CIRCADIAN RHYTHM OF ANDROSTENDIONE AND FREE TESTOSTERONE IN ASTHMATIC WOMEN WITH POSTMENOPAUSAL HORMONE THERAPY

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Objective of this study was to assess daily rhythm of androstendione (Δ^4A) and free testosterone (FT) levels in postmenopausal asthmatic women before and after hormonal replacement therapy and the influence of inhaled glucocorticosteroids (GC).

Methods. 54 asthmatic and 20 healthy postmenopausal women (aged 48-59) before and after 6 months of estrogen plus progestin therapy (EPT) were studied. Hormone concentrations in serum (Δ^4A and FT) were assessed with the use of RIA method. Statistical analysis of the circadian rhythm was performed with the use of cosinor test according to Halberg et al

Results. Cosinor analysis of Δ^4A and FT secretion during the day showed existence of daily rhythm in three studied groups before as well as after postmenopausal hormone therapy (HT). A statistically significant decrease of circadian concentrations of Δ^4A and FT in groups of patients treated with GC was observed. Changes in amplitude of Δ^4A and FT rhythm between studied groups were not observed. However, displacement of rhythm acrophase of studied hormones in asthmatic women in comparison to control group before and after HT was shown. No significant differences in circadian values of Δ^4A and FT concentrations before HT use compared to values after HT were shown.

Conclusions. Postmenopausal asthmatic women show diminished circadian concentrations of androstendione and free testosterone in serum caused among other things by inhalative GC. Postmenopausal hormone therapy did not influence any changes in function of studied endocrine organs.

Keywords: Androstendione - Testosterone - Circadian rhythm - Asthma bronchiale - Postmenopausal hormone therapy

Androgens play a number of important physiological roles in the human (Yialamas et al. 2003). Gonadal steroids influence mood, wellbeing, and genital physiology but evidence of actions is controversial. Hormone imbalance provokes symptoms that may also derive from other conditions (Ghizzani et al. 2003). In women testosterone is thought to influence bone density, muscle mass, erythropoesis, energy, sexual, cognitive function and mood. Testosterone deficiency in women may result from a variety of conditions, including e.g. oophorectomy, adrenal and pituitary disease and the use of high-dose corticosteroids. Simple aging and natural menopause may also contribute to androgen deficiency in some women. The impact of conventional postmenopausal hormone therapy (HT) on testosterone levels in naturally menopausal women is discussed, with the differences between oral and transdermal routes of estrogen delivery being emphasized (Mazer 2002). Recently, interest is increasing in the use of androgen replacement not only for women who have undergone premature or surgical menopause but also for those who experience natural menopause (Burd et al. 2001).

The role of androgens in pathogenesis of many diseases, especially of immunological origin, has been recently

raised. Decreased androgens synthesis, in women as well as in men was indicated to be a potential factor prior to occurrence of many diseases of immunological etiology. However, in the opinion of other investigators, the course of chronic diseases can predispose to a decrease of androgen synthesis (HALL et al. 1993, SEMPLE et al. 1987).

WEINSTEIN et al. (1996) suggested that an assessment of concentrations of sex steroids in postmenopausal, asthmatic women would be rationalized to confirm a connection between the humoral system and the pathophysiology of asthma. Assessment of hormone concentrations done once a day has little diagnostic value. A more precise method of endocrine system function assessment is an assay of circadian changes in hormones secretion. Such an assay is particularly appropriate in asthmatic patients due to a different symptom's intensification during the day.

Daily rhythm of androgens secretion in postmenopausal asthmatic women has not been studied so far. There is a lack of researches evaluating the influence of HT on androgens secretion in postmenopausal asthmatic women.

The aim of the study was to describe the circadian rhythm of androstendione (Δ^4A) and free testosterone (FT) in asthmatic women before and after postmenopausal hormone therapy and the influence of inhaled glucocorticosteroids.

Materials and Methods

Studies were performed in 54 asthmatic women with physiological menopause aged 48 to 59 (mean 53.87 ± 3.19), who were divided into two groups depending on the severity of asthma and administered treatment according to guidlines for the diagnosis and management of asthma (Guidlines for the diagnosis and menagement of asthma 1997):

Group I consisted of women using glucocorticoid drugs (GC) in inhalative form (Budesonide in mean doses 600-1600 μg/day). Studied women had not chronically used systemic GC in form of intramuscular or oral specimen for at least two years. Group II consisted of asthmatic women, not treated with GC. Group III-control group: consisted of 20 healthy postmenopausal women, who were not treated with any drugs except HT. Clinical characteristic of studied groups is presented in Table 1.

Women excluded from the study: who had more than 60 years, the last cycle more than 5 years ago, BMI> 26 kg/m^2, neoplastic diseases, nephropathy and hepatopathy (e.g. cholecystolithiasis), heart diseases and angiopathy (e.g. chronic hypertensive disease, new or past arterial and venous thrombus episodes), metabolic (e.g. diabetes), hematologic, endocrinological (which were not complications of asthma and glucocorticotherapy) and gynaecological (e.g. vaginal bleeding of unknown aetiology, endometriosis) diseases.

Clinical evaluation of patients was done on the basis of physical examination, spirometry, a frequency of asthma exacerbations, what was described in earlier studies (KOS-KUDLA et al. 1999, KOS-KUDLA et al. 2000). Contraindications to the use of HT were not documented in any of the women. No steroid drugs, other than these mentioned above, antidepressive medications or thyroid hormones were taken during the study. Local Ethic Committee approval was obtained for the study.

Studies of circadian rhythm of androstendione and free testosterone were performed before HT and after six, 28-days cycles of 17β-estradiol and medroxyprogesterone acetate therapy with the use of cyclic method. 17β-estradiol (Estraderm TTS - Novartis or Systen – Janssen-Cilag) was administered in a daily dose of 50 μg with transdermal patch twice a week (on Mondays and Thursdays) from 1st to 21st day of the cycle. Medroksyprogesterone acetate (Provera) was administrated orally from 11th to 22nd day of the cycle 10mg/day.

Circadian hormone concentration assessment after HT was done between the 18th and 20th day of the sixth cycle (venous blood was collected every 3 hours during the day). Δ^4A and FT concentrations in serum were assessed with RIA method with the use of DPC set (Diagnostic Products Corporation, Los Angeles, USA). The sensitivity of the method, defined as the smallest detectable concentration (detection limit for assays), was for FT: 0,15 pg/ml and for Δ^4A: 0.04 ng/ml. The intraassay coefficients of variation was for FT: 3,83 % and for Δ^4A: 5.74 %. The interassay coefficients of variation was for FT: 4.2 % and for Δ^4 A: 8.38 %.

Statistical evaluiation. Calculations were done with the use of personal computer (program CSS Statistica). Comparison of the means of examinated variables for two trials was done with the use of t-Student test or Wilcoxon matched pair test. Simple linear regression was noted in the study. The statistical analysis of the circadian rhythm was performed with the use of cosinor test according to HALBERG et al. (1967).

Results

Cosinor analysis of androstendione and free testosterone concentrations in serum during the day was shown existence of daily rhythm in three study groups before as well as after HT. Analysis of daily rhythm of Δ^4A and FT in asthmatic patients treated with GC is presented in Fig.1 and 2.

Table 1
Clinical characteristic of studied groups
(all mean values are given as mean±SD)

GROUP	NUMBER OF WOMEN	AGE [YEARS]	BMI	SEVERITY OF ASTHMA	ASTHMA DURATION [YEARS]	GC-TREATMENT DURATION [YEARS]
I	31	53.18 ± 3.67	22.91 ± 1.40	moderate	10.9	5.1
II	23	52.52 ± 3.67	24.37 ± 1.07	episodic and mild	11.6	0
III	20	52.70 ± 3.05	24.38 ± 1.35		0	0

I group - asthma patients with corticotherapy; II group - asthma patients without corticotherapy; III - control group

GROUP I

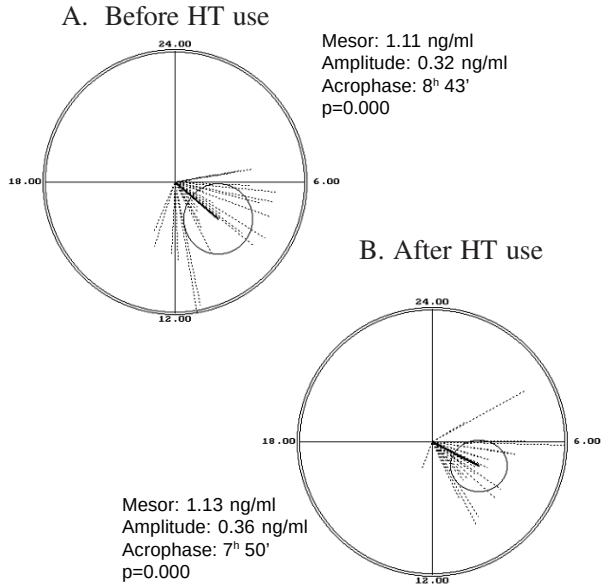


Fig. 1 Cosinor analysis of daily rhythm of androstendione in asthmatic women treated with glucocorticoids before and after HT use

GROUP I

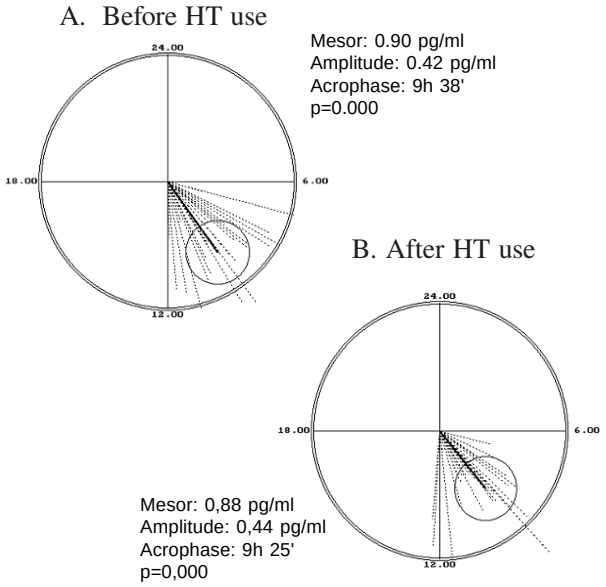


Fig. 2 Cosinor analysis of daily rhythm of free testosterone in asthmatic women treated with glucocorticoids before and after HT use

Prior to HT a statistically significant decrease of Δ^4A and FT mesor in the group of patients treated with GC in comparison to the control group was observed. Changes in amplitude of Δ^4A and FT rhythm between studied groups were not observed. Displacement of Δ^4A rhythm acrophase for about 1 hour in Group I was observed. FT displacement of acrophase averaging 1 hour 22 minutes in both asthmatic groups (in comparison to the control group) was observed before and after HT (Fig. 3 and 4). Changes of Δ^4A and FT concentrations during particular periods of the day before and after HT are presented in Fig.3 and 4.

Comparison of circadian secretion of Δ^4A after HT use between studied groups showed a statistically significant decrease of Δ^4A mesor values in patients treated with GC in comparison to patients not treated with GC and to the control group (Table 2). A statistically

Table 2

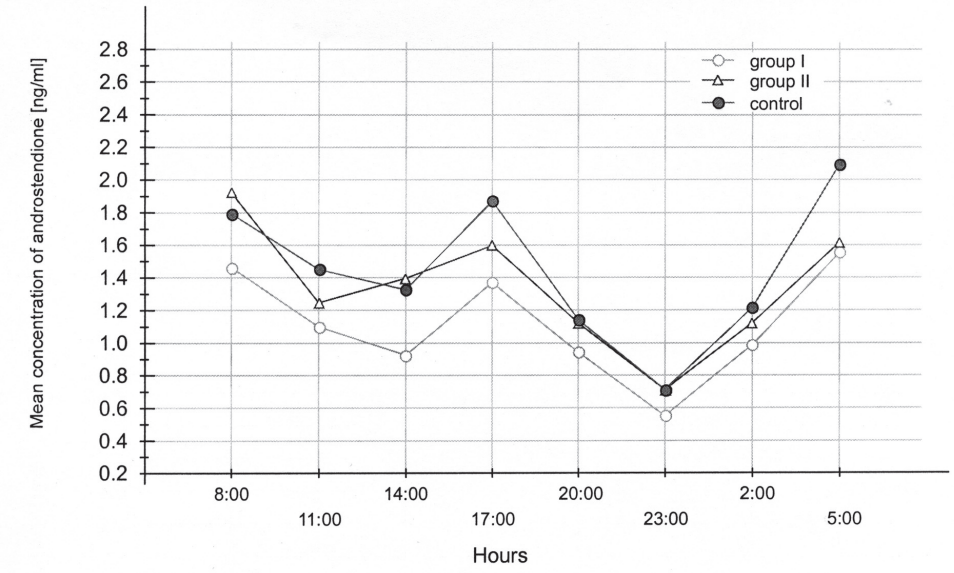
GROUP	Δ^4 A [ng/ml] ± SD	
	Before HT	**After HT**
I	1.11 ± 0.36 •	1.12 ± 0.29 + ∘
II	1.34 ± 0.29	1.36 ± 0.32
III - control	1.45 ± 0.38	1.37 ± 0.25

Comparison of circadian values of androstendione (Δ^4 A) in serum of asthmatic women treated with GC (group I) and not treated with GC (group II) and in control group before and after hormone replacement therapy (HT)

• - p≤ 0.05 group I vs. group III before HT
■ - p≤ 0.05 group I vs. group II after HT
∘ - p≤ 0.05 group I vs. group III after HT

A. Before HT use

HORMONE	GROUPS	MESOR [ng/ml] /±SD/	AMPLITUDE [ng/ml] /±SD/	ACROPHASE [h, min] /±SD/	COSINE (p)
Androstendione	I	1.11 ± 0.36	0.32 ± 0.14	$8^h43' \pm 2^h23'$	0.000
	II	1.34 ± 0.29	0.39 ± 0.16	$9^h53' \pm 2^h18'$	0.000
	III	1.45 ± 0.38	0.39 ± 0.22	$9^h18' \pm 1^h43'$	0.000



B. After HT use

HORMONE	GROUPS	MESOR [ng/ml] /±SD/	AMPLITUDE [ng/ml] /±SD/	ACROPHASE [h, min] /±SD/	COSINE (p)
Androstendione	I	1.13 ± 0.29	0.36 ± 0.16	$7^h50' \pm 2^h03'$	0.000
	II	1.36 ± 0.32	0.48 ± 0.21	$8^h55' \pm 2^h00'$	0.000
	III	1.38 ± 0.25	0.48 ± 0.12	$8^h43' \pm 1^h23'$	0.000

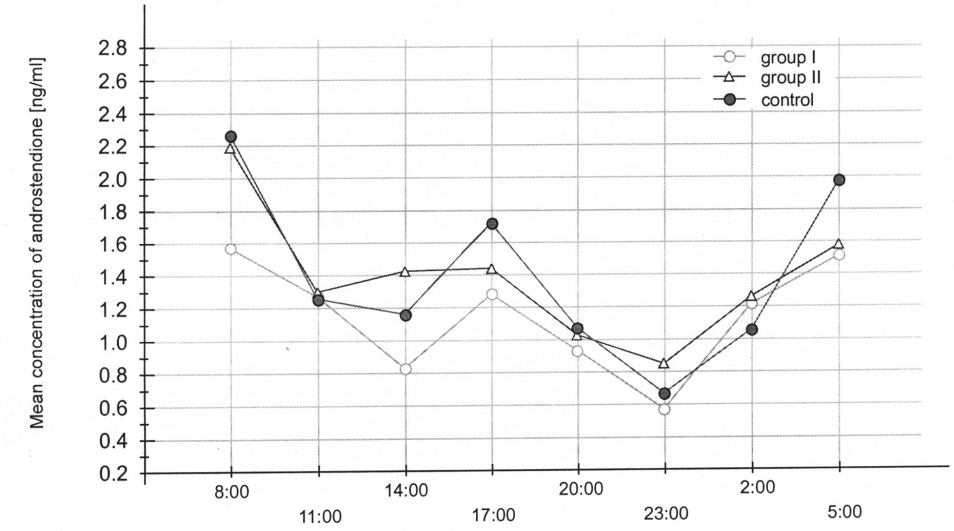
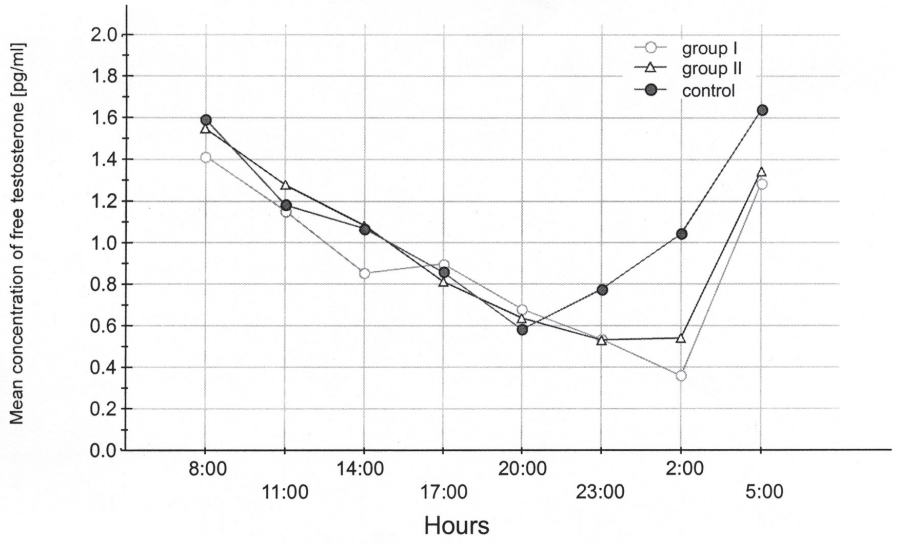


Fig. 3 Circadian oscillation of mean serum androstendione concentrations and chronobiological parameters of daily rhythm in asthmatic women treated with GC (group I), not treated with GC (group II) and in control group (III)

A. Before HT use

HORMONE	GROUPS	MESOR [pg/ml] /±SD/	AMPLITUDE [pg/ml] /±SD/	ACROPHASE [h, min] /±SD/	COSINE (p)
Free testosterone	I	0.90 ± 0.29	0.42 ± 0.15	$9^h38' \pm 1^h22'$	0.000
	II	0.97 ± 0.24	0.52 ± 0.21	$9^h23' \pm 1^h35'$	0.000
	III	1.09 ± 0.26	0.49 ± 0.18	$7^h31' \pm 1^h23'$	0.000



B. After HT use

HORMONE	GROUPS	MESOR [pg/ml] /±SD/	AMPLITUDE [pg/ml] /±SD/	ACROPHASE [h, min] /±SD/	COSINE (p)
Free testosterone	I	0.88 ± 0.30	0.44 ± 0.19	$9^h25' \pm 1^h29'$	0.000
	II	0.96 ± 0.24	0.53 ± 0.20	$9^h33' \pm 1^h10'$	0.000
	III	1.09 ± 0.25	0.43 ± 0.17	$7^h57' \pm 2^h03'$	0.000

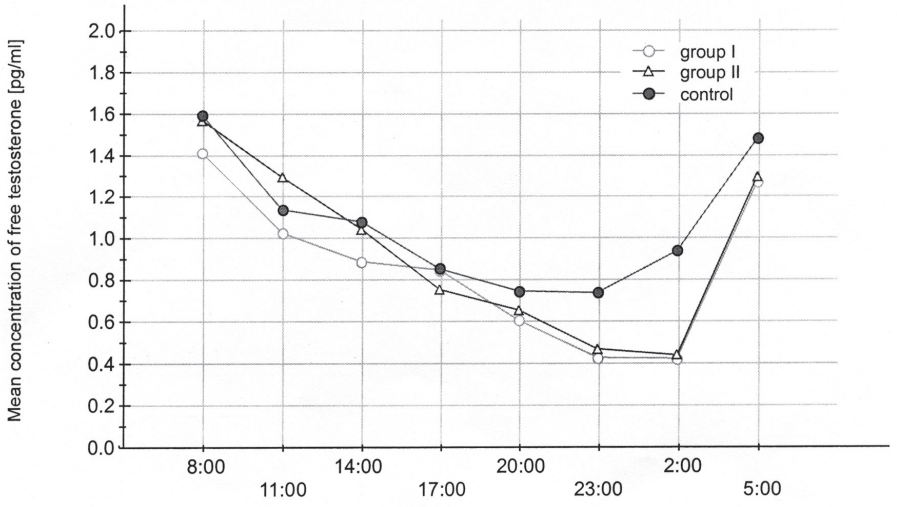


Fig.4 Circadian oscillation of mean serum free testosterone concentrations and chronobiological parameters of daily rhythm in asthmatic women treated with GC (group I), not treated with GC (group II) and in control group (III)

Table 3

GROUP	FT [pg/ml] ± SD	
	Before HT	After HT
I	0.90 ± 0.29 •	0.88 ± 0.30 ∘
II	0.97 ± 0.24	0.96 ± 0.24
III - control	1.09 ± 0.26	1.09 ± 0.25

Comparison of circadian values of free testosterone (FT) in serum of asthmatic women treated with GC (group I) and not treated with GC (group II) and in control group before and after hormone replacement therapy (HT)

• - p≤ 0.05 group I vs. group III before HT
∘ - p≤ 0.05 group I vs. group III after HT

significant decrease of FT concentrations in Group I in comparison to the control group was observed (table 3).

Significant differences in circadian concentrations of Δ^4A and FT before HT in comparison to values after HT have not been stated.

Discussion

Daily rhythm of Δ^4A and FT in postmenopausal asthmatic women has not been studied so far. Only Mileva et al. (1990) have shown substantial daily rhythm of testosterone secretion in asthmatic men not treated with GC.

Many investigators underline existence of daily rhythm of androstendione and testosterone (T) (Fiet et al. 1980; Goldman et al. 1985; Lejeune-Lenain et al. 1987; Ostrowska et al. 1996; Panico et al. 1990; Vermeulen 1976). These reports are compliant with results obtained in this study, where statistically significant daily rhythm of Δ^4A and FT secretion before as well as after HT use was demonstrated.

Rhythm of Δ^4A and testosterone secretion is dependent on oscillation of ACTH secretion from the hypophysis. This fact has been documented by measuring Δ^4A and cortisol secretions' changes in the blood over a 24-hour period (Godman et al. 1985; Lejeune-Lenain et al. 1987). This study also documents that daily oscillation of androgens concentrations, including Δ^4A and testosterone, maintain a period of minimal secretion in late evening hours and maximum secretion in early morning hours. Existence of additional afternoon acrophase of Δ^4A and testosterone secretions suggests the possibility of common stimulation of secretory activity of hypophyso–adrenal and hypophyso–gonadal axis (Lejeune-Lenain et al. 1987). Sluijmer et al. (1995) have shown a significant decrease of circulating Δ^4A and testosterone concentrations in postmenopausal women six weeks after ovariectomy in comparison to concentrations determined prior to surgery. This indicates the participation of ovarian steroidogenesis in the maintenance of adequate androgen concentrations in serum.

Vermeulen et al. (1976) and Fiet et al. (1980) have shown secretion of Δ^4A in daily rhythm in postmenopausal women - the highest concentrations of this hormone occurred in morning hours and the lowest about midnight.

Ostrowska et al. (1996) have also shown a statistically significant daily rhythm T with acrophase in hours between 8:01 and 9:25 in the morning in postmenopausal women. Similarly to the results of our study, acrophase of daily rhythm in healthy women as well as in asthmatic patients occured between 7:30 and 9:40.

Lonning et al. (1989) studies with healthy and postmenopausal women with breast cancer (differ from results of this study because they did not show daily rhythm of Δ^4A and FT secretion. Changes in Δ^4A and FT concentrations during the day have not shown statistically significant daily rhythm ($p>0.1$ in Friedman's test), but the values of these concentrations were higher at morning hours with a tendency to fall at evening hours.

The differences between obtained results can be caused by methodological and other conditions of performed studies, such as: number of study groups, frequency of blood collecting for assays during the day and in this more precise estimation of hormones concentrations oscillations as well as more precise statistical analysis of daily and episodic changes of concentration values (Lejeune-Lenain et al. 1987). In our study, a statistically significant decrease of circadian concentrations of Δ^4A and FT in group of asthmatic women treated with GC with comparison to the control group was observed before as well as after HT. Inhaled GC may also potentially inhibit ACTH secretion and lead to secondary adrenocortical insufficiency, which was shown in our own earlier studies (Kos-Kudla 1998). We found also a correlation between the time taken for return to normal adrenal cortex function and duration of bronchial asthma and of GC therapy (Kos-Kudla et al. 2003).

Similarly Weinstein et al. (1996) showed low normal concentrations of total testosterone in basic conditions in all postmenopausal asthmatic women to be lower in comparison to the group of healthy women. Use of exogenous GC influence ovulation cycle in women, as well as lower androgens' concentration in serum of

men. This effect can be interpreted with direct suppression of gonadal steroids secretion. Additionally chronic therapy with prednisolone inhibits pituitary secretion of gonadotropins in women (MacAdams et al. 1986; Stomati et al. 2000). Mac Adams et al. (1986) observed statistically significant decrease of concentration of bound as well as free testosterone in men treated with long-term GC (in doses smaller than 15 mg/24 h in conversion to prednisone) because of severe obstructive lung diseases. Interpreting this phenomenon the authors gave examples of many possible factors that could influence a decrease of T concentration. These were, among others, age of patient, severity of obstructive lung disease, use of additional obstructive drugs which could influence the effect of the chronic GC therapy on testosterone concentration in serum, and moreover direct influence of GC treatment on inhibition of GnRH secretion by hypothalamus. (MacAdams et al. 1986; Sekakura et al. 1975).

There is a lack of reports concerning suppresive influence of inhalative GC on androgens secretion. Mileva et al. (1990) confirmed an increase of concentration of basic androstendione secretion in 30% of asthmatic men not treated with GC. This result is different from our study in which circadian Δ^4A secretion in women not treated with GC did not differ from these from the control group. HT did not cause changes of circadian concentrations of Δ^4A and FT in serum of studied postmenopausal women. These observations are compliant with reports of Słowinska-Srzednicka et al. 1993) in which statistically significant changes of Δ^4A and testosterone secretion after half-year (six months) use of transdermal 17β estradiol (17β E_2) and chlormandinone acetate were not observed. Similarly, Castelo-Branco et al (1995) and Perrone et al (1994) did not show changes in Δ^4A and (testosterone concentrations in serum after 6-month cyclic administration of 17βE2 and medroksyprogesterone acetate.

In conclusion, we assume that the decrease of circadian Δ^4A and FT concentrations observed in postmenopausal asthmatic women treated with GC was a result of decreased androgenic secretory function of the adrenal glands, as well as of suppression of gonadal steroids secretion caused by inhalative GC administration. HT did not have any influence on changes of endocrine organs function in range of Δ^4A and FT secretion. Influence of specific hormones used in HT of asthmatic women needs confirmation in further studies.

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