered CIC 160 μg once daily in the evening plus salmeterol 50 μg twice daily. CIC320: CIC 320 μg·day^{-1}; CIC640: CIC 640 μg·day^{-1}; FP500: 500 μg·day^{-1}; FP1000: FP 1,000 μg·day^{-1}.

The secondary end-points of the present study compared the clinical effects of the different treatments with those seen with placebo. Although FEV_1 is often used as a marker for the clinical effect of anti-asthma drugs, this test cannot be used to establish the relative potency of ICS [14, 20]. This is confirmed in the present trial as the observed differences in FEV_1 between active and placebo treatments were very small. However, it has been suggested that challenges tests with methacholine [37] and adenosine [38] might be more appropriate to differentiate the effects of high and low doses of ICS. In previous studies, CIC, inhaled *via* a dry powder inhaler, has demonstrated dose-dependent improvements in adenosine challenge up to doses of 1,280 μg·day^{-1} [14, 38], and significant protective effects *versus* placebo at doses as low as 160 μg·day^{-1} may be expected [12]. Likewise, dose-dependent protective effects of FP against adenosine and methacholine challenge have been documented in previous studies [39].

With regard to the current study, the overall effects of the ICS on bronchial hyperresponsiveness against methacholine were small, with the observed changes ranging between 0.3 and 0.7 DC. This is in keeping with previously published data [15, 23, 39]. In the present study, in which FP and CIC were inhaled on top of a low maintenance dose of CIC, only the improvement by 0.6 and 0.7 DC with the two doses of FP reached statistical significance, a finding of little clinical relevance. These small increases did not allow us to establish the relative potency of the four treatments. Possibly, greater and more discriminative effects could have been obtained by prolonging each treatment to 52 weeks, a time-point at which the maximum effects of ICS on PC_{20} methacholine may be expected [40].

Adenosine-induced bronchoconstriction has been shown to be a sensitive marker of airway inflammation by promoting the release of a variety of inflammatory mediators [41], correlating with both exhaled nitric oxide and sputum, blood and bronchial tissue eosinophilia [42] and appears to be better suited to assess the anti-inflammatory effects of ICS than methacholine [43, 44]. In the present study, the room for

improvement in PC_{20} with both CIC and FP was larger with adenosine than with methacholine, a finding that is in line with a study in which a high dose of ICS increased PC_{20} adenosine by 3.1 DC and PC_{20} methacholine by only 1.5 DC [43]. Nevertheless, the absolute increases in PC_{20} adenosine with FP and CIC observed in the present study did not exceed two DC, when compared with placebo. This contrasts with previous data by PHILLIPS *et al.* [45] for FP and by TAYLOR *et al.* [38] and KANNIESS *et al.* [46] for CIC. Thus, CIC 400 $\mu g \cdot day^{-1}$ administered *via* a dry powder inhaler for 14 days increased PC_{20} adenosine by 2.0 DC and 2.4 DC according to TAYLOR *et al.* [38] and KANNIESS *et al.* [46], respectively, and 1,600 $\mu g \cdot day^{-1}$ increased PC_{20} adenosine by 3.4 DC [38, 46]. In a study with FP 1,000 $\mu g \cdot day^{-1}$, PC_{20} adenosine increased by ~4.5 DC [45].

Differences in methodology may largely explain the observed between-study differences in magnitude of effect. Firstly, the maximum treatment period of 10–12 days, chosen to avoid an overall study duration in excess of 6 months, may have limited the increase in PC_{20} adenosine, which requires up to 4 weeks to reach a maximum [37, 45, 47]. Moreover, the administration of a maintenance dose of CIC 160 $\mu g \cdot day^{-1}$ to preserve asthma control may have contributed to the unexpected, more than two-fold increase in PC_{20} adenosine, compared with PC_{20} values obtained at inclusion. Possibly, inclusion into the study improved adherence to treatment, which in daily life is known to be less than optimal in many asthma patients. This unexpected rise in DC limited room for further improvements in PC_{20} adenosine with any of the active treatments. As the overall improvements in bronchoprotection against adenosine were small (only the difference between CIC 320 $\mu g \cdot day^{-1}$ and FP 500 $\mu g \cdot day^{-1}$ reached statistical significance), the relative potencies of the different treatments could not be established.

In summary, results from the current study indicate that fluticasone propionate 500 $\mu g \cdot day^{-1}$ and 1,000 $\mu g \cdot day^{-1}$ exerted systemic effects in patients with moderate persistent asthma, whereas ciclesonide 320 $\mu g \cdot day^{-1}$ or 640 $\mu g \cdot day^{-1}$ did not affect either biochemical markers of bone formation or serum and urinary cortisol values, if administered in addition to a low ciclesonide dose. Although the long-term clinical meaning of these markers remains to be investigated, they do suggest that ciclesonide yields less systemic effects than fluticasone propionate in patients with moderate persistent asthma for a similar protective activity. The importance of this issue cannot be overestimated in the light of the currently accepted aims of asthma treatment [5], in which disease control with higher doses of inhaled corticosteroid features is the primary objective.

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