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Effect of the Systemic vs. Inhalatory Administration of Synthetic Glucocorticoids on the Urinary Steroid Profile

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INTRODUCTION

Glucocorticoids are a subclass of steroid hormones whose chemical and pharmacological properties are analogues to cortisol. Their physiological role is to regulate hormonal secretion in a time-dependent manner, according to circadian rhythms. Endogenous glucocorticoids are produced in the fascicular zone of the adrenal cortex under neuroendocrine control and are transported to their target receptors in the form of protein complexes with albumine and with the specific serum protein transcortin. Their mechanism of action depends on their binding to specific receptors, which are widely distributed in a great variety of biological districts, thus involving many different target tissues.

In this context, the goal of the present study was to verify (i) whether the administration of glucocorticoids could affect the urinary steroid profile, and especially the levels of endogenous glucocorticoids, androgens and their main metabolites, and (ii) whether these changes, if any, were dependent on the route of administration.

EXPERIMENTAL SECTION

Experiments have been carried out on patients undergoing treatment by corticosteroids. Two synthetic glucocorticoids (betamethasone and beclomethasone) were admistered either locally (inhaled beclomethasone, a single dose of 2 mg) and systemically (betamethasone: a single dose of 3 mg *per os* or a single dose of 4 mg i.m.). The subjects were from both sexes aged 30±5years. Baseline and circadian variability of the endogenous steroid profile was assessed in both groups before (20 males and 20 females) during (10 males and 10 females for oral administration, 10 females for intramuscolar administration and 10 males for inhalatory administration) and after treatment, by collecting urine samples for two months,

every two hours (from the first urine in the morning to the last urine in the night) four days per week.

The urines were prepared according to the screening procedure of conjugated anabolic steroids and analysed by GC/MS. The relative concentrations of the following steroids (glucuronate+free fraction) were measured before, during and after administration of synthetic glucocorticoids: testosterone (Testo), epitestosterone (epiT), androsterone (Andro), etiocholanolone (Etio), dehydroepiandrosterone (DHEA), 11β-hydroxyandrosterone (11OHA), 11β-hydroxyetiocholanolone (11OHE), 5α-androstane-3α,17β-diol (5a3a), 5β-androstane-3α,17β-diol (5b3a), cortisol (C), and tetrahydrocortisol (4HC). All concentration values were corrected for a value of the specific gravity of 1.020.

Volunteers and experimental protocol

Ten male and ten female for oral administration, ten female for intramuscular administration and ten male for inhalatory administration volunteers participated. Before the treatment, urine samples were collected from all volunteers for 30 days (men) or for one complete menstrual cycle (women); while during and after the treatment the urine samples were collected for ten days. Before, during and after the treatment the urines were collected every 2 hours, starting with the morning urine and finishing with the last urine in the night (usually around midnight).

GC/MS procedure

3 ml of urine and 50 µl of internal standard (17α-methyltestosterone) were loaded on a C18 column, washed with methanol and water, and eluted with methanol. After evaporation of the eluate to dryness, the residue was dissolved in 1 ml of 0.2M phosphate buffer pH=7.4. 30 µl of beta-glucuronidase from E. coli were added and hydrolysis was performed for 1 h at 50°C. The buffered solution was alkalinized with 1 ml of 0.1M potassium carbonate solution to pH 8-9 and the steroids were extracted with 10ml of tert-butylmethyl ether on a mechanical shaker for 5 minutes. After centrifugation, the etheral layer was transferred and evaporated to dryness under vacuum; the residue was derivatized by 50µL of MSTFA:NH_4I:Dithioerythrytol (1000:2:4 v/w/w) and 1 µL of the derivatized excract was injected directly into the injection port.

Quantitation by GC-MS of excreted steroids was performed on an Agilent 5890/ 5973A, in electron impact (70 eV), using a 17 m fused silica capillary column cross-linked

methyl silicone (HP1), ID 0.20mm, film thickness 0.11μm. The carrier gas was helium (flow rate: 1 ml/min, split ratio 1:10), and the temperature program was as follows: 180°C (hold 4.5 min), 3°C/min to 230°C, 20°C/min to 290°C, 30°C/min to 320°C; transfer line temperature: 280°C. Acquisition was carried out in selected ion monitoring (SIM) of the following fragments (m/z): 432 for testosterone and epitestosterone; 434 for androsterone and etiocholanolone; 552 for 11β-hydroxyandrosterone and 11β-hydroxyetiocholanolone; 241 for 5α-androstane-3α,17β-diol and 5β-androstane-3α,17β-diol; 432 for DHEA; 632 for cortisol; and 546 for tetrahydrocortisol. All values of urine concentration were calculated by the peak areas of the detected signals relative to the internal standard methyltestosterone (m/z 301). Samples with pH>7 were not included in the study. For calibration of the GC/MS instrument, the following reference mixture was used:

Reference substance	Working solution (μg/ml)	Concentration in urine (ng/ml)
Testosterone	4	40
Epitestosterone	4	40
Androsterone	200	2000
Etiocholanolone	200	2000
11β-hydroxyandrosterone	75	750
11β-hydroxyetiocholanolone	75	750
5α-androstane-3α,17β-diol	22.5	225
5β-androstane-3α,17β-diol	22.5	225
DHEA	8	80
Cortisol	4	40
Tetrahydrocortisol	50	500

RESULTS AND DISCUSSION

Baseline profile

In the preliminary longitudinal and circadian study, single concentration data for all hormones in each urine collection of each volunteer were compared with the corresponding concentration value of each hormone in the urine samples collected at 08.00 h. The variability of the urinary concentration values from urine samples collected from the same volunteer at the same time was very low; on the contrary, the absolute concentration values of each steroid showed marked interindividual differences. Nonetheless, the circadian trend was qualitatively the same for all volunteers: in both sexes the excretion of the endogenous glucocorticoids (cortisol and tetrahydrocortisol) was maximal in the morning and decreased significantly along the day (Figure 1), while more irregular variations were recorded for all the androgens (Figure 2).

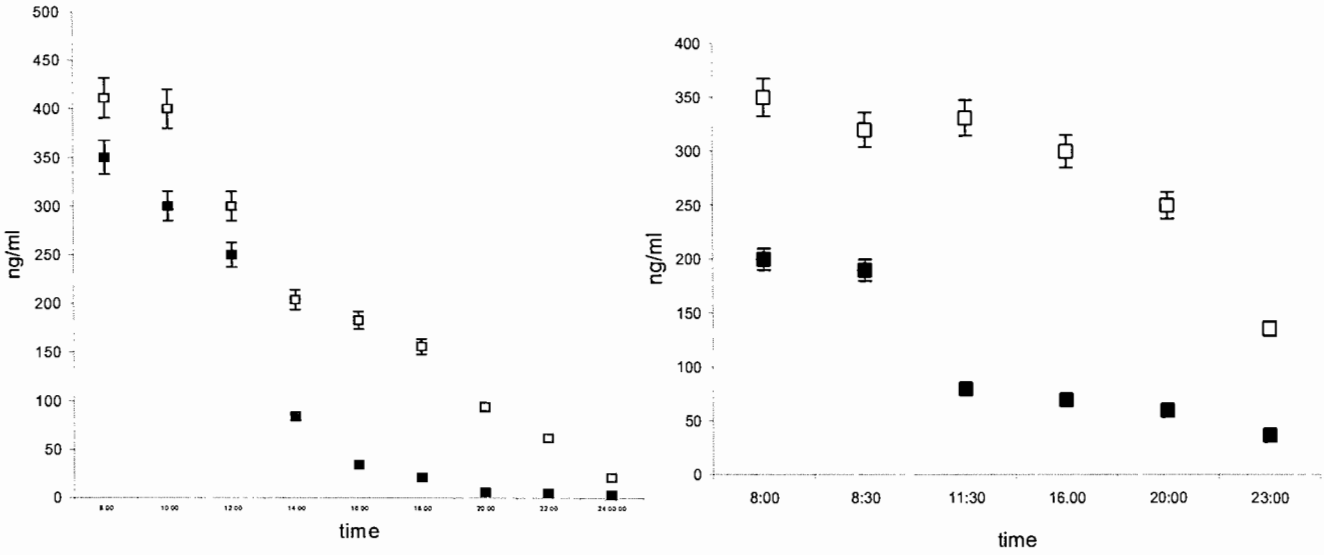

Figure 1. Circadian variability of the urinary concentration of endogenous glucocorticoids (■ cortisol; □ tetrahydrocortisol) in both sexes (left:males; right:females).

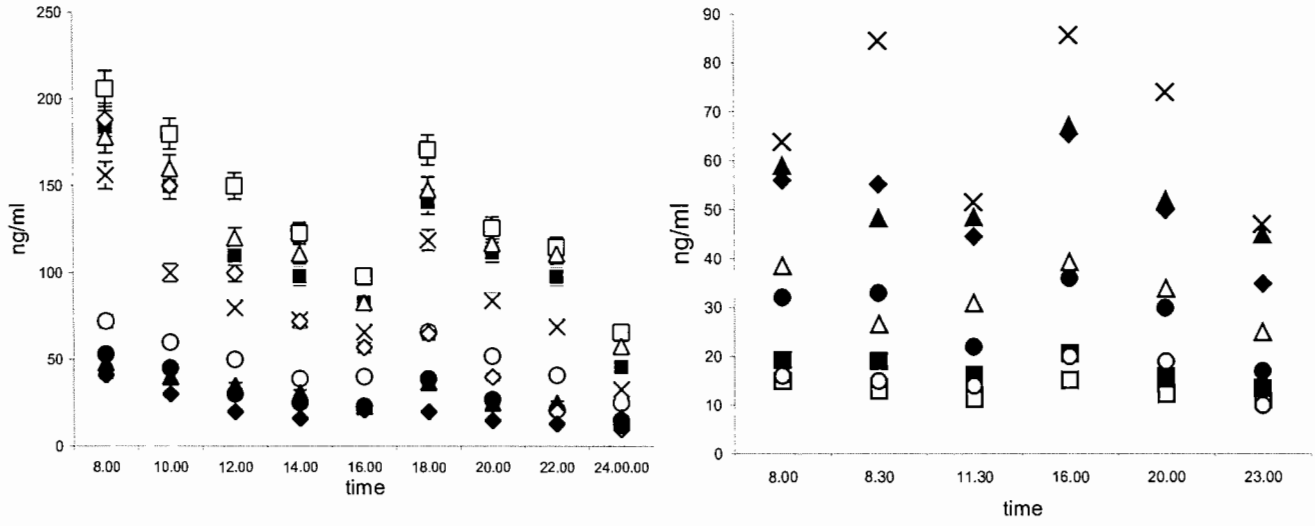

Figure 2. Circadian variability of the urinary concentration of androgens (□ testosterone; ■ epitestosterone; ○ androsterone; ● etiocholanolone; △ 5α-androstane-3α,17β-diol; σ 5β-androstane-3α,17β-diol; ◊ 11β-hydroxyandrosterone; ♦ 11β-hydroxyandrosterone; x DHEA) in both sexes (left:males; right:females).

In-treatment profile

Systemic administration

Oral administration

The effect of the oral administration of endogenous glucocorticoids and androgens was monitored on 10 male and on 10 female volunteers.

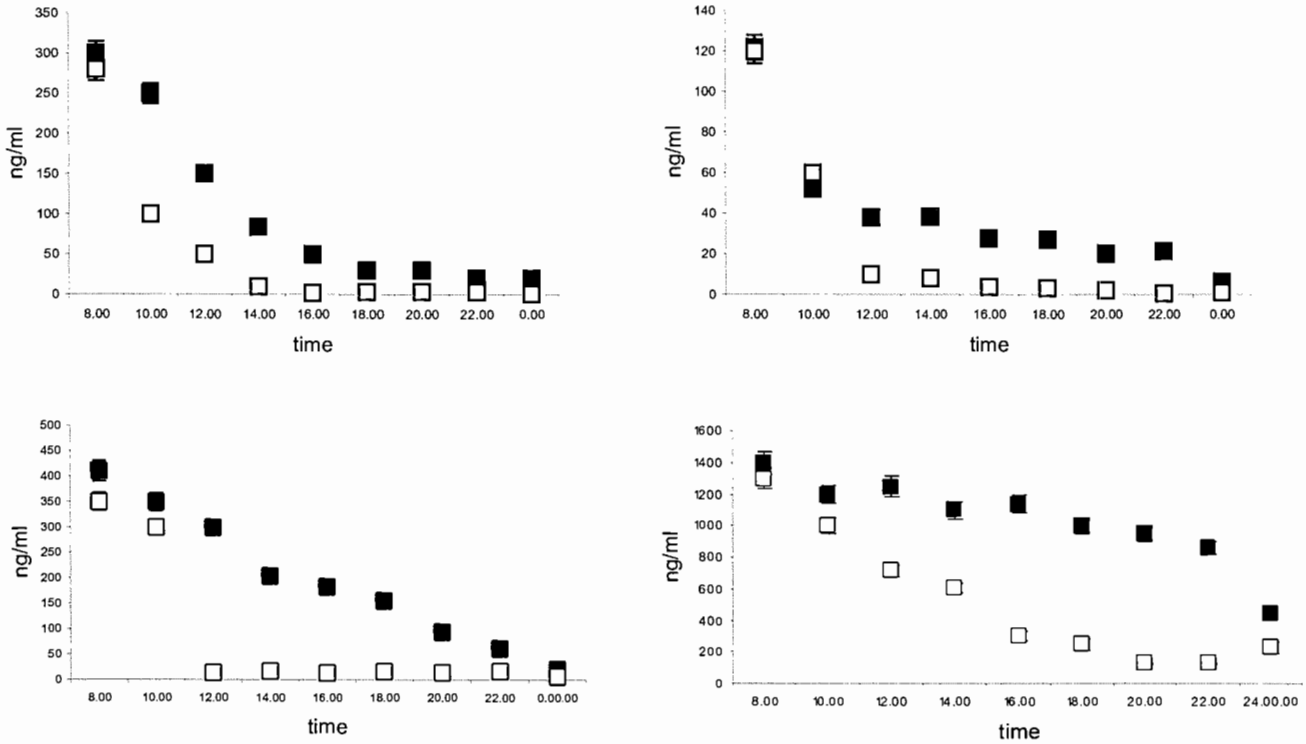

Figure 3. Urinary concentration of cortisol (above) and tetrahydrocortisol (below) before (■) and during (□) oral administration of betamethasone, in males (left) and females (right).

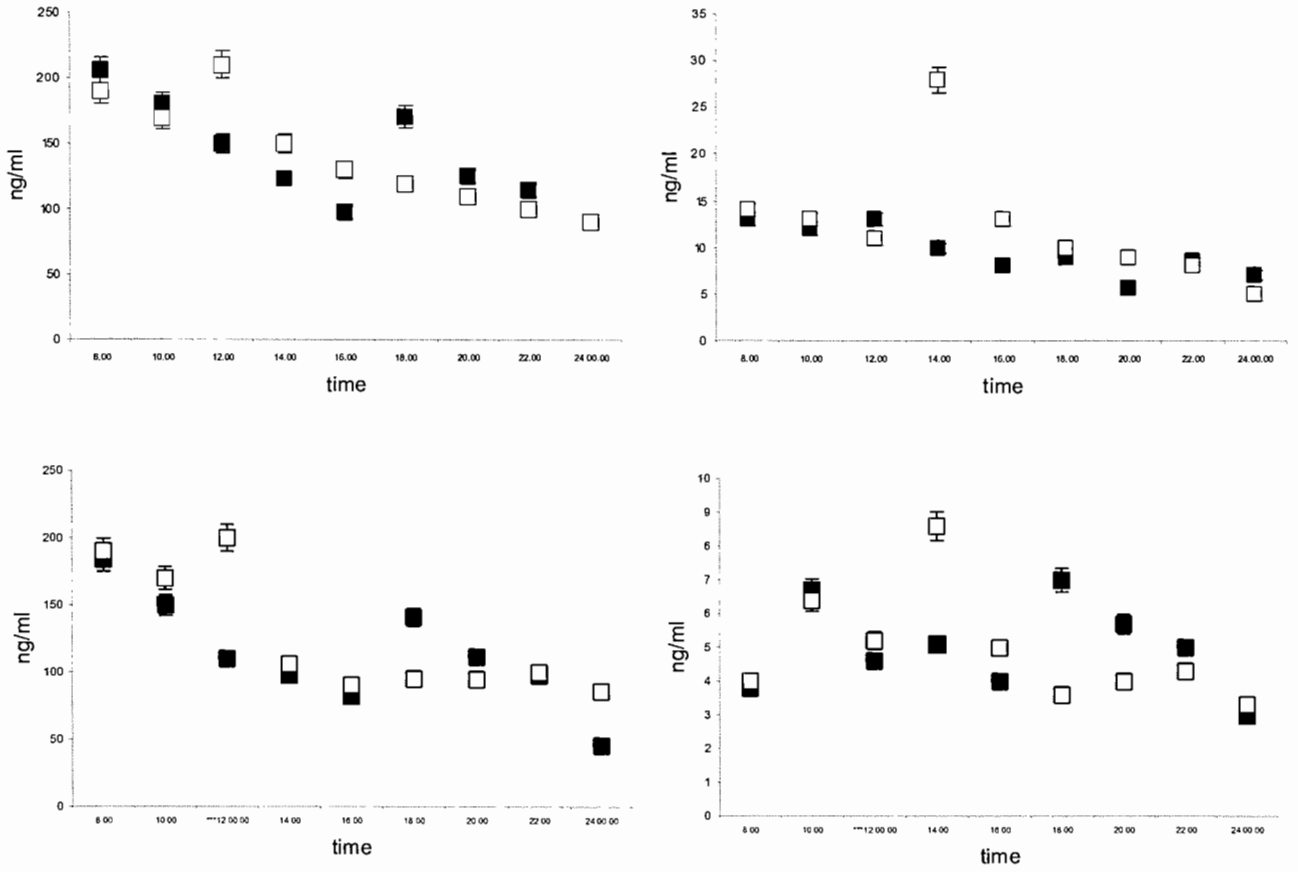

Figure 4. Urinary concentration of testosterone (above) and epitestosterone (below) before (■) and during (□) oral administration of betamethasone, in males (left) and females (right).

The urinary concentration of both cortisol and tetrahydrocortisol decreased significantly in all volunteers from four hours after the administration on (Figure 3); while the androgens showed a transient increase (Figure 4): data shown in the plots refer to the urinary concentrations of testosterone and epitestosterone only, but the observed trend was qualitatively the same for all androgens considered in this study.

Intramuscular administration

Intramuscular administration of betamethasone in females produced a marked decrease in the excretion of cortisol and tetrahydrocortisol (Figure 5, upper plots); while the effect on the androgens (Figure 5, lower plots) was similar to the one produced by the oral administration.

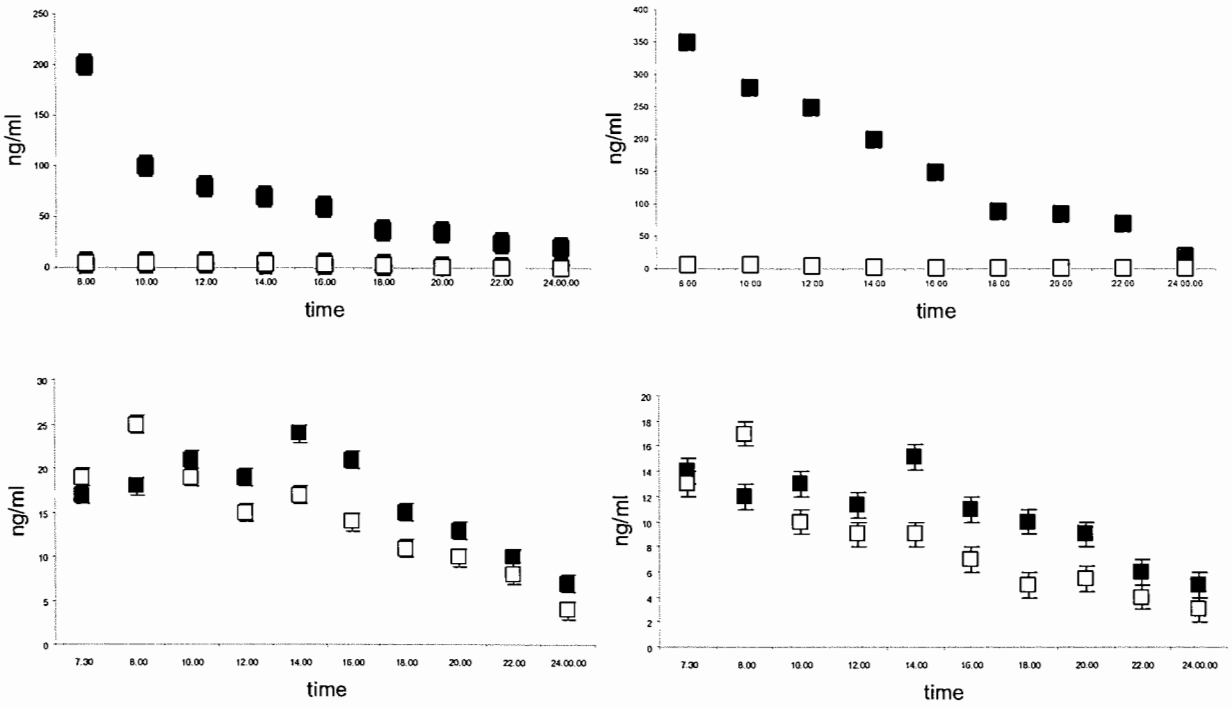

Figure 5. Circadian variability of the urinary concentration of glucocorticoids (above; left: cortisol, and right: tetrahydrocortisol) and androgens (below; left: testosterone, and right: epitestosterone), before (■) and during (□) intramuscular administration of betamethasone, in females.

Inhalatory administration

Inhalatory administration of beclomethasone in males did not produce any significant alteration of the steroid profile, as far as both the glucocorticoids and the androgens are concerned (Figure 6).

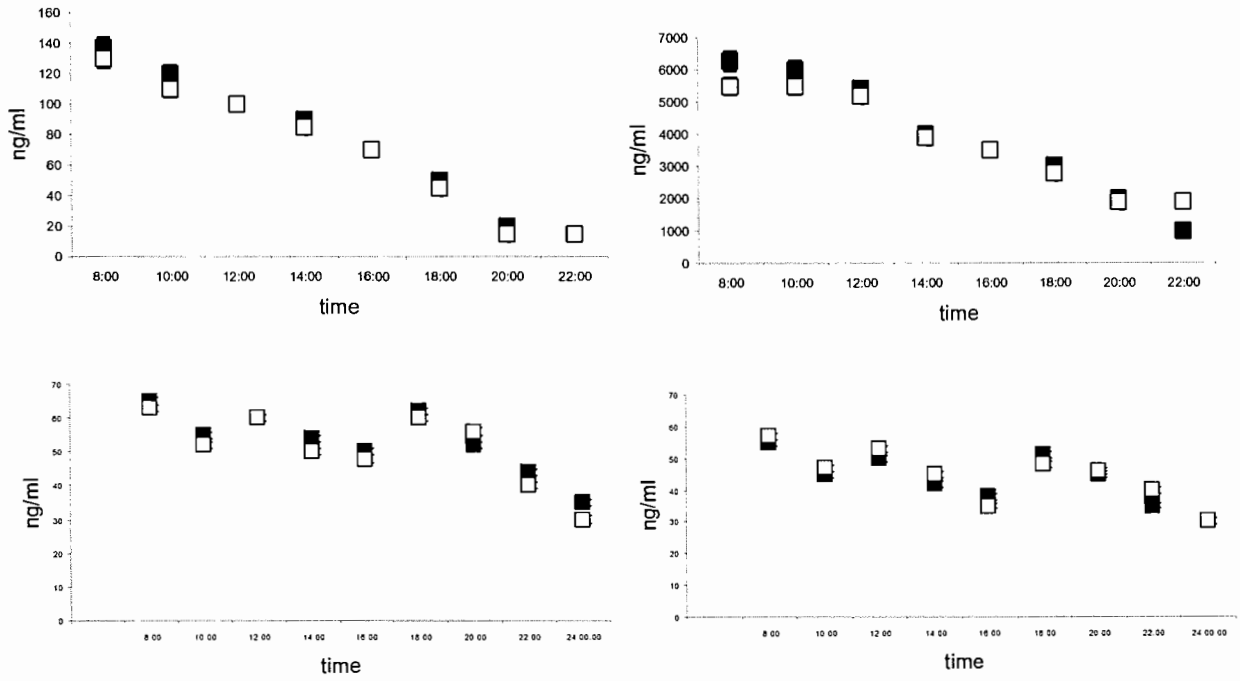

Figure 6. Circadian variability of the urinary concentration of glucocorticoids (above; left: cortisol, and right: tetrahydrocortisol) and androgens (below; left: testosterone, and right: epitestosterone), before (■) and during (□) inhalatory administration of beclomethasone, in males.

Post-treatment profile:

The follow up studies were carried out to verify whether the effect of synthetic glucocorticoids was maintained even after the suspension of the administration.

After the suspension of the oral administration of betamethasone, a reduced urinary concentration of both cortisol and tetrahydrocortisol was recorded for five days: this effect was recorded in all volunteers and was independent of the sex (data in Figure 7 refer to male subjects).

The same qualitative trend was recorded also after the suspension of the intramuscular administration of betamethasone: in this case the concentration of cortisol and tetrahydrocortisol returned to the baseline values after three days (Figure 8).

In both the above cases, no effect was seen on the urinary concentration of androgens (see data in Figures 9-10 respectively for the oral and the intramuscular administration).

Finally, no alteration of the urinary concentration of endogenous steroids was recorded after the suspension of the inhalatory administration of beclometasone (see Figures 11-12, respectively for cortisol/tetrahydrocortisol and testosterone/epitestosterone).

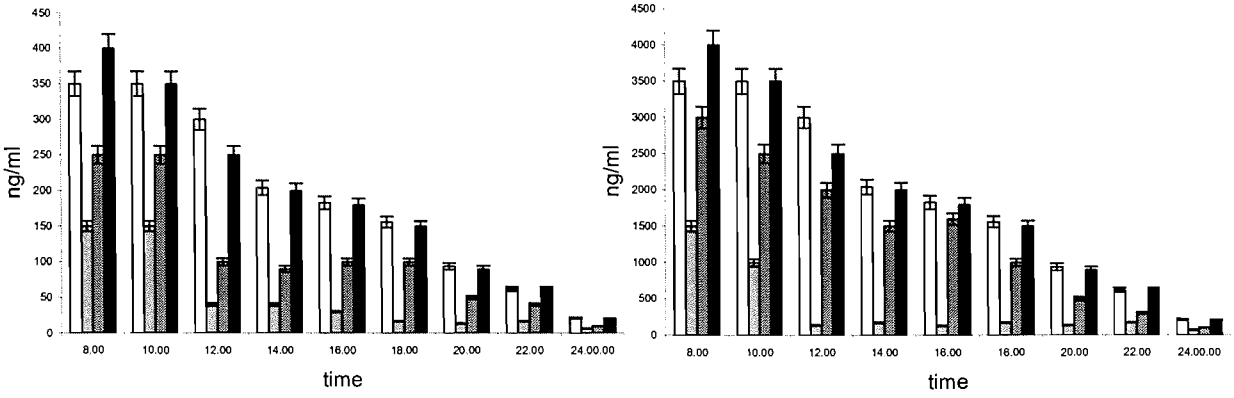

Figure 7. Circadian variability of the urinary concentration of cortisol (left) and tetrahydrocortisol (right), recorded before (open bar) and after 1 (dashed), 3 (grey), and 5 (black) days from the suspension of the treatment with oral betamethasone.

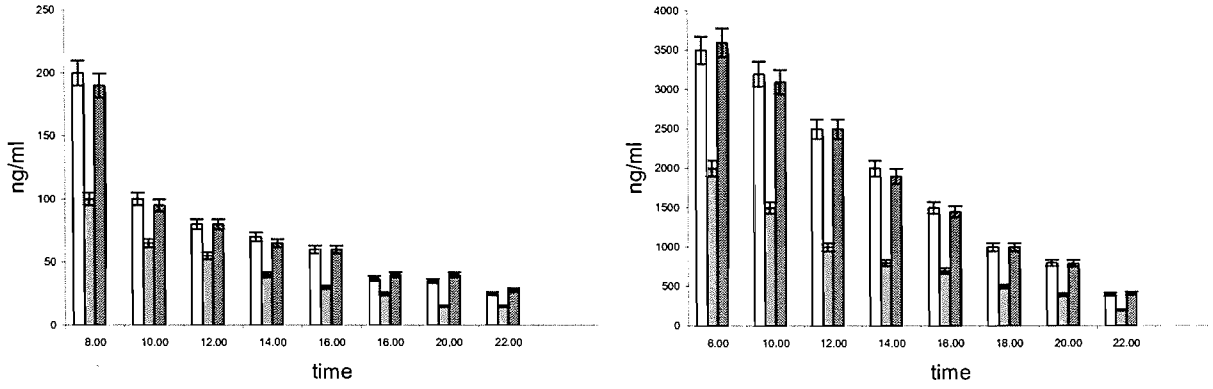

Figure 8. Circadian variability of the urinary concentration of cortisol (left) and tetrahydrocortisol (right), recorded before (open bar) and after 1 (dashed) and 3 (grey) days from the suspension of the treatment with intramuscular betamethasone

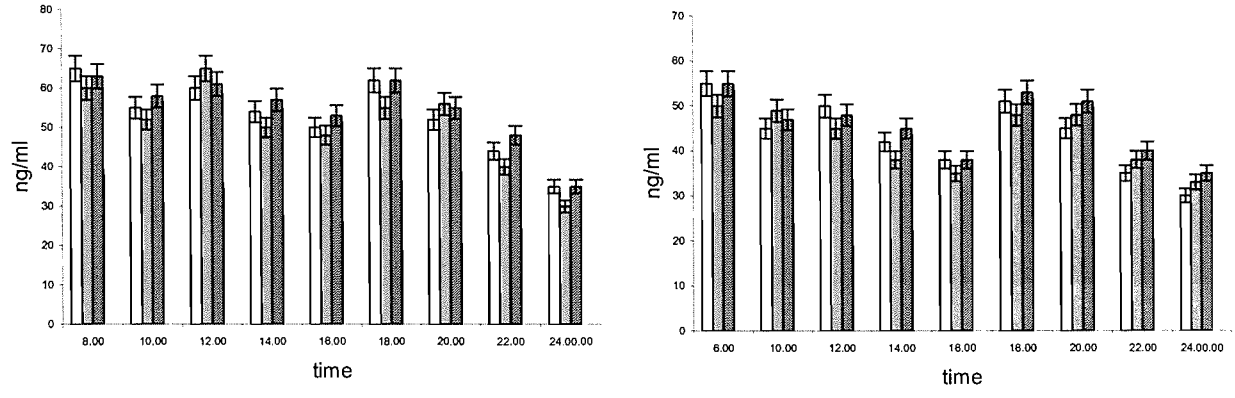

Figure 9. Circadian variability of the urinary concentration of testosterone (left) and epitestosterone (right), recorded before (open bar) and after 1 (dashed) and 3 (grey) days from the suspension of the treatment with oral betamethasone

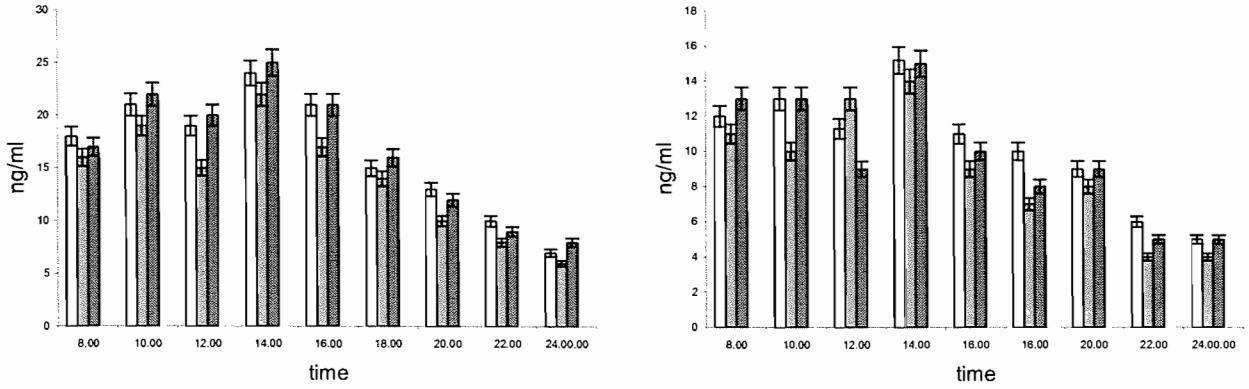

Figure 10. Circadian variability of the urinary concentration of of testosterone (left) and epitestosterone (right), recorded before (open bar) and after 1 (dashed) and 3 (grey) days from the suspension of the treatment with intramuscular betamethasone

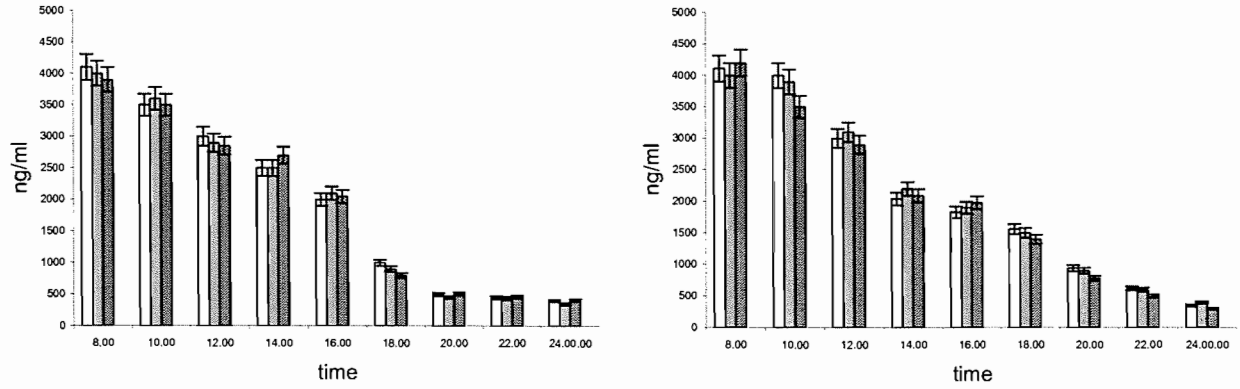

Figure 11. Circadian variability of the urinary concentration of cortisol (left) and tetrahydrocortisol (right), recorded before (open bar) and after 1 (dashed) and 3 (grey) days from the suspension of the treatment with inhaled beclomethasone.

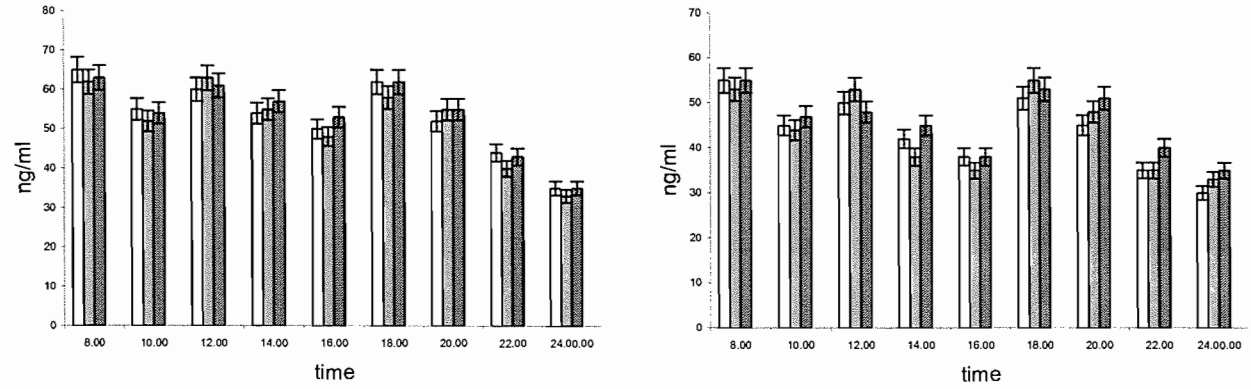

Figure 12. Circadian variability of the urinary concentration of testosterone (left) and epitestosterone (right), recorded before (open bar) and after 1 (dashed) and 3 (grey) days from the suspension of the treatment with inhaled beclomethasone.

CONCLUSIONS

- The significant decrease of urinary levels of endogenous glucocorticoids after oral and intramuscular administration can be explained by a mechanism of negative feedback.
- The transient increase of the urinary concentration of androgens after systemic (oral and intramuscular) administration of betamethasone can be explained by a shift of the synthetic pathway leading to the formation of androgens.
- Inhalatory administration does not lead to any significant variation, possibly as a consequence of the low circulating levels of drug.
- Additional data are necessary to further clarify the mechanims leading to the observed effects, especially at higher doses, and also to consider other topical routes.

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