

Altered pituitary-adrenal interaction in nocturnal asthma

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Background: Increased airway inflammation at night contributes to the nocturnal worsening of asthma, but the mechanisms regulating circadian variations in airway inflammation are unknown.

Objective: We hypothesized that altered hypothalamic-pituitary-adrenal axis function serves as an endogenous controller of inflammation in nocturnal asthma.

Methods: Patients with nocturnal asthma (n = 7), patients with nonnocturnal asthma (n = 13), and healthy control subjects (n = 11) adhered to a regular sleep-wake cycle for 1 week. Corticotropin and cortisol levels were assayed every 2 hours for 24 hours. Low-dose corticotropin stimulation was performed.

Circadian hormonal flux was analyzed by means of cosinor modeling and calculation of the area under the 24-hour curve.

Results: Corticotropin peak levels and areas under the 24-hour curve were significantly increased in patients with nocturnal asthma versus values in patients with nonnocturnal asthma and control subjects. Patients with nonnocturnal asthma demonstrated significantly increased areas under the 24-hour cortisol curve when compared with control subjects, but peak cortisol levels did not differ between groups. Cortisol levels after low-dose corticotropin stimulation did not differ between groups. Corticotropin and cortisol levels were not correlated with the degree of physiologic impairment.

Conclusion: Nocturnal asthma is marked by increased corticotropin levels that are not accompanied by commensurate increases in cortisol levels. This observation might indicate blunted adrenal responsiveness in the nocturnal asthma phenotype. Conversely, adrenal response to corticotropin might be enhanced in nonnocturnal asthma, attenuating nocturnal worsening of airway inflammation. (*J Allergy Clin Immunol* 2003;112:52-7.)

Key words: Corticotropin, cortisol, inflammation, nocturnal asthma

Nocturnal asthma is an asthma phenotype marked by nighttime increases in airway inflammation,¹ airway hyperresponsiveness,² and expiratory airflow limitation.³ These changes are associated with a nocturnal increase in symptoms, such as cough and dyspnea, and the frequen-

cy of nocturnal symptoms is an important factor in staging asthma severity and determining appropriate asthma pharmacotherapy.⁴ Although alterations in factors such as alveolar tissue inflammation,^{5,6} number and function of peripheral blood eosinophils,^{7,8} and glucocorticoid and β -adrenergic receptor affinity or activity⁹⁻¹¹ have been reported in nocturnal asthma, the mechanisms driving circadian changes in airway inflammation and function are not well understood. A portion of the circadian change in lung function might be controlled by the endogenous circadian pacemaker.¹²

A significant body of research has focused on the pathogenetic role of alterations in endogenous cortisol levels in patients with nocturnal asthma.¹³⁻¹⁷ In 1980, Barnes et al¹³ reported that there was no significant correlation between circadian variability in peak expiratory flow rates and plasma cortisol levels, and in 1991, Szeffler et al¹⁵ reported that serum cortisol levels did not vary between 4 AM and 4 PM in subjects with nocturnal asthma. Conversely, Haen et al¹⁴ reported in 1991 that asthmatic patients had significantly higher cortisol levels when compared with nonasthmatic control patients, and Kraft et al¹⁶ reported in 1998 that patients with nocturnal asthma had significantly lower cortisol levels than patients with nonnocturnal asthma and healthy control subjects at 8 PM. In 2002, Landstra et al¹⁷ reported that children with nocturnal asthma had significantly reduced serum cortisol levels when compared with control subjects at 12 AM, 8 AM, and 12 PM and that higher 24-hour mean serum cortisol levels were associated with higher airflow, as measured on the basis of FEV₁.

There is little information available to shed light on the importance of hypothalamic or pituitary control of cortisol secretion in nocturnal asthma. Exogenous administration of the hypothalamic hormone corticotropin-releasing hormone has been reported to improve overnight airflow in subjects with nocturnal asthma,¹⁸ but investigators have been unable to demonstrate significant differences between asthmatic and nonasthmatic control subjects in levels of the pituitary hormone corticotropin.¹⁹

The relationship between circadian rhythms and variations in lung function in healthy subjects was reported by Spengler and Shea¹² in 2000. Their research demonstrated that sleep deprivation did not affect pulmonary function, and they concluded that healthy adults demonstrate "small but significant" differences in spirometry over the 24-hour period caused, at least in part, by the endogenous circadian pacemaker. The role that the endogenous circadian pacemaker plays in the nocturnal worsening of airflow limitation

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in asthma remains to be determined, however, and it is likely that other physiologic and environmental processes also play an important role in the nocturnal asthma clinical phenotype.

We hypothesized that hypothalamic-pituitary-adrenal axis function differs between patients with nocturnal asthma and patients with nonnocturnal asthma and healthy control subjects and that this difference might have implications for the pathogenesis of nocturnal asthma.

METHODS

This study was approved by the National Jewish Medical and Research Center Institutional Review Board. All subjects provided written informed consent.

Classification of subjects

Nonsmoking healthy control subjects, patients with nocturnal asthma, and patients with nonnocturnal asthma were evaluated. Asthma was defined as follows: suggestive clinical history and either airway hyperresponsiveness indicated by a 20% or greater decrease in FEV₁ in response to inhalation of methacholine at 8 mg/mL or less or bronchodilator-responsive expiratory airflow limitation (FEV₁ of 55%-85% of predicted value, improving ≥200 mL and 12% in response to 180 µg of albuterol aerosol). Patients with nocturnal asthma met the above criteria and demonstrated an overnight decrease in peak flow of 20% or greater on at least 4 of 7 nights at home. Subjects were excluded if they had other lung diseases or other chronic illnesses, had used systemic or inhaled corticosteroids within 12 weeks, or had used leukotriene modifiers within 12 weeks. Antihistamine use was permitted.

Sleep-wake routine

Subjects maintained a regular sleep-wake routine for 7 sequential days before evaluation, sleeping at 10 PM and waking at 6 AM. No restrictions were placed on ambient light, bed position, or caloric intake. Sleep-wake cycle adherence was evaluated by using wrist actigraphy (ActiTrac; Individual Monitoring Systems Inc, Baltimore, Md).

In-laboratory evaluation

During a 24-hour period in which the sleep-wake cycle was maintained, an intravenous catheter was placed, and blood (5 mL) was withdrawn every 2 hours to obtain specimens for hormone analysis. Spirometry (Cybermedic, Louisville, Colo) was performed at bedtime and awakening.

Low-dose corticotropin stimulation

A subset of subjects in each group underwent low-dose corticotropin stimulation to assess adrenal cortisol production. Subjects returned to the laboratory on a different day at 7 AM without having repeated the minicostant routine. An intravenous catheter was placed, and blood was obtained for baseline serum cortisol measurement. One microgram of corticotropin (Organon Inc, West Orange, NJ) was then administered intravenously, and repeat blood sampling for cortisol was performed at 20 and 30 minutes after infusion.

Hormone analysis

Plasma corticotropin levels were assayed by means of immunochemiluminometric assay (Nichols Institute, San Juan Capistrano, Calif), and serum cortisol levels were assayed by means of enzyme immunoassay (Immunopharmacology laboratory, National Jewish Medical and Research Center, Denver, Colo).

Statistical analysis

Between-group differences in individual hormone levels were analyzed in 3 ways: (1) cosinor analysis of 24-hour pattern,²⁰ (2) pairwise comparisons of measured values at individual time points, and (3) comparison of the area under the 24-hour cosinor curve. Patterns of corticotropin and cortisol were obtained by fitting the following complex cosinor model by using a nonlinear mixed-effects model:

$$\text{ACTH}_k \text{ or cortisol}_k = \mu_k + \alpha_k \cos\left(\frac{2\pi \times \text{time}}{12} - \delta_{1k}\right) + 2\alpha_k \cos\left(\frac{2\pi \times \text{time}}{24} - \delta_{2k}\right).$$

This model was fitted to 3 groups (k = healthy control subjects, patients with nocturnal asthma, and patients with nonnocturnal asthma) simultaneously. Maximum and minimum corticotropin and cortisol levels and peak and trough times for these hormones were calculated and compared between groups.

All parameter estimations and comparisons were performed by using the SAS (v8.02; SAS Institute, Cary, NC) procedure NLMIXED.²¹ The area under the 24-hour curve was calculated by using subject-specific predictions of the above cosinor models and compared between the 3 groups by means of 1-way ANOVA. Results of corticotropin stimulation were analyzed by using 1-way ANOVA. A 2-tailed P value of less than .05 was considered statistically significant.

RESULTS

Subject characteristics

Seven patients with nocturnal asthma, 13 patients with nonnocturnal asthma, and 11 control subjects were evaluated. Demographic, spirometric, and corticosteroid use characteristics of subjects are presented in Table I. Subjects with nocturnal asthma had significantly lower bedtime percent predicted FEV₁, significantly lower morning absolute and percent predicted FEV₁, and greater overnight decrease in FEV₁ when compared with patients with nonnocturnal asthma and healthy control subjects. Median time since last use of inhaled or systemic corticosteroids ranged from 8 to 52 months.

Corticotropin

The 24-hour pattern in corticotropin level is shown in Fig 1. Peak corticotropin levels were highest in patients with nocturnal asthma (Table II), and pairwise comparisons revealed that corticotropin levels were significantly increased in patients with nocturnal asthma when compared with levels in both patients with nonnocturnal asthma and control subjects at 4 AM, 6 AM, 8 AM, and 10 AM (Fig 2). The area under the 24-hour corticotropin curve in patients with nocturnal asthma was significantly greater than that for patients with nonnocturnal asthma or control subjects (Table II). There were no significant between-group differences in time of peak or trough corticotropin levels (Table III).

Cortisol

The 24-hour circadian rhythm in cortisol is shown in Fig 2. Peak cortisol levels were highest in patients with nonnocturnal asthma, but this difference did not achieve significance (Table II). However, pairwise comparisons

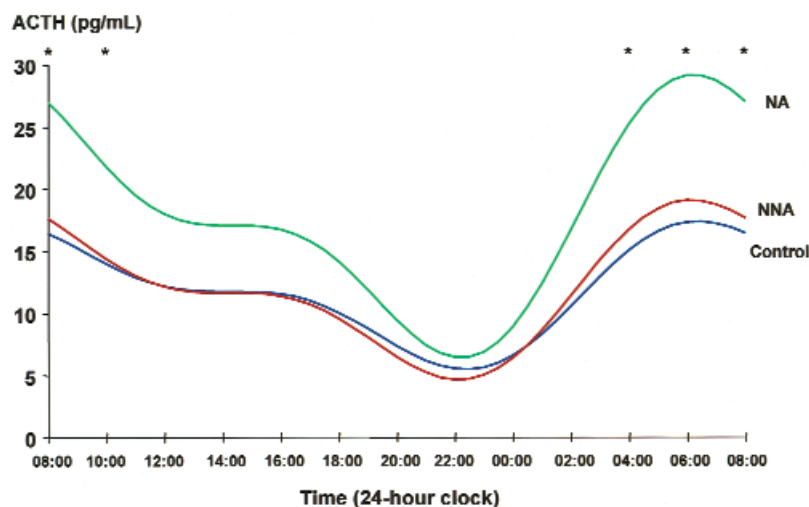


FIG 1. Modeled 24-hour corticotropin levels in patients with nocturnal asthma (green), patients with non-nocturnal asthma (red), and control subjects (blue). *Patients with nocturnal asthma > patients with non-nocturnal asthma and control subjects: $P < .05$ for both comparisons at 2 AM, 4 AM, 6 AM, 8 AM, and 10 AM.

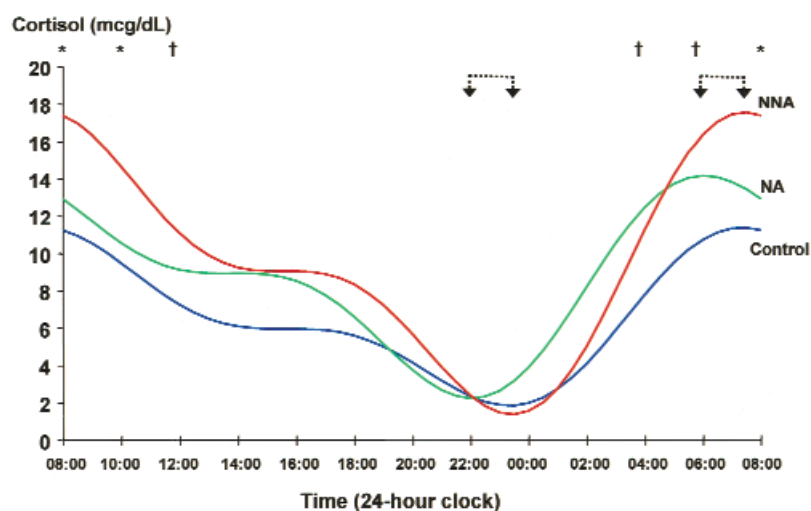


FIG 2. Modeled 24-hour cortisol levels in patients with nocturnal asthma (green), patients with non-nocturnal asthma (red), and control subjects (blue). Connected arrows indicate a phase difference in peak and trough cortisol times in patients with nocturnal asthma versus patients with non-nocturnal asthma and control subjects (see text). *Patients with non-nocturnal asthma > patients with nocturnal asthma and control subjects: $P < .05$ for both comparisons at 8 AM and 10 AM. †Patients with non-nocturnal asthma > control subjects: $P < .05$ at 4 AM, 6 AM, and 12 PM.

revealed that cortisol levels were significantly increased in patients with nonnocturnal asthma when compared with levels in patients with nocturnal asthma at 8 AM and 10 AM and with levels in control subjects at 4 AM, 6 AM, 8 AM, 10 AM, and 12 PM. Patients with nocturnal asthma had higher cortisol levels than control subjects at 2 AM and 4 AM. The area under the 24-hour cortisol curve in patients with nonnocturnal asthma was significantly greater versus that in control subjects but not that in patients with nocturnal asthma (Table II). The time of peak and trough cortisol levels was advanced approximately 90 minutes in patients with nocturnal asthma (Table III).

Low-dose corticotropin stimulation

A subset of subjects underwent low-dose corticotropin stimulation (Table IV) at 7 AM. To ensure that the subsets' hormonal rhythms reflected those of the groups as a whole, cosinor analysis was repeated to ensure that the subsets' circadian patterns did not differ from those of the complete groups. There were no significant between-group differences in baseline cortisol levels, 20- and 30-minute levels, or 20- and 30-minute percentage changes in response to low-dose corticotropin stimulation (Table IV).

TABLE I. Subject characteristics

	Patients with nocturnal asthma (n = 7)	Patients with nonnocturnal asthma (n = 13)	Control subjects (n = 11)	P value
Age (y)	35.7 ± 3.3	33.2 ± 2.5	32.3 ± 2.7	.72*
Sex (F/M)	3/4	8/5	7/4	.72†
Bedtime FEV ₁ (L, mean ± SEM)	2.84 ± 0.32	3.51 ± 0.24	3.84 ± 0.26	.071*
Bedtime FEV ₁ (% predicted)	69.7 ± 5.3	92.1 ± 3.5	97.8 ± 2.7	.0001*
Morning FEV ₁ (L, mean ± SEM)	2.40 ± 0.34	3.32 ± 0.25	3.89 ± 0.27	.008*
Morning FEV ₁ (% predicted)	53.1 ± 6.1	87.3 ± 3.7	98.9 ± 2.8	<.0001*
Overnight % change in FEV ₁ (mean ± SEM)	-18.8 ± 2.3	-5.1 ± 2.1	1.5 ± 2.3	<.0001*
Prior inhaled steroid use (n)	3	5	0	1.0†
Last inhaled steroid use (mo, median)	8	13	NA	.67‡
Prior use of systemic corticosteroids (n)	4	4	1	.09†
Most recent systemic steroid use (mo, median)	27.5	10.5	52	.44‡

*One-way ANOVA.

†Fisher exact test.

‡Kruskal-Wallis test.

TABLE II. Peak observed hormone levels and areas under the 24-hour cosinor curve (mean ± SEM) in patients with nocturnal asthma, patients with nonnocturnal asthma, and healthy control subjects

	Patients with nocturnal asthma (n = 7)	Patients with nonnocturnal asthma (n = 13)	Control subjects (n = 11)
Peak corticotropin level (pg/mL)	29.1 ± 2.6*	19.0 ± 1.9	17.3 ± 2.1
Corticotropin AUC (pg·h ⁻¹ ·mL ⁻¹)	420.3 ± 46.4†	283.5 ± 41.3	276.0 ± 24.0
Peak cortisol level (μg/dL)	14.1 ± 1.5	16.3 ± 1.1	10.7 ± 1.2
Cortisol AUC (μg·h ⁻¹ ·dL ⁻¹)	201.9 ± 25.0	225.4 ± 25.5‡	152.7 ± 11.6

AUC, Area under the 24-hour curve.

*P = .001 for patients with nocturnal asthma versus control subjects, P = .004 for patients with nocturnal asthma versus patients with nonnocturnal asthma.

†P = .02 for patients with nocturnal asthma versus control subjects and patients with nocturnal asthma versus patients with nonnocturnal asthma.

‡P = .019 for comparison of patients with nonnocturnal asthma versus control subjects, P > .05 for comparison of patients with nonnocturnal asthma versus patients with nocturnal asthma.

Correlation of spirometry and peak hormone levels

Simple linear regression was performed to evaluate the relationship between peak measured corticotropin and cortisol levels and FEV₁ percent predicted at both bedtime and awakening to determine whether differences in hormone levels were correlated with asthma severity rather than asthma phenotype. Bedtime FEV₁ was not significantly correlated with peak corticotropin (Pearson $r = 0.18$, $P = .32$) or cortisol (Pearson $r = 0.12$, $P = .54$) levels. A similar lack of correlation was seen between morning FEV₁ and peak corticotropin (Pearson $r = 0.12$, $P = .51$) or cortisol (Pearson $r = -0.06$, $P = .76$) levels.

DISCUSSION

Our results suggest that the nocturnal asthma phenotype is marked by increased levels of corticotropin but that this increase in corticotropin is not accompanied by a commensurate increase in cortisol. Despite the increase of corticotropin in patients with nocturnal asthma, the area under the 24-hour cortisol curve was greatest in patients with nonnocturnal asthma. These differences appear to be specific to the nocturnal asthma phenotype because peak hormone levels are not correlated with the degree of physiologic impairment. Low-dose corti-

cotropin stimulation at 7 AM was unable to detect differences between the 3 groups of subjects.

Enhanced nighttime airway inflammation in nocturnal asthma might provide a stimulus for increased endogenous anti-inflammatory activity in the form of increased serum cortisol levels. To achieve this, the anterior pituitary gland would be expected to provide appropriate hormonal signaling through the release of corticotropin. Our observation of increased corticotropin peak levels and areas under the 24-hour curve in nocturnal asthma suggests an appropriate pituitary response. However, despite the increase in corticotropin, the adrenal gland does not respond with increases in serum cortisol levels in subjects with nocturnal asthma. A number of scenarios could explain this. First, there might be inhibition of cortisol secretion or release in response to corticotropin in nocturnal asthma, or alternatively, there might be an enhanced and therefore protective response to corticotropin in nonnocturnal asthma, such that disproportionate increases in corticotropin levels are not required. Additionally, there might be defects at the intra-adrenal level, with inhibition of the adrenal cortex or decreased direct stimulation of adrenocortical steroidogenesis by the adrenal medulla.²²

Low-dose corticotropin stimulation testing has been promoted as a sensitive method for detecting adrenal suppression in patients receiving corticosteroid thera-

Asthma, rhinitis,
other respiratory
diseases

TABLE III. Calculated time of peak and trough hormone levels (24-hour clock time) in patients with nocturnal asthma, patients with nonnocturnal asthma, and healthy control subjects

	Patients with nocturnal asthma (n = 7)	Patients with nonnocturnal asthma (n = 13)	Control subjects (n = 11)
Corticotropin peak time	06:14	06:09	06:23
Corticotropin trough time	22:14	22:09	22:23
Cortisol peak time	06:03*	07:26	07:21
Cortisol trough time	22:03†	23:26	23:21

*P = .019 for comparison with control subjects.

†P = .002 for comparison with patients with nonnocturnal asthma.

TABLE IV. Results of low-dose corticotropin stimulation in a subset of patients with nocturnal asthma, patients with nonnocturnal asthma, and healthy control subjects

	Patients with nocturnal asthma (n = 3)	Patients with nonnocturnal asthma (n = 8)	Control subjects (n = 7)
Baseline cortisol (μg/dL)	15.6 ± 2.2	15.0 ± 1.4	14.0 ± 1.9
20-min cortisol value (μg/dL)	21.3 ± 2.8	22.0 ± 1.7	20.5 ± 2.4
20-min change (%)	40.6 ± 17.4	50.5 ± 10.7	51.5 ± 15.1
30-min cortisol value (μg/dL)	22.7 ± 3.1	22.8 ± 1.9	21.4 ± 2.7
30-min change (%)	51.1 ± 18.4	55.6 ± 11.2	57.1 ± 15.9

P > .05 for all comparisons.

py,²³ and formal dose-response testing might also enhance detection of adrenal suppression.²⁴ Despite our use of the single low-dose test, we were unable to detect differences in adrenal response to corticotropin at 7 AM. Although the reasons for this are unclear, the 90-minute phase advance in cortisol peak and trough seen in patients with nocturnal asthma might affect the response to corticotropin stimulation in the early morning hours. Alternatively, the increase in endogenous corticotropin seen in patients with nocturnal asthma at 7 AM might already be fully stimulating the adrenal gland, preventing detection of any between-group differences. Additionally, even though the 1-μg dose of corticotropin used in these experiments is a fraction of that used in routine corticotropin stimulation, it likely results in serum corticotropin concentrations greater than the *in vivo* range of 5 to 30 pg/mL that we observed in these experiments. This might serve to further diminish the ability to detect differences between the groups.

A number of methodological features distinguish these experiments from prior research in this area. First, attempts were made to control subjects' sleep-wake cycles in an attempt to standardize any environmental cues that might affect nocturnal worsening of asthma. Although a formal "constant routine" protocol²⁵ would have more rigorously controlled these cues, such a protocol differs so dramatically from typical human experience that the clinical relevance of our results would be difficult to interpret. Second, we sampled hormone levels at 2-hour intervals, a frequency twice that of many other previous investigations. Despite this increased sampling frame, cortisol secretion has been reported to occur in episodic bursts,²⁶ and it is possible that even 2-hour intervals were inadequate for description of the details of pituitary-adrenal interaction. Finally, the cosinor analysis

used in this study allows comparisons at individual time points but also facilitates modeling of the 24-hour pattern of hormone flux, allowing comparison of peak and trough times and calculation of the area under the curve. This analytic technique more accurately reflects events over the entire 24-hour period and allows inferences to be made not from changes between individual time points or from 24-hour mean levels¹⁷ but from modeled changes over the course of time.

Our results have implications for both the clinical management of nocturnal asthma and the direction of future research. The phase advance of cortisol peak and trough time in nocturnal asthma demonstrated by our research might partly explain why chronotherapeutic dosing of corticosteroids improves lung function and reduces airway inflammation^{27,28} in these patients. The observation of higher nighttime levels of cortisol in nonnocturnal asthma raises the possibility that exogenous corticosteroids might be a less effective therapeutic intervention in nonnocturnal asthma versus nocturnal asthma. Additional research will be required to determine whether adrenal or extra-adrenal factors explain the disproportionate increase of corticotropin in nocturnal asthma.

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REFERENCES

1. Martin RJ. Location of airway inflammation in asthma and the relationship to circadian change in lung function. *Chronobiol Int* 1999;16:623-30.
2. Martin RJ, Cicutto LC, Ballard RD. Factors related to the nocturnal worsening of asthma. *Am Rev Respir Dis* 1990;141:33-8.
3. Van Keimpema AR, Ariaansz M, Tamminga JJ, Nauta JJ, Postmus PE. Nocturnal waking and morning dip of peak expiratory flow in clinically

- stable asthma patients during treatment. Occurrence and patient characteristics. *Respiration* 1997;64:29-34.
4. Guidelines for the diagnosis and management of asthma. Bethesda, Md: National Heart, Lung, and Blood Institute; 1997.
 5. Kraft M, Djukanovic R, Wilson S, Holgate ST, Martin RJ. Alveolar tissue inflammation in asthma. *Am J Respir Crit Care Med* 1996;154:1505-10.
 6. Kraft M, Martin RJ, Wilson S, Djukanovic R, Holgate ST. Lymphocyte and eosinophil influx into alveolar tissue in nocturnal asthma. *Am J Respir Crit Care Med* 1999;159:228-34.
 7. Frick WE, Sedgwick JB, Busse WW. The appearance of hypodense eosinophils in antigen-dependent late phase asthma. *Am Rev Respir Dis* 1989;139:1401-6.
 8. Calhoun WJ, Bates ME, Schrader L, Sedgwick JB, Busse WW. Characteristics of peripheral blood eosinophils in patients with nocturnal asthma. *Am Rev Respir Dis* 1992;145:577-81.
 9. Kraft M, Hamid Q, Chrousos GP, Martin RJ, Leung DY. Decreased steroid responsiveness at night in nocturnal asthma. Is the macrophage responsible? *Am J Respir Crit Care Med* 2001;163:1219-25.
 10. Kraft M, Vianna E, Martin RJ, Leung DYM. Nocturnal asthma is associated with reduced glucocorticoid receptor binding affinity and decreased steroid responsiveness at night. *J Allergy Clin Immunol* 1999;103:66-71.
 11. Turki J, Pak J, Green SA, Martin RJ, Liggett SB. Genetic polymorphisms of the beta 2-adrenergic receptor in nocturnal and nonnocturnal asthma. Evidence that Gly16 correlates with the nocturnal phenotype. *J Clin Invest* 1995;95:1635-41.
 12. Spengler CM, Shea SA. Endogenous circadian rhythm of pulmonary function in healthy humans. *Am J Respir Crit Care Med* 2000;162:1038-46.
 13. Barnes P, Fitzgerald G, Brown M, Dollery C. Nocturnal asthma and changes in circulating epinephrine, histamine, and cortisol. *N Engl J Med* 1980;303:263-7.
 14. Haen E, Hauck R, Emslander HP, Langenmayer I, Liebl B, Schopohl J, et al. Nocturnal asthma. Beta 2-adrenoceptors on peripheral mononuclear leukocytes, cAMP- and cortisol-plasma concentrations. *Chest* 1991;100:1239-45.
 15. Szeffler SJ, Ando R, Cicutto LC, Surs W, Hill MR, Martin RJ. Plasma histamine, epinephrine, cortisol, and leukocyte beta-adrenergic receptors in nocturnal asthma. *Clin Pharmacol Ther* 1991;49:59-68.
 16. Kraft M, Pak J, Martin RJ. Serum cortisol in asthma: marker of nocturnal worsening of symptoms and lung function? *Chronobiol Int* 1998;15:85-92.
 17. Landstra AM, Postma DS, Boezen HM, van Aalderen WM. Role of serum cortisol levels in children with asthma. *Am J Respir Crit Care Med* 2002;165:708-12.
 18. Georges G, Kraft M, Vianna EO, Martin RJ. Human corticotropin-releasing hormone improves overnight FEV1 in nocturnal asthma. *J Asthma* 1998;35:261-5.
 19. Morrish DW, Sproule BJ, Aaron TH, Outhet D, Crockford PM. Hypothalamic-pituitary-adrenal function in extrinsic asthma. *Chest* 1979;75:161-6.
 20. Halberg F, Tong YL, Johnson EA. Circadian systems phase—an aspect of temporal morphology: procedures and illustrative examples. In: von Mayersbach H, editor. *The cellular aspects of biorhythms*. Berlin: Springer Verlag; 1967. p. 20-48.
 21. SAS/STAT user's guide: version 8. Cary, NC: SAS Institute, Inc; 1999. p. 2419-504.
 22. Bornstein SR, Chrousos GP. Clinical review 104: Adrenocorticotropin (ACTH)- and non-ACTH-mediated regulation of the adrenal cortex: neural and immune inputs. *J Clin Endocrinol Metab* 1999;84:1729-36.
 23. Broide J, Soferman R, Kivity S, Golander A, Dickstein G, Spirer Z, et al. Low-dose adrenocorticotropin test reveals impaired adrenal function in patients taking inhaled corticosteroids. *J Clin Endocrinol Metab* 1995;80:1243-6.
 24. Laue L, Peck GL, Loriaux DL, Gallucci W, Chrousos GP. Adrenal androgen secretion in postadolescent acne: increased adrenocortical function without hypersensitivity to adrenocorticotropin. *J Clin Endocrinol Metab* 1991;73:380-4.
 25. Czeisler CA, Allan JS, Strogatz SH, Ronda JM, Sanchez R, Rios CD, et al. Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. *Science* 1986;233:667-71.
 26. Hellman L, Nakada F, Curti J, Weitzman ED, Kream J, Roffwarg H, et al. Cortisol is secreted episodically by normal man. *J Clin Endocrinol Metab* 1970;30:411-22.
 27. Beam WR, Weiner DE, Martin RJ. Timing of prednisone and alterations of airways inflammation in nocturnal asthma. *Am Rev Respir Dis* 1992;146:1524-30.
 28. Pincus DJ, Szeffler SJ, Ackerson LM, Martin RJ. Chronotherapy of asthma with inhaled steroids: the effect of dosage timing on drug efficacy. *J Allergy Clin Immunol* 1995;95:1172-8.